

The Ministry of Health, Labour and Welfare Ministerial Notification No. 285

Pursuant to Paragraph 1, Article 41 of the Pharmaceutical Affairs Law (Law No. 145, 1960), the Japanese Pharmacopoeia (hereinafter referred to as “new Pharmacopoeia”), which has been established as follows*, shall be applied on April 1, 2006, and the Ministry of Health, Labour and Welfare Ministerial Notification No. 111 (Matter of Establishing the Japanese Pharmacopoeia; hereinafter referred to as “previous Pharmacopoeia”), issued in 2001, shall be abolished on March 31, 2006. However, in the case of drugs which are listed in the new Pharmacopoeia (limited to those listed in the previous Pharmacopoeia) and drugs which have been approved as of April 1, 2006 as prescribed under Paragraph 1, Article 14 of the same law [including drugs the Minister of Health, Labour and Welfare specifies (the Ministry of Health and Welfare Ministerial Notification No. 104, 1994) as those exempted from marketing approval pursuant to Paragraph 1, Article 14 of the Pharmaceutical Affairs Law (hereinafter referred to as “drugs exempted from approval”)], the Name and Standards established in the previous Pharmacopoeia (limited to part of the Name and Standards for the drugs concerned) may be accepted to conform to the Name and Standards established in the new Pharmacopoeia before and on September 30, 2007. In the case of drugs which are listed in the new Pharmacopoeia (excluding those listed in the previous Pharmacopoeia) and drugs which have been approved as of April 1, 2006 as prescribed under Paragraph 1, Article 14 of the same law (including those exempted from approval), they may be accepted as those being not listed in the new Pharmacopoeia before and on September 30, 2007.

Jiro Kawasaki

The Minister of Health, Labour and Welfare

March 31, 2006

Referring the next title to this book: “The Japanese Pharmacopoeia”.

(The text referred to by the term “as follows” are omitted here. All of them are made available for public exhibition at the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, at each Regional Bureau of Health and Welfare, and at each Prefectural Office in Japan).

*The term “as follows” here indicates the contents of the Japanese Pharmacopoeia Fifteenth Edition from General Notices to Ultraviolet-visible Reference Spectra (pp. 1 - 1654).

CONTENTS

Preface	i
The Japanese Pharmacopoeia, Fifteenth Edition	1
General Notices	1
General Rules for Crude Drugs	5
General Rules for Preparations	7
General Tests, Processes and Apparatus	17
1. Chemical Methods	
1.01 Alcohol Number Determination.....	17
1.02 Ammonium Limit Test.....	19
1.03 Chloride Limit Test.....	20
1.04 Flame Coloration Test.....	20
1.05 Mineral Oil Test.....	20
1.06 Oxygen Flask Combustion Method.....	20
1.07 Heavy Metals Limit Test.....	21
1.08 Nitrogen Determination (Semimicro- Kjeldahl Method).....	22
1.09 Qualitative Tests.....	23
1.10 Iron Limit Test.....	28
1.11 Arsenic Limit Test.....	29
1.12 Methanol Test.....	30
1.13 Fats and Fatty Oils Test.....	31
1.14 Sulfate Limit Test.....	33
1.15 Readily Carbonizable Substances Test.....	33
2. Physical Methods	
Chromatographys	
2.01 Liquid Chromatography.....	33
2.02 Gas Chromatography.....	35
2.03 Thin-layer Chromatography.....	37
Spectroscopic Methods	
2.21 Nuclear Magnetic Resonance Spectroscopy.....	38
2.22 Fluorometry.....	40
2.23 Atomic Absorption Spectrophotometry.....	40
2.24 Ultraviolet-visible Spectrophotometry.....	41
2.25 Infrared Spectrophotometry.....	43
Other Physical Methods	
2.41 Loss on Drying Test.....	44
2.42 Congealing Point Determination.....	44
2.43 Loss on Ignition Test.....	45
2.44 Residue on Ignition Test.....	45
2.45 Refractive Index Determination.....	46
2.46 Residual Solvents Test.....	46
2.47 Osmolarity Determination.....	46
2.48 Water Determination (Karl Fischer Method).....	47
2.49 Optical Rotation Determination.....	50
2.50 Endpoint Detection Methods in Titrimetry.....	51
2.51 Conductivity Measurement.....	53
2.52 Thermal Analysis.....	54
2.53 Viscosity Determination.....	56
2.54 pH Determination.....	59
2.55 Vitamin A Assay.....	60
2.56 Determination of Specific Gravity and Density.....	61
2.57 Boiling Point and Distilling Range Test.....	63
2.58 X-Ray Powder Diffraction Method.....	64
2.59 Test for Total Organic Carbon.....	65
2.60 Melting Point Determination.....	66
3. Powder Property Determinations	
3.01 Determination of Bulk and Tapped Densities.....	68
3.02 Specific Surface Area by Gas Adsorption.....	69
3.03 Powder Particle Density Determination.....	71
3.04 Particle Size Determination.....	72
4. Biological Tests/Biochemical Tests/ Microbial Tests	
4.01 Bacterial Endotoxins Test.....	77
4.02 Microbial Assay for Antibiotics.....	81
4.03 Digestion Test.....	84
4.04 Pyrogen Test.....	87
4.05 Microbial Limit Test.....	88
4.06 Sterility Test.....	93
5. Tests for Crude Drugs	
5.01 Crude Drugs Test.....	97
5.02 Microbial Limit Test for Crude Drugs....	100
6. Tests for Preparations	
6.01 Test for Metal Particles in Ophthalmic Ointments.....	106
6.02 Uniformity of Dosage Units.....	106
6.03 Particle Size Distribution Test for Preparations.....	109
6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines.....	109
6.05 Test for Extractable Volume of Parenteral Preparations.....	110
6.06 Foreign Insoluble Matter Test for Injections.....	110
6.07 Insoluble Particulate Matter Test for Injections.....	111
6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions.....	113
6.09 Disintegration Test.....	114
6.10 Dissolution Test.....	116

7. Tests for Containers and Packing Materials	
7.01 Test for Glass Containers for Injections..	120
7.02 Test Methods for Plastic Containers.....	121
7.03 Test for Rubber Closure for Aqueous Infusions.....	127
8. Other Methods	
8.01 Sterilization and Aseptic Manipulation, and Reverse Osmosis-Ultrafiltration....	128
9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, Thermometers, etc.	
Reference Standards	
9.01 Reference Standards.....	129
Standard Solutions	
9.21 Standard Solutions for Volumetric Analysis.....	132
9.22 Standard Solutions.....	143
9.23 Matching Fluids for Color.....	145
Reagents, Test Solutions, etc.	
9.41 Reagents, Test Solutions.....	146
9.42 Solid Supports/Column Packing for Chromatography.....	259
9.43 Filter Paper, Filters for Filtration, Test Papers, Crucibles, etc.....	261
9.44 Reference Particles, etc.....	261
Measuring Instruments and Appliances, Thermometers, etc.	
9.61 Optical Filters for Wavelength and Transmission Rate Calibration.....	262
9.62 Measuring Instruments, Appliances.....	262
9.63 Thermometers.....	263
Official Monographs.....	265
Crude Drugs.....	1251
Infrared Reference Spectra.....	1373–1518
Ultraviolet-visible Reference Spectra.....	1519–1654
General Information	
1. Amino Acid Analysis.....	1655
2. Aristolochic Acid.....	1662
3. Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia.....	1663
4. Capillary Electrophoresis.....	1675
5. Decision of Limit for Bacterial Endotoxins.....	1680
6. Disinfection and Sterilization Methods.....	1680
7. Guideline for Residual Solvents, Residual Solvents Test, and Models for the Test in Monographs.....	1682
8. International Harmonization Implemented in the Japanese Pharmacopoeia Fifteenth Edition.....	1684
9. Isoelectric Focusing.....	1708
10. Laser Diffraction Measurement of Particle Size.....	1710
11. Media Fill Test.....	1712
12. Microbial Attributes of Nonsterile Pharmaceutical Products.....	1715
13. Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products.....	1717
14. Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products.....	1721
15. Peptide Mapping.....	1724
16. pH Test for Gastrointestinal Medicine.....	1727
17. Plastic Containers for Pharmaceutical Products.....	1728
18. Powder Flow.....	1729
19. Preservatives-Effectiveness Tests.....	1732
20. Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards.....	1734
21. Quality Control of Water for Pharmaceutical Use.....	1736
22. Rapid Identification of Microorganisms Based on Molecular Biological Method...	1740
23. SDS-Polyacrylamide Gel Electrophoresis....	1742
24. Solid and Particle Densities.....	1746
25. Sterility Assurance for Terminally Sterilized Pharmaceutical Products.....	1748
26. Tablet Friability Test.....	1751
27. Terminal Sterilization and Sterilization Indicators.....	1752
28. Test for Trace Amounts of Aluminum in Trans Parenteral Nutrition (TPN) Solutions.....	1754
29. Total Protein Assay.....	1756
30. Validation of Analytical Procedures.....	1760
Appendix	
Atomic Weight Table (2004).....	1763
Standard Atomic Weights 2004.....	1764
Index.....	1765
Index in Latin name.....	1777
Index in Japanese.....	1779

PREFACE

The 14th Edition of the Japanese Pharmacopoeia (JP) was promulgated by Ministerial Notification No. 111 of the Ministry of Health, Labour and Welfare (MHLW) on March 30, 2001. To keep pace with progress in medical and pharmaceutical sciences, in November 2001, the Committee on JP established the basic principles for the preparation of the JP 15th Edition, setting out the characteristics and roles of the JP, the definite measures for the revision, the date of the revision, and the organization of the JP expert committees.

At the above Committee, the five basic principles of JP, which we refer to as the “Five Pillars of JP” were established as follows.:

- 1) Making the JP more substantial by including all drugs which are important from the viewpoint of health care and medical treatment;
- 2) Making prompt partial revision as necessary and facilitating smooth administrative operation;
- 3) Promoting international harmonization;
- 4) Ensuring transparency regarding the revision and dissemination to the public of the JP;
- 5) Promoting the introduction of new analytical technology and appropriate modifications to existing science and technology, and promoting the improvement of reference standards.

It was agreed that the Committee on JP should make efforts, on the basis of the Five Pillars of JP, to ensure that the JP is used effectively in health care and medical treatment, by seeking the understanding and cooperation for interested parties.

The JP should provide an official standard, being required to assure the quality of medicines in Japan in response to the progress of science and technology and medical demands at the time. It should define the standards for specifications, as well as the methods of testing to assure the overall quality of every drug in principle, and it should have a role in clarifying the criteria for quality assurance of drugs that are recognized to be essential for public health and medical treatment.

The JP has been prepared with the aid of the knowledge and experience of many professionals in the pharmaceutical field. Therefore, JP should have the characteristics of an official standard, which might be widely used by all parties concerned. It should provide information and understanding about the quality of drugs to the public, and it should be conducive to

smooth and effective regulatory control of the quality of drugs, as well as promoting and maintaining international consistency and harmonization of technical requirements.

It was also agreed that JP articles should cover drugs which are important from the viewpoint of health care and medical treatment based on demand, frequency of use and clinical results. It should ensure prompt pharmacopoeial listing after the introduction of drugs to the market.

A definite rule for selection of articles and a clarification of criteria for selection were also established. The rule was described by the Pharmaceutical Affairs and Food Sanitation Council (PAFSC) in an article entitled “What the future Japanese Pharmacopoeia should be” on December 2002. The target date for the publication of JP 15th Edition (the Japanese edition) was set as April 2006.

At the first stage, JP Expert Committees were organized into the following twelve Panels and two sub-committees: Panel on the Principles of Revisions; Panel on the Selection of Articles; Panel on Nomenclature; Panel on Pharmaceutical Excipients; First Panel on Chemical Drugs, Second Panel on Chemical Drugs; Panel on Biologicals; Panel on Biological Tests; Panel on Physico-Chemical Methods; Panel on Physical Methods; Panel on Preparations; Panel on Crude Drugs; First Sub-committee on the Principles of Revisions; First Sub-committee on Crude Drugs.

In November 2001, the JP Expert Committees were reorganized into the following eleven Panels in accordance with the recommendation of the PAFSC: Panel on the Principles of Revisions; Panel on Nomenclature; Panel on Pharmaceutical Excipients; Panel on Physico-Chemical Methods; Panel on Medicinal Chemicals; Panel on Biologicals; Panel on Biological Tests; Panel on Antibiotics; Panel on Crude Drugs; Sub-committee on the Principles of Revisions; Panel on International Harmonization (Pharmacopoeial Discussion Group (PDG) related Panel). Thereafter, the Panel on Pharmaceutical Water, Panel on Reference Standards, and three working groups under the Panel on Medicinal Chemicals were established to expedite discussion of revision drafts of Monographs.

In the Committee on JP, Mitsuru Uchiyama took the role of chairman from January 2001 to December 2002, Tadao Terao from January to June 2003, and Takao Hayakawa from July 2003 to March 2006.

In addition to the regular revision every five years in line with the basic principles for the preparation of the JP, it was agreed that partial revision should be done as necessary to take account of recent progress of science and in the interests of international harmonization.

In accordance with the above principles, the expert committees initiated deliberations on selection of articles, and revisions for General Notices, General Rules for Preparations, General Tests, Monographs and so on.

Draft revisions covering subjects, the addition to General Notices of a sentence “In principle, unless otherwise specified, animals used as a source of materials for preparing pharmaceutical preparations must be healthy” and the deletion of a monograph “Phenacetin” were examined by the Committee on JP in November 2001, followed by the PAFSC in December 2001, and then submitted to the MHLW.

The revision was promulgated on March 29, 2002 by Ministerial Notification No.151 of the MHLW.

For draft revisions covering subjects in General Notices, General Rules for Preparations, General Rules for Crude Drugs, General Tests and Monographs, deliberations were continued between June 2000 and February 2002, prepared for a supplement to the JP 14, examined by the Committee on JP in September 2002, followed by the PAFSC in December 2002, and then submitted to the Minister of MHLW. The supplement was named “Supplement I to the JP 14th Edition” and promulgated on December 27, 2002 by Ministerial Notification No. 395 of MHLW.

The numbers of meetings in the above process to prepare the supplement drafts were as follows: Panel on the Principles of Revisions (6 times); Panel on the Selection of Articles (2); Panel on Nomenclature (11); Panel on Pharmaceutical Excipients (10); First Committee on Chemical Drugs (10); Second Committee on Chemical Drugs (16); Committee on Biologicals (8); Committee on Biological Methods (9); Committee on Physico-Chemical Methods (8); Committee on Physical Methods (8); Committee on Pharmaceutical Preparations (5); Committee on Crude Drugs (6); First Sub-committee on the Principles of Revisions (27); First Sub-committee on Crude Drugs (7). The numbers of meetings of newly reorganized Panels were as follows, Panel on Nomenclature (2), Panel on Physico-Chemical Methods (1), Panel on Crude Drugs (3), and Sub-panel on the Principles of Revisions (1).

In consequence of the above revision, the JP 14th Edition with Supplement I carries 881 articles in Part I, including 31 articles newly added and 8 articles deleted; and 481 articles in Part II, including 15 articles newly added and 3 articles deleted. It should be noted that in the preparation of the drafts for the Supplement I,

generous cooperation was given by the Technical Committee of the Pharmaceutical Manufacturer’s Association of Osaka (OPMA) and of Tokyo (PMAT), the Tokyo Crude Drugs Association (TCDA), the Japan Pharmaceutical Excipients Council (JPEC), the Japan Kampo Medicine Manufacturers’ Association (JKMA), the Japan Antibiotics Research Association (JARA), the Japan Flavor and Fragrance Materials Association (JFFMA), the Japan Medical Plants Federation (JMPF), the Japan Pharmaceutical Manufacturers Association (JPMA), the Japanese Society of Hospital Pharmacists (JSHP), the Japan Pharmaceutical Association (JPA), and the Japan Oilseeds Processors Association (JOPA).

Draft revisions covering subjects in General Rules for Preparations, General Tests and Monographs, for which discussions were completed between March 2002 and December 2003, were prepared for a supplement to the JP 14. They were examined by the Committee on JP in September 2004, followed by the PAFSC in December 2004, and then submitted to the Minister of Health, Labour and Welfare.

The supplement was named “Supplement II to the JP 14th Edition” and promulgated on December 28, 2004 by Ministerial Notification No.461 of MHLW.

The numbers of meetings in the expert committees to prepare the supplement drafts were as follows: Panel on the Principles of Revisions (9); Panel on Nomenclature (10); Panel on Pharmaceutical Excipients (11); Panel on Physico-Chemical Methods (30); Panel on Medicinal Chemicals (containing the WG) (24); Panel on Biologicals (11); Panel on Biological Tests (10); Panel on Antibiotics (19); Committee on Crude Drugs (19); Sub-panel on the Principles of Revisions (12); Panel on International Harmonization (PDG related Panel) (9); Panel on Pharmaceutical Water (2); Panel on Reference Standards (3).

In consequence of this revision, the JP 14th Edition including the Supplement I and II carries 907 articles in Part I, including 27 articles newly added and 1 article deleted; and 484 articles in Part II, including 12 articles newly added and 9 articles deleted. It should be noted that in the preparation of the drafts for the Supplement II, generous cooperation was given by the Technical Committee of OPMA and PMAT, TCDA, JPEC, JKMA, JARA, JFFMA, JMPF, JPMA, JSHP, JPA, and JOPA.

Draft revisions covering subjects, the addition to General Notices of a sentence “The items in General Test, Monographs (excipients) or General Information in JP, which reflect text signed-off by all three pharmacopoeias, JP, EP and USP (referred to as “the signed-off item”), are stated at the front”, the addition of a test to General Tests “Test for Extractable Volume

of Parenteral Preparations” and the revisions in General Notices for Preparations and Monographs due to the addition of the test, were examined by the Committee on JP in June 2005, followed by the PAFSC, and then submitted to the Minister MHLW.

The revision was promulgated on July 21, 2005 by Ministerial Notification No. 344 of MHLW.

Draft revisions covering subjects in General Notices, General Rules for Crude Drugs, General Rules for Preparations, General Tests and Monographs, for which discussions were finished between January 2004 and August 2005, were decided as a supplement to the JP 15. They were examined by the Committee on JP in October 2005, followed by the PAFSC in December 2005, and then submitted to the Minister of MHLW.

The numbers of meetings in the expert committees to prepare the supplement drafts were as follows: Panel on the Principles of Revisions (2); Panel on Nomenclature (2); Panel on Pharmaceutical Excipients (3); Panel on Physico-Chemical Methods (6); Panel on Medicinal Chemicals (including the working groups) (17); Panel on Biologicals (3); Panel on Biological Tests (2); Panel on Antibiotics (6); Panel on Crude Drugs (6); Panel on International Harmonization (PDG) (2); Panel on Pharmaceutical Water (2); Panel on Reference Standards (2).

In April 2004, the Pharmaceuticals and Medical Devices Agency (PMDA) was established, and a part of the organization for JP preparation was moved from MHLW to PMDA. Since July 2004, the numbers of discussions in the expert committees to prepare the supplement drafts were as follows: Panel on the Principles of Revisions (6); Panel on International Harmonization (PDG related)(3); Panel on Pharmaceutical Water (7); Panel on Reference Standards (4); Panel on Physico-Chemical Methods (6); Panel on Drug Formulation (7); Panel on Physical Methods (9); Panel on Medicinal Chemicals (containing the WG) (32); Panel on Biologicals (6); Panel on Antibiotics (9); Panel on Biological Tests (6); Panel on Crude Drugs (12); Panel on Nomenclature (8); Panel on Pharmaceutical Excipients (7). It should be noted that in the preparation of the drafts for the supplement, generous cooperation was given by OPMA and PMAT, TCDA, JPEC, JKMA, JARA, JFMA, JMPF, JPMA, ISHP, JPA, and JOPA.

In consequence of this revision, the JP 15th Edition carries 1483 articles, owing to the addition of 102 articles and the deletion of 8 articles.

The principles of description and the salient points of the revision in this volume are as follows:

1. The JP 15th Edition comprises the following items, in order: Notification of MHLW; Contents;

Preface; General Notices; General Rules for Crude Drugs; General Rules for Preparations; General Tests, Processes and Apparatus; Official Monographs; then followed by Infrared Reference Spectra; Ultraviolet-visual Reference Spectra; General Information; Table of Atomic Mass as an appendix; and a Cumulative Index.

2. The articles in General Rules for Preparations, Official Monographs, Infrared Reference Spectra Official and Ultraviolet-visible Reference Spectra are respectively placed in alphabetical order.

3. The following items in each monograph are put in the order shown below, except that unnecessary items are omitted depending on the nature of the drug:

- (1) English title
- (2) Commonly used name(s)
- (3) Latin title (only for crude drugs)
- (4) Title in Japanese
- (5) Structural formula or empirical formula
- (6) Molecular formula and molecular mass
- (7) Chemical name
- (8) Origin
- (9) Limits of the content of the ingredient(s) and/or the unit of potency
- (10) Labeling requirements
- (11) Method of preparation
- (12) Description/Description of crude drugs
- (13) Identification tests
- (14) Specific physical and/or chemical values
- (15) Purity tests
- (16) Loss on drying, loss on ignition, and/or water
- (17) Residue on ignition, total ash, and/or acid-insoluble ash
- (18) Tests being required for pharmaceutical preparations and other special tests
- (19) Isomer ratio
- (20) Assay or the content of the ingredient(s)
- (21) Containers and storage
- (22) Expiration date
- (23) Others

4. In each monograph, the following physical and chemical values representing the properties and quality of the drug are given in the order indicated below, except that unnecessary items are omitted depending on the nature of the drug:

- (1) Alcohol number
- (2) Absorbance
- (3) Congealing point
- (4) Refractive index
- (5) Osmolarity
- (6) Optical rotation
- (7) Viscosity

- (8) pH
- (9) Specific gravity
- (10) Boiling point
- (11) Melting point
- (12) Acid value
- (13) Saponification value
- (14) Ester value
- (15) Hydroxyl value
- (16) Iodine value

5. Identification tests comprise the following items, which are generally put in the order given below:

- (1) Coloration reactions
- (2) Precipitation reactions
- (3) Decomposition reactions
- (4) Derivatives
- (5) Visible, ultraviolet or infrared spectra
- (6) Special reactions
- (7) Cations
- (8) Anions

6. Purity tests comprise the following items, which are generally put in the order given below, except that unnecessary items are omitted depending on the nature of the drug:

- (1) Color
- (2) Odor
- (3) Clarity and/or color of solution
- (4) Acidity or alkalinity
- (5) Acidity
- (6) Alkalinity
- (7) Chloride
- (8) Sulfate
- (9) Sulfite
- (10) Nitrate
- (11) Nitrite
- (12) Carbonate
- (13) Bromide
- (14) Iodide
- (15) Soluble halide
- (16) Thiocyanide
- (17) Selenium
- (18) Cationic salts
- (19) Ammonium
- (20) Heavy metals
- (21) Iron
- (22) Manganese
- (23) Chromium
- (24) Bismuth
- (25) Tin
- (26) Aluminum
- (27) Zinc
- (28) Cadmium
- (29) Mercury
- (30) Copper

- (31) Lead
- (32) Silver
- (33) Alkaline earth metals
- (34) Arsenic
- (35) Foreign matter
- (36) Related substances
- (37) Residual solvent
- (38) Other mixtures
- (39) Readily carbonizable substances

7. In accordance with the deletion of the prescription on composition of JP by the Pharmaceutical Affairs Law, the composition of Official Monographs was prescribed in General Notices.

8. The following items in General Notices were revised:

- (1) The criteria for conformity of drugs by Description tests in monograph was defined by paragraph 5.
- (2) The atomic mass table used for JP was changed to the Standard Atomic Weights 2004 by paragraph 8.
- (3) A provision “the temperature used for the tests or in storage is described, in principle, in specific figures” was added by paragraph 15.
- (4) The containers of well-closed, tight and hermetic were defined by paragraphs 38, 39 and 40, respectively.
- (5) Renumbering for paragraph, and other rearrangements were done.

9. The following items of the General Rules for Preparations were revised:

- (1) General Notices for Preparations
- (2) Extracts
- (3) Capsules
- (4) Transdermal Systems (newly added)
- (5) Tablets
- (6) Injections
- (7) Plasters and Pressure Sensitive Adhesives Tapes
- (8) Arrangements for others

10. The provision of the criteria for conformity of crude drugs was revised by paragraphs 4 and 5 of General Rules for Crude Drugs.

11. In the General Tests, Processes and Apparatus, “Content Uniformity Tests” and “Mass Variation Tests” were combined and renamed as “Uniformity of Dosage Units”.

12. The following items of the General Tests, Processes and Apparatus were revised:

- (1) Ammonium Limit Test
- (2) Arsenic Limit Test
- (3) Boiling Point and Distilling Range Test

- (4) Crude Drugs Test
- (5) Disintegration Test
- (6) Dissolution Test
- (7) Insoluble Particulate Matter Test for Injections
- (8) Melting Point Determination
- (9) Microbial Assay for Antibiotics
- (10) Microbial Limit Test for Crude Drugs
- (11) Powder Particle Size Determination
- (12) Residue on Ignition Test
- (13) Specific Surface Area Determination
- (14) Test Methods for Plastic Containers

13. The following items of the General Tests, Processes and Apparatus were deleted:

- (1) Absorbance Ratio Method
- (2) Methoxyl Assay
- (3) Paper Chromatography
- (4) Volatile Contaminations in Ethanol

14. The following Reference Standards were newly added:

Cilostazol
 Ethyl Icosapentate
 Ginsenoside Rb₁
 Ginsenoside Rg₁
 Gonadorelin Acetate
 Human Menopausal Gonadotrophin
 Interleukin-2
 Isoflurane
 Limaprost
 Low-molecular Mass Heparin
 Melting Point Standard (Acetanilide)
 Melting Point Standard (Acetophenetidin)
 Melting Point Standard (Caffeine)
 Melting Point Standard (Sulfanilamide)
 Melting Point Standard (Sulfapyridine)
 Melting Point Standard (Vanillin)
 Methylergometrine Maleate
 Oxytocin
 Phytonadione
 Pravastatin 1,1,3,3-Tetramethylbutylammonium
 Ritodrine Hydrochloride
 Roxatidine Acetate Hydrochloride
 Vasopressin
 Warfarin Potassium

15. The following Reference Standards were deleted.

Benzylpenicilline Sodium
 Cefaloridin
 Cefamandole Lithium
 Cefetamet Pivoxil Hydrochloride
 Cefoselis Sulfate
 Cefoxitin
 Cefradine

Posterior Pituitary
 Secretin
 Ticarcillin Sodium

16. English and Latin titles of drugs were based, in principle, on the International Nonproprietary Names for Pharmaceutical Substances, and the chemical names were based on the Rules of the International Union of Pure and Applied Chemistry (IUPAC).

17. Molecular formulas of organic compounds begin with C and then H, followed by other involved elements in the alphabetical order of the symbols of the elements.

18. Structural formulas of drugs represent, as far as possible, steric configurations.

19. The test procedures in monographs were written in full, except within the same monograph and in the monographs for preparations having a corresponding monograph of their principal material substances.

20. The following articles were newly added to Official Monographs:

Alacepril
 Alacepril Tablets
 Amphotericin B for Injection
 Amphotericin B Syrup
 Amphotericin B Tablets
 Arbekacin Sulfate Injection
 L-Arginine
 L-Aspartic Acid
 Atenolol
 Benincasa Seed
 Betahistine Mesilate Tablets
 Betamethasone Tablets
 Betamethasone Valerate and Gentamicin Sulfate Cream
 Betamethasone Valerate and Gentamicin Sulfate Ointment
 Bezafibrate
 Bezafibrate Sustained Release Tablets
 Bucillamine
 Cefaclor Capsules
 Cefaclor Compound Granules
 Cefaclor Fine Granules
 Cefcapene Pivoxil Hydrochloride Fine Granules
 Cefcapene Pivoxil Hydrochloride Tablets
 Cefdinir Capsules
 Cefdinir Fine Granules
 Cefditoren Pivoxil Fine Granules
 Cefditoren Pivoxil Tablets
 Cefotiam Hydrochloride for Injection
 Cefozopran Hydrochloride for Injection

- Cefteram Pivoxil Fine Granules
 Celmoleukin (Genetical Recombination)
 Chenodeoxycholic Acid
 Cilastatin Sodium
 Cilostazol
 Cilostazol Tablets
 Clarithromycin Tablets
 Clindamycin Hydrochloride Capsules
 Croscarmellose Sodium
 Daiokanzoto Extract
 Dolichos Seed
 Doxifluridine
 Doxifluridine Capsules
 Eleutherococcus Senticosus Rhizome
 Ethyl Icosapentate
 Etidronate Disodium
 Etidronate Disodium Tablets
 Etodolac
 Faropenem Sodium for Syrup
 Faropenem Sodium Tablets
 Fosfomycin Sodium for Injection
 Gonadorelin Acetate
 Haloperidol Tablets
 Hochuekkito Extract
 Human Menopausal Gonadotrophin
 Idarubicin Hydrochloride for Injection
 Imipenem and Cilastatin for Injection
 Isoflurane
 Kakkonto Extract
 Kamishoyosan Extract
 Limaprost Alfadex
 Lisinopril Hydrate
 Lisinopril Tablets
 Mefloquine Hydrochloride
 Metformin Hydrochloride
 Metformin Hydrochloride Tablets
 Metoprolol Tartrate
 Metoprolol Tartrate Tablets
 Metronidazole Tablets
 Nelumbo Seed
 Nicergoline
 Nicergoline Powder
 Nicergoline Tablets
 Nitrendipine
 Nitrendipine Tablets
 Parnaparin Sodium
 Piperacillin Sodium for Injection
 Polygonatum Rhizome
 Pravastatin Sodium
 Propranolol Hydrochloride Tablets
 Pullulan
 Rifampicin Capsules
 Ritodrine Hydrochloride
 Ritodrine Hydrochloride Tablets
 Roxatidine Acetate Hydrochloride
 Roxatidine Acetate Hydrochloride Extended-release Capsules
 Ryokeijutsukanto Extract
 Saccharin
 Saireito Extract
 Sulpiride Capsules
 Sulpiride Tablets
 Tamsulosin Hydrochloride
 Taurine
 Teceleukin (Genetical Recombination)
 Teceleukin for Injection (Genetical Recombination)
 Timolol Maleate
 Trimetazidine Hydrochloride Tablets
 Urapidil
 Vancomycin Hydrochloride for Injection
 Verapamil Hydrochloride Tablets
 Voglibose
 Voglibose Tablets
 Zaltoprofen
 Zaltoprofen Tablets
- 21.** The following monographs were revised:
- Achyranthes Root
 Aclarubicin Hydrochloride
 Acrinol Hydrate
 Actinomycin D
 Akebia Stem
 Alisma Rhizome
 Powdered Alisma Rhizome
 Alprostadil Alfadex
 Amikacin Sulfate
 Amomum Seed
 Powder Amomum Seed
 Amoxicillin Hydrate
 Amphotericin B
 Ampicillin Hydrate
 Anhydrous Ampicillin
 Ampicillin Sodium
 Arbekacin Sulfate
 L-Arginine Hydrochloride
 Asiasarum Root
 Aspoxicillin Hydrate
 Astragalus Root
 Astromicin Sulfate
 Atractylodes Lancea Rhizome
 Powdered Atractylodes Lancea Rhizome
 Aztreonam
 Bacitracin
 Bekanamycin Sulfate
 Benidipine Hydrochloride Tablets
 Benzyl Alcohol
 Benzylpenicillin Potassium
 Berberine Tannate

Betahistine Mesilate
Betamethasone
Bethanechol Chloride
Bleomycin Hydrochloride
Bleomycin Sulfate
Bromazepam
Bupleurum Root
Burdock Fruit
Butyl Parahydroxybenzoate
Calcium Gluconate Hydrate
Calcium Para-aminosalicylate Granules
Calcium Para-aminosalicylate Hydrate
L-Carbocysteine
Carumonam Sodium
Cefaclor
Cefadroxil
Cefalotin Sodium
Cefatrizine Propylene Glycolate
Cefazolin Sodium
Cefazolin Sodium Hydrate
Cefcapene Pivoxil Hydrochloride Hydrate
Cefdinir
Cefditoren Pivoxil
Cefepime Dihydrochloride Hydrate
Cefixime
Cefmenoxime Hydrochloride
Cefmetazole Sodium
Cefminox Sodium Hydrate
Cefotetan
Cefotiam Hexetil Hydrochloride
Cefotiam Hydrochloride
Cefozopran Hydrochloride
Cefpiramide Sodium
Cefpodoxime Proxetil
Cefroxadine Hydrate
Cefsulodin Sodium
Ceftazidime Hydrate
Ceftibuten Hydrate
Ceftizoxime Sodium
Ceftriaxone Sodium Hydrate
Cefuroxime Axetil
Microcrystalline Cellulose
Powdered Cellulose
Chloramphenicol
Chloramphenicol Palmitate
Chlorpheniramine Maleate
Chlorpheniramine Maleate Injection
Chlorpheniramine Maleate Powder
Chlorpheniramine Maleate Tablets
d-Chlorpheniramine Maleate
Chlorpromazine Hydrochloride Tablets
Ciclacillin
Cinnamon Bark
Powdered Cinnamon Bark
Anhydrous Citric Acid
Citric Acid Hydrate
Citrus Unshiu Peel
Clindamycin Hydrochloride
Clindamycin Phosphate
Clove
Cloxacillin Sodium Hydrate
Colistin Sodium Methanesulfonate
Colistin Sulfate
Coptis Rhizome
Powdered Coptis Rhizome
Corn Starch
Cornus Fruit
Cortisone Acetate
Cyanamide
Cyanocobalamin
Cyanocobalamin Injection
Cycloserine
Daunorubicin Hydrochloride
Demethylchlortetracycline Hydrochloride
Dexamethasone
Dibekacin Sulfate
Dicloxacillin Sodium Hydrate
Dimercaprol
Dimercaprol Injection
Doxorubicin Hydrochloride
Doxycycline Hydrochloride Hydrate
Enflurane
Enviomycin Sulfate
Epirubicin Hydrochloride
Erythromycin
Erythromycin Ethylsuccinate
Erythromycin Lactobionate
Erythromycin Stearate
Ethanol
Anhydrous Ethanol
Ethanol for Disinfection
Ethyl Parahydroxybenzoate
Etilefrine Hydrochloride
Famotidine Powder
Famotidine Tablets
Faropenem Sodium Hydrate
Powdered Fennel
Flomoxef Sodium
Flopropione Capsules
Foeniculated Ammonia Spirit
Fosfomycin Sodium
Fradiomycin Sulfate
Gardenia Fruit
Gentamicin Sulfate
Ginger
Powdered Ginger
Ginseng
Powdered Ginseng

Glycyrrhiza
Powdered Glycyrrhiza
Griseofulvin
Haloperidol
Hemp Fruit
Idarubicin Hydrochloride
Imipenem Hydrate
Insulin
Insulin Human (Genetical Recombination)
Insulin Injection
Insulin Zinc Injection (Aqueous Suspension)
Insulin Zinc Protamine Injection (Aqueous Suspension)
Isophane Insulin Injection (Aqueous Suspension)
Amorphous Insulin Zinc Injection (Aqueous Suspension)
Crystalline Insulin Zinc Injection (Aqueous Suspension)
Isepamicin Sulfate
Japanese Angelica Root
Powdered Japanese Angelica Root
Josamycin
Josamycin Propionate
Jujube
Jujube Seed
Kanamycin Monosulfate
Kanamycin Sulfate
Ketoprofen
Kitasamycin
Kitasamycin Acetate
Kitasamycin Tartrate
Lactose Hydrate
Anhydrous Lactose
Latamoxef Sodium
Lenampicillin Hydrochloride
Lincomycin Hydrochloride Hydrate
Loquat Leaf
Meropenem Hydrate
Methylcellulose
Methylergometrine Maleate
Methylergometrine Maleate Tablets
Methyl Parahydroxybenzoate
Metronidazole
Micronomicin Sulfate
Midecamycin
Midecamycin Acetate
Minocycline Hydrochloride
Mitomycin C
Moutan Bark
Powdered Moutan Bark
Mupirocin Calcium Hydrate
Nalidixic Acid
Netilmicin Sulfate
Nicotinamide
Nystatin
Ophiopogon Tuber
Oxytetracycline Hydrochloride
Oxytocin
Oxytocin Injection
Panipenem
Powdered Peach Kernel
Peony Root
Powdered Peony Root
Peplomycin Sulfate
Perilla Herb
Phellodendron Bark
Powdered Phellodendron Bark
Phenethicillin Potassium
Phytonadione
Pimaricin
Pinellia Tuber
Pirarubicin
Pivmecillinam Hydrochloride
Plantago Seed
Platycodon Root
Powdered Platycodon Root
Polygala Root
Powdered Polygala Root
Polymixin B Sulfate
Poria Sclerotium
Powdered Poria Sclerotium
Potassium Clavulanate
Potato Starch
Povidone
Processed Aconite Root
Powdered Processed Aconite Root
Propranolol Hydrochloride
Propyl Parahydroxybenzoate
Pueraria Root
Pyrrolnitrin
Red Ginseng
Rehmannia Root
Rhubarb
Powdered Rhubarb
Ribostamycin Sulfate
Rifampicin
Rokitamycin
Roxithromycin
Saccharin Sodium Hydrate
Safflower
Santonin
Schisandra Fruit
Scutellaria Root
Powdered Scutellaria Root
Senna Leaf
Powdered Senna Leaf
Siccanin
Sisomicin Sulfate

Sodium Citrate Hydrate
 Sodium Fusidate
 Spectinomycin Hydrochloride Hydrate
 Spiramycin Acetate
 Streptomycin Sulfate
 Sulbenicillin Sodium
 Sulpiride
 Swertia Herb
 Talampicillin Hydrochloride
 Teicoplanin
 Tetracycline Hydrochloride
 Tobramycin
 Tranexamic Acid Capsules
 Tranexamic Acid Tablets
 Trichomycin
 Trichosanthes Root
 Trimetazidine Hydrochloride
 Uncaria Hook
 Vancomycin Hydrochloride
 Vasopressin Injection
 Warfarin Potassium
 Water
 Water for Injection
 Purified Water
 Sterile Purified Water
 Wine
 Wheat Starch
 Zinostatin Stimalamer

22. The following monographs were revised in Japanese titles:

Acebutolol Hydrochloride
 Acetylcholine Chloride for Injection
 Acetylspiramycin
 Aclarubicin Hydrochloride
 Acrinol Hydrate
 Adrenaline
 Adrenaline Injection
 Adrenaline Solution
 Alimemazine Tartrate
 Alprenolol Hydrochloride
 Aluminum Potassium Sulfate Hydrate
 Amantadine Hydrochloride
 Ambenonium Chloride
 Amikacin Sulfate
 Aminophylline Hydrate
 Amitriptyline Hydrochloride
 Amitriptyline Hydrochloride Tablets
 Amoxicillin Hydrate
 Ampicillin Hydrate
 Arbekacin Sulfate
 L-Arginine Hydrochloride
 L-Arginine Hydrochloride Injection
 Arotinolol Hydrochloride

Aspoxicillin Hydrate
 Astromicin Sulfate
 Atropine Sulfate Hydrate
 Atropine Sulfate Injection
 Bacampicillin Hydrochloride
 Bamethan Sulfate
 Beclometasone Dipropionate
 Bekanamycin Sulfate
 Benidipine Hydrochloride
 Benidipine Hydrochloride Tablets
 Benserazide Hydrochloride
 Benzalkonium Chloride
 Benzalkonium Chloride Concentrated Solution 50
 Benzalkonium Chloride Solution
 Benzethonium Chloride
 Benzethonium Chloride Solution
 Benzylpenicillin Benzathine Hydrate
 Berberine Chloride Hydrate
 Betahistine Mesilate
 Betamethasone Dipropionate
 Betamethasone Sodium Phosphate
 Betamethasone Valerate
 Bethanechol Chloride
 Biperiden Hydrochloride
 Bleomycin Hydrochloride
 Bleomycin Sulfate
 Bromhexine Hydrochloride
 Bromocriptine Mesilate
 Bromovalerylurea
 Bucumolol Hydrochloride
 Bufetolol Hydrochloride
 Bufexamac Cream
 Bunazosin Hydrochloride
 Bupranolol Hydrochloride
 Butropium Bromide
 Caffeine Hydrate
 Calcium Chloride Hydrate
 Calcium Gluconate Hydrate
 Calcium Lactate Hydrate
 Calcium Para-aminosalicylate Hydrate
 Camostat Mesilate
 Carbazochrome Sodium Sulfonate Hydrate
 Carbidopa Hydrate
 Carteolol Hydrochloride
 Cefcapene Pivoxil Hydrochloride Hydrate
 Cefepime Dihydrochloride for Injection
 Cefepime Dihydrochloride Hydrate
 Cefmenoxime Hydrochloride
 Cefminox Sodium Hydrate
 Cefotiam Hexetil Hydrochloride
 Cefotiam Hydrochloride
 Cefozopran Hydrochloride
 Cefpirome Sulfate
 Cefpodoxime Proxetil

Cefroxadine Hydrate
 Ceftazidime Hydrate
 Cefteram Pivoxil
 Ceftibuten Hydrate
 Ceftriaxone Sodium Hydrate
 Cefuroxime Axetil
 Cellacefate
 Cetraxate Hydrochloride
 Chloramphenicol Palmitate
 Chloramphenicol Sodium Succinate
 Chlorhexidine Gluconate Solution
 Chlorhexidine Hydrochloride
 Chlormadinone Acetate
 Chlorphenesin Carbamate
 Chlorpheniramine Maleate
 Chlorpheniramine Maleate Injection
 Chlorpheniramine Maleate Powder
 Chlorpheniramine Maleate Tablets
d-Chlorpheniramine Maleate
 Chlorpromazine Hydrochloride
 Chlorpromazine Hydrochloride Injection
 Chlorpromazine Hydrochloride Tablets
 Citric Acid Hydrate
 Clemastine Fumarate
 Clindamycin Hydrochloride
 Clindamycin Phosphate
 Clocapramine Hydrochloride Hydrate
 Clofedanol Hydrochloride
 Clomifene Citrate
 Clomifene Citrate Tablets
 Clomipramine Hydrochloride
 Clonidine Hydrochloride
 Cloperastine Hydrochloride
 Cloxacillin Sodium Hydrate
 Cocaine Hydrochloride
 Codeine Phosphate Hydrate
 1% Codeine Phosphate Powder
 10% Codeine Phosphate Powder
 Codeine Phosphate Tablets
 Colistin Sulfate
 Cortisone Acetate
 Croconazole Hydrochloride
 Cyclopentolate Hydrochloride
 Cyclophosphamide Hydrate
 Cyproheptadine Hydrochloride Hydrate
 Dantrolene Sodium Hydrate
 Daunorubicin Hydrochloride
 Deferoxamine Mesilate
 Demethylchlortetracycline Hydrochloride
 Dextromethorphan Hydrobromide Hydrate
 Dibasic Calcium Phosphate Hydrate
 Dibasic Sodium Phosphate Hydrate
 Dibekacin Sulfate
 Dibucaine Hydrochloride
 Dicloxacillin Sodium Hydrate
 Diethylcarbamazine Citrate
 Diethylcarbamazine Citrate Tablets
 Difenidol Hydrochloride
 Dihydrocodeine Phosphate
 1% Dihydrocodeine Phosphate Powder
 10% Dihydrocodeine Phosphate Powder
 Dihydroergotamine Mesilate
 Dihydroergotoxine Mesilate
 Dilazep Hydrochloride Hydrate
 Diltiazem Hydrochloride
 Dimemorfan Phosphate
 Diphenhydramine and Bromovalerylurea Powder
 Diphenhydramine Hydrochloride
 Disodium Edetate Hydrate
 Distigmine Bromide
 Distigmine Bromide Tablets
 Dobutamine Hydrochloride
 Dopamine Hydrochloride
 Dopamine Hydrochloride Injection
 Doxapram Hydrochloride Hydrate
 Doxorubicin Hydrochloride
 Doxycycline Hydrochloride Hydrate
 Ecothiopate Iodide
 Edrophonium Chloride
 Edrophonium Chloride Injection
 Enoxacin Hydrate
 Enviomycin Sulfate
 Eperisone Hydrochloride
 Ephedrine Hydrochloride
 Ephedrine Hydrochloride Injection
 10% Ephedrine Hydrochloride Powder
 Ephedrine Hydrochloride Tablets
 Epirubicin Hydrochloride
 Ergometrine Maleate
 Ergometrine Maleate Injection
 Ergometrine Maleate Tablets
 Ergotamine Tartrate
 Erythromycin Ethylsuccinate
 Erythromycin Lactobionate
 Erythromycin Stearate
 Estradiol Benzoate
 Estradiol Benzoate Injection
 Estradiol Benzoate Injection (Aqueous Suspension)
 Ethambutol Hydrochloride
 Ethyl L-Cysteine Hydrochloride
 Ethylmorphine Hydrochloride Hydrate
 Etilefrine Hydrochloride
 Etilefrine Hydrochloride Tablets
 Faropenem Sodium Hydrate
 Fentanyl Citrate
 Ferrous Sulfate Hydrate
 Flavoxate Hydrochloride
 Fluphenazine Enanthate

Flurazepam Hydrochloride
 Formoterol Fumarate Hydrate
 Fosfomycin Calcium Hydrate
 Fradiomycin Sulfate
 Fursultiamine Hydrochloride
 Gabexate Mesilate
 Gentamicin Sulfate
 Guanabenz Acetate
 Guanethidine Sulfate
 Homatropine Hydrobromide
 Homochlorcyclizine Hydrochloride
 Human Chorionic Gonadotrophin
 Human Chorionic Gonadotrophin for Injection
 Hydralazine Hydrochloride
 Hydralazine Hydrochloride for Injection
 Hydralazine Hydrochloride Powder
 Hydralazine Hydrochloride Tablets
 Hydrocortisone Acetate
 Hydrocortisone Butyrate
 Hydrocortisone Sodium Phosphate
 Hydrocortisone Sodium Succinate
 Hydrocortisone Succinate
 Hydrocotarnine Hydrochloride Hydrate
 Hydroxocobalamin Acetate
 Hypromellose Phthalate
 Hydroxyzine Hydrochloride
 Hydroxyzine Pamoate
 Idarubicin Hydrochloride
 Ifenprodil Tartrate
 Imipenem Hydrate
 Imipramine Hydrochloride
 Imipramine Hydrochloride Tablets
 Indenolol Hydrochloride
 Ipratropium Bromide Hydrate
 Isepamicin Sulfate
l-Isoprenaline Hydrochloride
 Josamycin Propionate
 Kainic Acid Hydrate
 Kanamycin Monosulfate
 Kanamycin Sulfate
 Ketamine Hydrochloride
 Ketotifen Fumarate
 Kitasamycin Acetate
 Kitasamycin Tartrate
 Lactose Hydrate
 Lenampicillin Hydrochloride
 Levallorphan Tartrate
 Levallorphan Tartrate Injection
 Levomepromazine Maleate
 Levothyroxine Sodium Hydrate
 Lincomycin Hydrochloride Hydrate
 Loxoprofen Sodium Hydrate
L-Lysine Hydrochloride
 Lysozyme Hydrochloride
 Magnesium Sulfate Hydrate
 Maltose Hydrate
 Maprotiline Hydrochloride
 Meclofenoxate Hydrochloride
 Mepenzolate Bromide
 Mepivacaine Hydrochloride
 Mepivacaine Hydrochloride Injection
 Mercaptopurine Hydrate
 Meropenem Hydrate
 Metenolone Acetate
 Metenolone Enanthate
 Metenolone Enanthate Injection
 Methamphetamine Hydrochloride
 Methylbenactyzium Bromide
 Methyldopa Hydrate
dl-Methylephedrine Hydrochloride
 10% *dl*-Methylephedrine Hydrochloride Powder
 Methylergometrine Maleate
 Methylergometrine Maleate Tablets
 Methylprednisolone Succinate
 Methylrosanilinium Chloride
 Mexiletine Hydrochloride
 Miconazole Nitrate
 Micronomicin Sulfate
 Midecamycin Acetate
 Minocycline Hydrochloride
 Monobasic Calcium Phosphate Hydrate
 Morphine Hydrochloride Hydrate
 Morphine Hydrochloride Injection
 Morphine Hydrochloride Tablets
 Mupirocin Calcium Hydrate
 Naloxone Hydrochloride
 Naphazoline Hydrochloride
 Naphazoline Nitrate
 Neostigmine Methylsulfate
 Neostigmine Methylsulfate Injection
 Netilmicin Sulfate
 Nicardipine Hydrochloride
 Nicardipine Hydrochloride Injection
 Noradrenaline
 Noradrenaline Injection
 Nortriptyline Hydrochloride
 Noscapine Hydrochloride Hydrate
 Opium Alkaloids Hydrochlorides
 Opium Alkaloids Hydrochlorides Injection
 Orciprenaline Sulfate
 Oxapium Iodide
 Oxprenolol Hydrochloride
 Oxybuprocaine Hydrochloride
 Oxycodone Hydrochloride Hydrate
 Oxytetracycline Hydrochloride
 Pancuronium Bromide
 Papaverine Hydrochloride
 Papaverine Hydrochloride Injection

Penbutolol Sulfate
Pentoxifyverine Citrate
Peplomycin Sulfate
Perphenazine Maleate
Perphenazine Maleate Tablets
Pethidine Hydrochloride
Pethidine Hydrochloride Injection
Phenylephrine Hydrochloride
Pilocarpine Hydrochloride
Pipemidic Acid Hydrate
Piperazine Adipate
Piperazine Phosphate Hydrate
Piperazine Phosphate Tablets
Pirenzepine Hydrochloride Hydrate
Pivmecillinam Hydrochloride
Polymixin B Sulfate
Prednisolone Acetate
Prednisolone Sodium Succinate for Injection
Prednisolone Succinate
Procainamide Hydrochloride
Procainamide Hydrochloride Injection
Procainamide Hydrochloride Tablets
Procaine Hydrochloride
Procaine Hydrochloride Injection
Procarbazine Hydrochloride
Procaterol Hydrochloride Hydrate
Prochlorperazine Maleate
Prochlorperazine Maleate Tablets
Promethazine Hydrochloride
Propantheline Bromide
Propranolol Hydrochloride
Protamine Sulfate
Protamine Sulfate Injection
Protirelin Tartrate Hydrate
Pyrantel Pamoate
Pyridostigmine Bromide
Pyridoxine Hydrochloride
Pyridoxine Hydrochloride Injection
Quinidine Sulfate Hydrate
Quinine Ethyl Carbonate
Quinine Hydrochloride Hydrate
Quinine Sulfate Hydrate
Ranitidine Hydrochloride
Retinol Acetate
Retinol Palmitate
Riboflavin Butyrate
Riboflavin Sodium Phosphate
Riboflavin Sodium Phosphate Injection
Ribostamycin Sulfate
Saccharin Sodium Hydrate
Salbutamol Sulfate
Scopolamine Butylbromide
Scopolamine Hydrobromide Hydrate
Sisomicin Sulfate
Sodium Acetate Hydrate
Sodium Carbonate Hydrate
Sodium Citrate Hydrate
Sodium Picosulfate Hydrate
Sodium Prasterone Sulfate Hydrate
Sodium Thiosulfate Hydrate
Sorbitan Sesquioleate
Spectinomycin Hydrochloride Hydrate
Streptomycin Sulfate
Sucralfate Hydrate
Sulfamonomethoxine Hydrate
Sulpyrine Hydrate
Sultamicillin Tosilate Hydrate
Suxamethonium Chloride for Injection
Suxamethonium Chloride Hydrate
Suxamethonium Chloride Injection
Talampicillin Hydrochloride
Terbutaline Sulfate
Testosterone Enanthate
Testosterone Enanthate Injection
Testosterone Propionate
Testosterone Propionate Injection
Tetracaine Hydrochloride
Tetracycline Hydrochloride
Thiamine Chloride Hydrochloride
Thiamine Chloride Hydrochloride Injection
Thiamine Chloride Hydrochloride Powder
Thiamine Nitrate
Thioridazine Hydrochloride
Tiaramide Hydrochloride
Tiaramide Hydrochloride Tablets
Ticlopidine Hydrochloride
Timepidium Bromide Hydrate
Tipepidine Hibenstate
Tipepidine Hibenstate Tablets
Tizanidine Hydrochloride
Tocopherol Acetate
Tocopherol Calcium Succinate
Tocopherol Nicotinate
Todralazine Hydrochloride Hydrate
Tolperisone Hydrochloride
Trihexyphenidyl Hydrochloride
Trihexyphenidyl Hydrochloride Tablets
Trimebutine Maleate
Trimetazidine Hydrochloride
Trimetoquinol Hydrochloride Hydrate
Tubocurarine Chloride Hydrochloride Hydrate
Tubocurarine Chloride Hydrochloride Injection
Tulobuterol Hydrochloride
Vancomycin Hydrochloride
Verapamil Hydrochloride
Vinblastine Sulfate
Vinblastine Sulfate for Injection
Vincristine Sulfate

Zinc Sulfate Hydrate

23. The following three monographs in the JP 14th Edition were replaced by a new monograph “Hympromellose”, and were deleted due to this revision:

Hydroxypropylmethylcellulose 2208
Hydroxypropylmethylcellulose 2906
Hydroxypropylmethylcellulose 2910

24. The following monographs were deleted:

Cefaloridine
Cefamandole Sodium
Cefetamet Pivoxil Hydrochloride
Cefoselis Sulfate
Cefoxitin Sodium
Cefradine
Secretin
Ticarcillin Sodium

Those who were engaged in the preparation of the JP 15th Edition are as follows:

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Shigeru Matsuki	Fumitoshi Shincho
Hayashi Matsukura	Osamu Shirota
Eiichi Mikami	Kouichi Shudo
Satoshi Minobe	Hisashi Sonobe
Katsutoshi Mise	Shoko Sueyoshi
Hiroto Miyamoto	Shinji Sugaya
Naoki Miyata	Hisakazu Sunada
Michinao Mizugaki	Hideyo Suzuki
Taiichi Mizuta	Senji Suzuki
Kaoru Morikawa	Yukio Tabuchi
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Takashi Morita	Tadahiro Takeda
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Hiroaki Nagashige	Toshihiro Tanaka
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Haruo Watanabe	Takeshi Yamazaki
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Takehiko Yajima	Hikaru Yoden
Teruhide Yamaguchi	Chikako Yomota
Keiichi Yamamoto	Hitoo Yoshida
Keiji Yamamoto	Kazumasa Yoshikawa
Tosuke Yamamoto	Sumie Yoshioka

*: Chairman, the Committee on JP

** : Acting Chairman, the Committee on JP

GENERAL NOTICES

1. The official name of this pharmacopoeia is 第十五改正日本薬局方, and may be abbreviated as 日局十五, 日局15, JP XV or JP 15.

2. The English name of this pharmacopoeia is The Japanese Pharmacopoeia, Fifteenth Edition.

3. Among drugs, the Japanese Pharmacopoeia Drugs (the JP Drugs) are those specified in the monographs. The title names and the commonly used names adopted in the monographs should be used as official names. The distinction of the preparations name of Fine Granules and Powders follows according to the definition in the section of “Powders” of General Rules for Preparations. In the drug monographs, in addition to English name, chemical names or Latin names can be mentioned in the titles, as appropriate.

4. “Crude Drugs and related drugs” are placed together in the posterior part of the Official Monographs. These include: Crude Drugs being applied the requirements of the General Rules for Crude Drugs, or Powders, Extracts, Tinctures, Syrups, Spirits, Lini-ments or Suppositories containing Crude Drugs as the active ingredient, combination preparations containing Crude Drugs as the principal active ingredient.

5. Drugs are to be tested according to the provisions given in the pertinent monographs, General Notices, General Rules for Crude Drugs, General Rules for Preparations, and General Tests for their conformity to the Japanese Pharmacopoeia. However, the items of “Description” and “Storage” under Containers and storage in the monographs on preparations are given for information, and should not be taken as indicating standards for conformity.

6. In principle, unless otherwise specified, animals used for preparing the JP Drugs or their source materials must be healthy.

7. In this English version, the JP Drugs described in the monographs begin with a capital letter.

8. The molecular formulas or constitution formulas in parentheses () after the name of drugs or chemicals designate chemically pure substances. Atomic masses adopted in the Japanese Pharmacopoeia conform to the table of “Standard Atomic Weights 2004”. Molecular masses are indicated to two decimal places rounded from three decimals.

9. The following abbreviations are used for the principal units.

meter	m
centimeter	cm
millimeter	mm
micrometer	μm
nanometer	nm
kilogram	kg
gram	g
milligram	mg
microgram	μg
nanogram	ng
picogram	pg
Celsius degree	$^{\circ}\text{C}$
square centimeter	cm^2
liter	L
milliliter	mL
microliter	μL
megahertz	MHz
per centimeter	cm^{-1}
newton	N
kilopascal	kPa
pascal	Pa
mole per liter	mol/L
millipascal second	$\text{mPa}\cdot\text{s}$
square millimeter second	mm^2/s
lux	lx
mass per cent	%
mass parts per million	ppm
mass parts per billion	ppb
volume per cent	vol%
volume parts per million	vol ppm
mass per volume per cent	w/v%
hydrogen ion concentration	pH
endotoxin unit	EU

Note: “ppm” used in the Nuclear Magnetic Resonance Spectroscopy indicates the chemical shift, and “w/v%” is used in the formula or composition of preparations.

10. The unit used for expressing the potency of drug is recognized as the quantity of drug. Usually it is expressed by a definite quantity of a definite standard substance which shows a definite biological activity, and differs according to each drug. The units are determined, in principle, by comparison with each reference standard by means of biological methods. The term “Unit” used for the JP articles indicates the unit defined in the Japanese Pharmacopoeia.

11. The statement “Being specified separately.” in the monographs means that the tests are to be specified when the drugs are granted approval based on the Pharmaceutical Affairs Law.

12. When an assurance that a product is of the JP Drug quality is obtained consistently from data derived from the manufacturing process validation studies, and from the records of appropriate manufacturing process control and of the test results of the quality control, some of the test items in the monograph being performed for the release of a product may be omitted as occasion demands.

13. The test methods specified in the Japanese Pharmacopoeia can be replaced by alternative methods which give better accuracy and precision. However, where a difference in test results is suspected, only the result obtained by the procedure given in the Pharmacopoeia is effective for the final judgment.

14. The details of the biological test methods may be changed insofar as they do not affect the essential qualities of the test.

15. The temperature for the tests or storage is described, in principle, in specific figures. However, the following expressions may be used instead.

Standard temperature, ordinary temperature, room temperature, and lukewarm are defined as 20°C, 15 – 25°C, 1 – 30°C, and 30 – 40°C, respectively. A cold place, unless otherwise specified, shall be a place having a temperature of 1 – 15°C.

The temperature of cold water, lukewarm water, warm water, and hot water are defined as not exceeding 10°C, 30 – 40°C, 60 – 70°C, and about 100°C, respectively.

The term “heated solvent” or “hot solvent” means a solvent heated almost to the boiling point of the solvent, and the term “warmed solvent” or “warm solvent” usually means a solvent heated to a temperature between 60°C and 70°C. The term “heat on or in a water bath” indicates, unless otherwise specified, heating with a boiling water bath or a steam bath at about 100°C.

Cold extraction and warm extraction are usually performed at temperatures of 15 – 25°C and 35 – 45°C, respectively.

16. To measure the number of drops, a dropping device which delivers 20 drops of Purified Water weighing 0.90 – 1.10 g at 20°C shall be used.

17. The term “in vacuum” indicates, unless otherwise specified, a pressure not exceeding 2.0 kPa.

18. The acidity or alkalinity of a solution, unless otherwise specified, is determined by blue or red litmus papers. To indicate these properties more precisely, pH values are used.

19. The terms in Table 1 are used to express the degree of cutting of Crude Drugs or fineness of powder Drugs.

Table 1

Sieve No.	4	6.5	8.6	18	50	100	200
Nominal Designation of sieve	4750 μm	2800 μm	2000 μm	850 μm	300 μm	150 μm	75 μm
Names of the drugs which pass through the respective sieves	Coarse cutting	Moderately fine cutting	Fine cutting	Coarse powder	Moderately fine powder	Fine powder	Very fine powder

20. Unless otherwise specified, the water to be used in the tests of drugs shall be Purified Water.

21. As for wording “solution of a solute”, where the name of the solvent is not stated, the term “solution” indicates a solution in water.

22. For solution an expression such as “(1 in 3)”, “(1 in 10)”, or “(1 in 100)” means that 1 g of a solid is dissolved in, or 1 mL of a liquid is diluted with the solvent to make the total volume of 3 mL, 10 mL or 100 mL, respectively. For the liquid mixture an expression such as “(10:1)” or “(5:3:1)” means that the respective numbers of parts, by volume, of the designated liquids are to be mixed.

23. The term “weigh accurately” means to weigh down to the degree of 0.1 mg, 0.01 mg or 0.001 mg by taking into account the purpose of the test and using a relevant weighing device. The term “weigh exactly” means to weigh to the given decimal places.

24. A value of “n” figures in a test of a JP Drug shall be obtained by rounding off a value of “n + 1” figures.

25. Unless otherwise specified, all tests of the drugs shall be performed at the ordinary temperature and observations of the results shall follow immediately after the operations. However, the judgment for a test which is affected by temperature should be based on the conditions at the standard temperature.

26. The terms “immediately”/“at once” used in the test of a JP Drug mean that the procedure is to be performed within 30 seconds after the preceding procedure.

27. In the section under the heading Description, the term “white” is used to indicate white or practically white, and “colorless” is colorless or practically colorless. Unless otherwise specified, the test of color is carried out by placing 1 g of a solid drug on a sheet of white paper or in a watch glass placed on white paper. A liquid drug is put into a colorless test tube of 15-mm internal diameter and is observed in front of a white

background through a layer of 30 mm. For the test of clarity of liquid drugs the same procedure is applied with either a black or white background. For the observation of fluorescence of a liquid drug, only a black background shall be used.

28. In the section under the heading Description, the term “odorless” is used to indicate odorless or practically odorless. Unless otherwise specified, the test of odor shall be carried out by placing 1 g of a solid drug or 1 mL of a liquid drug in a beaker.

29. In the section under the heading Description, solubilities are expressed by the terms in Table 2. Unless otherwise specified, solubility means the degree of dissolution of a JP Drug, previously powdered in the case of a solid drug, within 30 minutes in a solvent at $20 \pm 5^\circ\text{C}$, by vigorous shaking for 30 seconds each time at 5-minute intervals.

Table 2

Descriptive term	Volume of solvent required for dissolving 1 g or 1 mL of solute
Very soluble	Less than 1 mL
Freely soluble	From 1 mL to less than 10 mL
Soluble	From 10 mL to less than 30 mL
Sparingly soluble	From 30 mL to less than 100 mL
Slightly soluble	From 100 mL to less than 1000 mL
Very slightly soluble	From 1000 mL to less than 10000 mL
Practically insoluble, or insoluble	10000 mL and over

30. In the test of a drug, the term “dissolve” or “miscible” indicates that it dissolves in, or mixes in arbitrary proportion with the solvent to form a clear solution or mixture. Insoluble materials other than the drug including fibers should not be detected or practically invisible, if any.

31. Identification is the test necessary to identify the active ingredient(s) of the drug based upon its specific property.

32. Purity is the test to detect impurities/contaminants in drugs, and it, as well as other requirements in each monograph, specifies the purity of the drug usually by limiting the kind/nature and quantity of the impurities/contaminants. The impurities/contaminants subject to the purity test are those supposed to generate/contaminate during the manufacturing process or storage, including hazardous agents such as heavy metals, arsenic, etc. If any foreign substances are used or supposed to be added, it is necessary to perform tests to detect or limit the presence of such

substances.

33. The term “constant mass” in drying or ignition, unless otherwise specified, means that the mass difference after an additional 1 hour of drying or ignition is not more than 0.10% of the preceding mass of the dried substance or ignited residue. For crude drugs, the difference is not more than 0.25%. However, when the difference does not exceed 0.5 mg in a chemical balance, 0.05 mg in a semi-microbalance, or 0.005 mg in a microbalance, the difference is considered as negligible and constant mass has been attained.

34. Assay is the test to determine the composition, the content of the active ingredients, and the potency unit of medicine by physical, chemical or biological procedures.

35. In stating the appropriate quantities to be taken for assay, the use of the word “about” indicates a quantity within 10% of the specified mass. The word “dry” in respect of the sample indicates drying under the same conditions, as described in Loss on drying in the monograph.

36. For the content of an ingredient determined by Assay in the monographs, if it is expressed simply as “not less than a certain percentage” without indicating its upper limit, 101.0% is understood as the upper limit.

37. The container is the device which holds drugs. The stopper or cap, etc., is considered as part of the container. The containers have no physical and chemical reactivity affecting the specified description and quality of the contents.

38. A well-closed container protects the contents from extraneous solids and from loss of the drug under ordinary or customary conditions of handling, shipment, and storage.

Where a well-closed container is specified, it may be replaced by a tight container.

39. A tight container protects the contents from extraneous solids or liquids, from loss of the contents, and from efflorescence, deliquescence, or evaporation under ordinary or customary conditions of handling, shipment, and storage.

Where a tight container is specified, it may be replaced by a hermetic container.

40. A hermetic container is impervious to air or any other gas under ordinary or customary conditions of handling, shipment, and storage.

41. The term “light-resistant” means that it can prevent transmittance of light affecting in the specified properties and quality of the contents and protect the contained medicament from the light under ordinary or customary conditions of handling, shipment, and storage.

42. For the JP Drugs, the contents or potency in terms of units of the active ingredient(s), or the specified expiration date in the monographs have to be shown on the immediate container or wrapping of them.

43. The origin, numerical value or physical properties of the JP Drugs, being stipulated by the special labeling requirements in the monographs, have to be shown on the immediate container or wrapping of them.

44. The harmonized General Tests and Monographs among the Japanese Pharmacopoeia, the European Pharmacopoeia and the United States Pharmacopoeia are preceded by the statement as such.

The parts of the text, being not harmonized, are surrounded by the symbols (♦ ♦).

GENERAL RULES FOR CRUDE DRUGS

1. Crude drugs in the monographs include medicinal parts obtained from plants or animals, cell inclusions and secretes separated from the origins, their extracts, and minerals. General Rules for Crude Drugs and Crude Drugs Test are applicable to the following:

Acacia, Achyranthes Root, Agar, Akebia Stem, Alisma Rhizome, Aloe, Alpinia Officinarum Rhizome, Amomum Seed, Anemarrhena Rhizome, Angelica Dahurica Root, Apricot Kernel, Areca, Artemisia Capillaris Flower, Asiasarum Root, Asparagus Tuber, Astragalus Root, Atractylodes Lancea Rhizome, Atractylodes Rhizome, Bear Bile, Bearberry Leaf, Belladonna Root, Benincasa Seed, Benzoin, Bitter Cardamon, Bitter Orange Peel, Bupleurum Root, Burdock Fruit, Calumba, Capsicum, Cardamon, Cassia Seed, Catalpa Fruit, Chrysanthemum Flower, Cimicifuga Rhizome, Cinnamon Bark, Citrus Unshiu Peel, Clematis Root, Clove, Cnidium Monnieri Fruit, Cnidium Rhizome, Coix Seed, Condurango, Coptis Rhizome, Cornus Fruit, Corydalis Tuber, Cyperus Rhizome, Digenea, Dioscorea Rhizome, Dolichos Seed, Eleutherococcus Senticosus Rhizome, Ephedra Herb, Epimedium Herb, Eucommia Bark, Evodia Fruit, Fennel, Forsythia Fruit, Fritillaria Bulb, Gambir, Gardenia Fruit, Gastrodia Tuber, Gentian, Geranium Herb, Ginger, Ginseng, Glehnia Root, Glycyrrhiza, Gypsum, Hemp Fruit, Honey, Houத்துynia Herb, Immature Orange, Imperata Rhizome, Ipecac, Japanese Angelica Root, Japanese Gentian, Japanese Valerian, Jujube, Jujube Seed, Lindera Root, Lithospermum Root, Longgu, Lonicera Leaf and Stem, Loquat Leaf, Lycium Bark, Lycium Fruit, Magnolia Bark, Magnolia Flower, Mallotus Bark, Mentha Herb, Moutan Bark, Mulberry Bark, Nelumbo Seed, Notopterygium Rhizome, Nuphar Rhizome, Nux Vomica, Ophiopogon Tuber, Oriental Bezoar, Oyster Shell, Panax Japonicus Rhizome, Peach Kernel, Peony Root, Perilla Herb, Pharbitis Seed, Phellodendron Bark, Picrasma Wood, Pinellia Tuber, Plantago Herb, Plantago Seed, Platycodon Root, Polygala Root, Polygonatum Rhizome, Polygonum Root, Polyporus Sclerotium, Poria Sclerotium, Powdered Acacia, Powdered Agar, Powdered Alisma Rhizome, Powdered Aloe, Powdered Amomum Seed, Powdered Atractylodes Lancea Rhizome, Powdered Atractylodes Rhizome, Powdered

Calumba, Powdered Capsicum, Powdered Cinnamon Bark, Powdered Clove, Powdered Cnidium Rhizome, Powdered Coix Seed, Powdered Coptis Rhizome, Powdered Cyperus Rhizome, Powdered Dioscorea Rhizome, Powdered Fennel, Powdered Gambir, Powdered Gardenia Fruit, Powdered Gentian, Powdered Geranium Herb, Powdered Ginger, Powdered Ginseng, Powdered Glycyrrhiza, Powdered Ipecac, Powdered Japanese Angelica Root, Powdered Japanese Gentian, Powdered Japanese Valerian, Powdered Magnolia Bark, Powdered Moutan Bark, Powdered Oyster Shell, Powdered Panax Japonicus Rhizome, Powdered Peach Kernel, Powdered Peony Root, Powdered Phellodendron Bark, Powdered Picrasma Wood, Powdered Platycodon Root, Powdered Polygala Root, Powdered Polyporus Sclerotium, Powdered Poria Sclerotium, Powdered Processed Aconite Root, Powdered Rhubarb, Powdered Rose Fruit, Powdered Scutellaria Root, Powdered Senega, Powdered Senna Leaf, Powdered Smilax Rhizome, Powdered Sophora Root, Powdered Sweet Hydrangea Leaf, Powdered Swertia Herb, Powdered Tragacanth, Powdered Zanthoxylum Fruit, Processed Aconite Root, Processed Ginger, Prunella Spike, Pueraria Root, Red Ginseng, Rehmannia Root, Rhubarb, Rice Starch, Rose Fruit, Rosin, Safflower, Saffron, Saposhnikovia Root, Sappan Wood, Sausurea Root, Schisandra Fruit, Schizonepeta Spike, Scopolia Rhizome, Scutellaria Root, Senega, Senna Leaf, Sinomenium Stem, Smilax Rhizome, Sophora Root, Sweet Hydrangea Leaf, Swertia Herb, Termeric, Toad Venom, Tragacanth, Tribulus Fruit, Trichosanthes Root, Uncaria Hook, Zanthoxylum Fruit, Zedoary.

2. Crude drugs are usually used in the forms of whole crude drugs, cut crude drugs or powdered crude drugs.

Whole crude drugs are the medicinal parts or their ingredients prepared by drying and/or simple processes, as specified in the monographs.

Cut crude drugs are small pieces or small blocks prepared by cutting or crushing of the whole crude drugs, and also coarse, medium or fine cutting of the crude drugs in whole, and, unless otherwise specified, are required to conform to the specifications of the whole

crude drugs used as original materials.

Powdered crude drugs are coarse, medium, fine or very fine powder prepared from the whole crude drugs or the cut crude drugs; usually powdered crude drugs as fine powder are specified in the monographs.

3. Unless otherwise specified, crude drugs are used in dried form. The drying is usually carried out at a temperature not exceeding 60°C.

4. The origin of crude drugs is to serve as the criteria. Such statements as 'other species of the same genus' and 'allied plants' or 'allied animals' appearing in the origin of crude drugs usually indicate plants or animals which may be used as materials for crude drugs containing the same effective constituents.

5. Description in each monograph for crude drugs usually covers the crude drug derived from its typical original plant or animal and includes statements of characteristic properties of the crude drug. As for the color, odor and solubility, apply correspondingly to the prescription of the General Notices, except the odor which is to serve as the criteria. The taste and aspects obtained by microscopic observation are to

serve as the criteria.

6. Powdered crude drugs, otherwise specified, may be mixed with diluents so as to attain proper content and potency.

7. Powdered crude drugs do not contain fragments of tissues, cells, cell inclusions or other foreign matter alien to the original crude drugs or cut crude drugs.

8. Crude drugs are as free as possible from contaminants and other impurities due to molds, insects and other animals and from other foreign matters, and are required to be kept in a clean and hygienic state.

9. Crude drugs are preserved under protection from moisture and insect damage, unless otherwise specified. In order to avoid insect damage, suitable fumigants may be used to preserve crude drugs, provided that the fumigants are so readily volatilized as to be harmless at the usual dosage of the crude drugs, and such fumigants that may affect the therapeutic efficacy of the crude drugs or interfere with the testing are precluded.

10. Crude drugs are preserved in well-closed containers unless otherwise specified.

GENERAL RULES FOR PREPARATIONS

1. General Notices for Preparations

(1) General notices for preparations present general rules and definitions for pharmaceutical dosage forms.

(2) Pharmaceutical excipients are substance(s) other than drug substance(s) contained in preparations which are used to increase the utility of the preparation, to enable manufacturing of drug products easy, to keep product's integrity, to improve the appearance of a formulation and so forth. For these purposes, suitable excipients such as diluents, stabilizers, preservatives, buffering agents, corrigents, suspending agents, emulsifiers, aromatics, solubilizers, coloring agents, and viscous agents may be added. The excipients used, however, must be non-toxic, harmless and pharmacologically inactive in the amount administered and must not interfere with the therapeutic efficacy or the quality test of the preparations.

(3) Vegetable oils used for pharmaceutical preparations usually indicate the edible oils listed in the Pharmacopoeia. When starch is called for, any kind of starch incorporated in the Pharmacopoeia may be used, unless otherwise specified.

Moreover, ethanol specified in vol% is prepared by adding purified water or water for injection to ethanol at the specified vol%.

(4) To pharmaceutical preparations, functions which control the releasing rate of drug substance(s), leading to the modified absorption or transfer into the body may be added for the purpose of controlling the onset and duration of therapeutic effects and/or decreasing adverse or side effects. However, modified release preparations must meet the corresponding requirements of dissolution test etc. which specify the releasing rate, unless otherwise specified. In addition, the functional modification of releasing rate must be displayed on the pack insert and direct container or package of the preparation, unless otherwise specified.

(5) Immediate-release and modified-release preparations exist in oral dosage forms which show different release characteristics, respectively. Immediate-release dosage forms are preparations showing a release of drug substance(s), which is not intentionally modified and generally dependent on the intrinsic physicochemi-

cal properties of the drug substance. Modified-release dosage forms are preparations showing a release of drug substance(s) which is suitably modified by a specific formulation design and/or manufacturing method. Modified-release dosage forms include enteric-coated and extended release preparations. Enteric coated preparations are designed to release the majority drug substance(s) in small intestines rather than in stomachs in order to prevent the degradation or decomposition of drug substance(s) in stomach or to decrease the irritant effect of drug substance(s) on stomachs. Enteric coated preparations are generally prepared by applying enteric films to preparations. Extended release preparations are designed to control the releasing rate and time of drug substance(s) and the release sites in gastrointestinal tracts in order to decrease the dosing times and/or to reduce adverse or side effects. Extended release preparations are generally prepared using suitable agents that prolong the drug release. Capsules, tablets, powders, granules, and pills of oral dosage forms can be coated with appropriate coating agents, such as sugar, sugar alcohol, or high-molecular-mass materials to enable the ingestion easy or to prevent degradation of drug substance(s).

(6) When a high level of sterility assurance is maintained consistently, based on the records derived from validation studies of the manufacturing process and the in-process controls, the sterility test usually required for the release of the product may be omitted (Parametric release).

(7) Unless otherwise specified, preserve pharmaceutical preparations at room temperature.

2. Aerosols

(1) Aerosols are preparations for use by expelling a solution or suspension of drug substance(s) under a pressure of liquefied or compressed gas filled in a common or different container.

Aerosols are used for topical application, space spray, inhalation, oral administration, etc. Modes of expelling are available in vapor, powder, foam and paste, depending on the purpose of use.

(2) Hermetic containers are used for preservation.

3. Aromatic Waters

(1) Aromatic Waters are clear saturated solutions of essential oils or other volatile substances in water.

(2) Unless otherwise specified, Aromatic Waters may be usually prepared by the following process. Shake thoroughly 2 mL of an essential oil or 2 g of a volatile substance with 1000 mL of lukewarm purified water for 15 minutes, set the mixture aside for 12 hours or longer, filter through moistened filter paper, and add purified water to make 1000 mL. Alternatively, incorporate thoroughly 2 mL of an essential oil or 2 g of volatile substances with sufficient refined siliceous earth or pulped filter-paper, add 1000 mL of purified water, agitate thoroughly for 10 minutes, and then filter the mixture. To obtain a clear filtrate, repeat the filtration, and add sufficient water through the filter paper to make 1000 mL.

(3) Aromatic Waters have odor and taste derived from the drug substance(s) and excipients used.

(4) Tight containers are used for preservation.

4. Capsules

(1) Capsules are preparations in which liquefied, suspended, semi-solid, powdered or granulated drugs or preparations are enclosed in capsules or wrapped with capsule bases. There are two kinds of capsules, which are:

(i) Hard capsules (ii) Soft capsules

(2) Capsules are usually prepared by the following methods.

(i) Hard capsules: Drug substance(s) or uniform mixtures of drug substance(s) with diluents and other suitable excipients, or granules or preparations prepared by a suitable method, are filled as they are or prepared lightly formed and into hard capsules. Extended-release or enteric-coated capsules can be prepared by filling extended-release or enteric-coated preparations into capsules or by changing the components of capsule shells or coating the capsule with suitable coating agents.

(ii) Soft capsules: Drug substance(s) or mixtures of drug substance(s) with suitable diluents, etc. are enclosed by a suitable capsule such as gelatin plasticized by addition of glycerin, sorbitol, etc., and molded in a suitable shape. If necessary, coloring agents, preservatives, etc. may be added to capsule agents. By changing the components of capsule shells or applying suitable coating agents to capsules, extended-release or enteric-coated capsules can be prepared.

(3) Unless otherwise specified, Capsules meet the

requirements of the Dissolution Test <6.10> or the Disintegration Test <6.09>.

(4) Unless otherwise specified, Capsules meet the requirements of the Uniformity of Dosage Units <6.02>.

(5) Well-closed or tight containers are used for preservation.

5. Cataplasms/Gel Patches

(1) Cataplasms/Gel Patches are generally pasty preparations containing the mixture of drug substance(s) and water or those prepared by spreading the mixture on cloth, which are intended for external use.

(2) Unless otherwise specified, Cataplasms/Gel Patches are usually prepared by mixing drug substance(s) with glycerin, water, or other suitable liquid materials, or with high-molecular-mass materials(s) which are soluble in water or absorbent of water until homogeneity is attained.

(3) Pasty cataplasms which have separated out one or more of their components during storage are rehomogenized before use unless the substances have deteriorated.

(4) Tight containers are used for preservation.

6. Elixirs

(1) Elixirs are usually clear, sweetened, and aromatic liquid preparations, containing ethanol, intended for oral use.

(2) Elixirs are usually prepared by dissolving drugs or their extractives in ethanol and purified water, adding aromatic agents and sucrose, other sugars or sweetening agents, and clarifying by filtration or other procedures.

(3) Tight containers are used for preservation.

7. Extracts

(1) Extracts are prepared by evaporating the extractives of crude drugs. There are two kinds of Extracts which are:

(i) viscous extracts (ii) dry extracts

(2) Unless other-wise specified, Extracts are prepared as follows.

(i) Crude drugs pulverized in suitable sizes, are usually extracted for a certain period of time with suitable solvents by cold extraction or warm extraction, or by percolation as directed in (2) under Tinctures.

The extractive is filtered, and the filtrate is concentrated or dried in a suitable method to produce a millet jelly-like consistency in the case of a viscous extract, and to make crushable solid masses, granules or powder in the case of a dry extract.

Extracts for which the content of the drug substance(s) is specified are prepared by assaying the drug substance(s) in a sample portion and adjusting, if necessary, with suitable diluents to the specified strength.

(ii) Weigh crude drugs pulverized in suitable sizes according to the prescription and heat after adding 10 – 20 times water to them. After liquid-solid separation by centrifuge etc., the filtrate is concentrated or dried in a suitable method to produce a millet jelly-like consistency in the case of a viscous extract, and to make crushable solid masses, granules or powder in the case of a dry extract.

(3) Extracts have odor and taste derived from the crude drugs used.

(4) Unless otherwise specified, Extracts meet the requirements of the Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 0.3 g of Extracts to ash, warm with 3 mL of the dilute hydrochloric acid, and filter. Wash the residue with two 5 mL portions of water. Neutralize the combined filtrate and washings (indicator: a drop of phenolphthalein TS) by adding ammonia TS until the color of the solution changes to pale red, filter, if necessary, and add 2 mL of the dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid in the same manner as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for preservation.

8. Fluidextracts

(1) Fluidextracts are liquid percolates of crude drugs, usually prepared so that each mL contains soluble constituents from 1 g of the crude drugs.

(2) Fluidextracts are usually prepared by the percolation process. Mix well 1000 g of coarse powder or fine cutting of the crude drugs with the first solvent to moisten it, close the container, and allow it to stand for about 2 hours at room temperature. Transfer the content to a suitable percolator, stuff it as tightly as possible, open the lower opening of the percolator, and slowly pour the second solvent to cover the crude drugs. Close the lower opening when the solvent begins

to drop, and allow the mixture to stand for 2 to 3 days at room temperature. Open the lower opening, and allow the percolate to run out at the rate of 0.5 to 1.0 mL per minute.

Set aside the first 850 mL of the percolate as the first percolate. Add the second solvent to the percolator, then drip the percolate, and use it as the second percolate.

The time of maceration and the flow rate during percolation may be varied depending on the kind and the amount of the crude drugs used. The flow rate is usually regulated as follows, depending on the amount of the crude drugs used.

Mass of crude drug	Volume of solution running per minute
Not more than 1000 g	0.5 – 1.0 mL
Not more than 3000 g	1.0 – 2.0 mL
Not more than 10,000 g	2.0 – 4.0 mL

Concentrate the second percolate, taking care not to lose the volatile substances of the crude drug, mix with the first percolate, and use it as (A). To (A) add the second solvent to make 1000 mL, and allow the mixture to stand for 2 days. Decant the supernatant liquid or filter the liquid to obtain a clear solution.

Fluidextracts for which the content of the drug substance(s) is specified are obtained by adjusting the content of the drug substance(s) with a sufficient amount of the second solvent, as required on the basis of the result of the assay made with a portion of (A).

Use the specified solvent only in cases where there is no distinction between the first and the second solvent.

(3) Fluidextracts have odor and taste derived from the crude drugs used.

(4) Unless otherwise specified, Fluidextracts meet the requirements of the Heavy Metals Limit Test <1.07> when the test solution and the control solution are prepared as follows.

Test solution: Ignite 1.0 g of Fluidextracts to ash, warm with 3 mL of dilute hydrochloric acid, filter, and wash the residue with two 5 mL portions of water. Neutralize the combined filtrate and washings by adding ammonia TS, filter, if necessary, and add 2 mL of dilute acetic acid and water to make 50 mL.

Control solution: Proceed with 3 mL of dilute hydrochloric acid as directed in the preparation of the test solution, and add 3.0 mL of Standard Lead Solution and water to make 50 mL.

(5) Tight containers are used for preservation.

9. Granules

(1) Granules are prepared in a form of granules using drug substance(s) or a mixture of drug substance(s) and excipients.

(2) Granules are made, usually, from drug substance(s) or a uniform mixture of drug substance(s) with diluents, binders, disintegrators or other suitable excipients. The granules are prepared by a suitable method so that the finished granules are preferably equal in size. Extended-release or enteric coated granules can also be prepared by a suitable method.

(3) When the Particle Size Distribution Test <6.03> is performed with granules, all the granules pass through a No. 10 (1700 μm) sieve, not more than 5% of total granules remain on a No. 12 (1400 μm) sieve, and not more than 15% of total granules pass through a No. 42 (355 μm) sieve.

(4) Unless otherwise specified, Granules comply with the Dissolution Test <6.10> or the Disintegration Test <6.09>, provided that this provision does not apply to granules not more than 5% of which remain on a No. 30 (500 μm) sieve when shaken with a No. 30 sieve as directed under Particle Size Distribution Test <6.03>.

(5) Unless otherwise specified, Granules for single-dose use meet the requirements of the Uniformity of Dosage Units.

(6) Well-closed or tight containers are used for preservation.

10. Infusions and Decoctions

(1) Infusions and Decoctions are liquid preparations usually obtained by macerating crude drugs in purified water.

(2) Infusions and Decoctions are usually prepared by the following method. Cut crude drugs as directed below, and transfer 50 g to an infusion or decoction apparatus.

Leaves, flowers, and whole plants: Coarse cutting

Woods, stems, barks, roots, and rhizomes: Medium cutting

Seeds and fruits: Fine cutting

Infusions: Damp an amount of crude drugs with 50 mL of purified water for about 15 minutes, pour 900 mL of hot purified water, and heat for 5 minutes with several shakings. Filter through cloth after cooling.

Decoctions: Heat, with several stirrings, an amount of crude drugs with 950 mL of purified water for 30 minutes, and filter through cloth while warm.

Sufficient purified water is further added to the filtrate through the residue to make 1000 mL of an

infusion or decoction.

Prepare Infusions or Decoctions before use.

(3) Infusions and Decoctions have odor and taste derived from the crude drugs used.

(4) Tight containers are used for preservation.

11. Injections

(1) Injections are solutions, suspensions or emulsions of drugs or other preparations that contain drugs to be dissolved or suspended before use. They are sterile preparations to be administered directly into the skin or the body through the skin, or mucous membrane.

(2) Unless otherwise specified, Injections are prepared by dissolving, suspending or emulsifying drug substance(s) in a prescribed volume of the solvent, or by distributing drug substance(s) in hermetic containers for Injections. Every care should be taken to prevent contamination. The entire process of preparing Injections from the preparation of drug solution to the sterilization should be completed as rapidly as possible by taking into consideration the composition of Injection and the storage condition. The concentration of Injections expressed as % indicates w/v%.

Water for injection prepared by Reverse Osmosis-Ultrafiltration shall be sterilized by heating before use. This provision does not apply to Injections and attached solvent, if they are sterilized by heating in the process of manufacture.

Drugs to be dissolved or suspended before use and designated in the title as "for injection" may be accompanied by a suitable solvent.

(3) Solvents used in the preparation of Injections or attached to Injections must be harmless in the amounts usually administered and must not interfere with the therapeutic efficacy or with quality testing.

The solvents are classified into the following two major groups. They should meet the following requirements.

(i) Aqueous vehicles: As the solvent of aqueous injections, water for injection is usually used. Unless otherwise specified, isotonic sodium chloride solution, Ringer's solution, or other suitable aqueous solutions may be used instead. Unless otherwise specified, these aqueous vehicles other than those exclusively for intracutaneous, subcutaneous or intramuscular administration meet the requirements of the Bacterial Endotoxins Test <4.01>.

When the Bacterial Endotoxins Test <4.01> is not applicable to aqueous vehicles, the Pyrogen Test <4.04> may be used.

(ii) Non-aqueous vehicles: Vegetable oils are usually used as solvents for nonaqueous injections. These oils, unless otherwise specified, are clear at 10°C and have no odor or taste suggesting rancidity. The acid value is not more than 0.56, iodine value is between 79 and 137, and the saponification value falls in the range between 185 and 200. They meet the requirements of the Mineral Oil Test <1.05>.

Several suitable organic solvents other than the vegetable oils may be used as nonaqueous vehicles.

(4) The usual size of particles observed in suspensions for injection is not larger than 150 μm , and that of particles in emulsions for injection is not larger than 7 μm . As a rule, suspensions for injection are not to be injected into the vessels or spinal cord, and emulsions for injection, not into the spinal cord.

(5) Unless otherwise specified, any coloring agent must not be added solely for the purpose of coloring the preparations.

(6) Unless otherwise specified, sodium chloride or other suitable excipients may be added to aqueous injections to render them isotonic with blood or other body fluids. Acids or alkalis may be added to them to adjust the pH.

(7) Unless otherwise specified, sufficient amounts of suitable preservatives to prevent the growth of microorganisms are added to Injections filled in multiple dose containers.

(8) Unless otherwise specified, Injections other than those used exclusively for intracutaneous, subcutaneous or intramuscular administration meet the requirements of the Bacterial Endotoxins Test <4.01>.

When the Bacterial Endotoxins Test <4.01> is not applicable to Injections, the Pyrogen Test <4.04> may be used.

(9) Unless otherwise specified, Injections and solvents attached to Injections meet the requirements of the Sterility Test <4.06>.

(10) Usual containers of Injections are colorless and meet the requirements of the Glass Containers for Injections <7.01>. Where specified in individual monographs, these containers may be replaced by colored containers meeting the requirements of the Glass Containers for Injections <7.01> or by plastic containers for aqueous injections meeting the requirements of the Test Methods for Plastic Containers <7.02>.

(11) Unless otherwise specified, rubber stoppers used for glass containers of 100 mL or more of aqueous infusions meet the requirements of the Rubber Closures for Aqueous Infusions <7.03>.

(12) Unless otherwise specified, Injections meet the requirements of the Foreign Insoluble Matter Test for Injections <6.06>.

(13) Unless otherwise specified, Injections meet the requirements of the Insoluble Particulate Matter Test for Injections <6.07>.

(14) Unless otherwise specified, the actual volume of an injection contained in a single-dose container meets the requirements of Test for Extractable Volume of Parenteral Preparations <6.05>.

(15) Unless otherwise specified, Injections to be dissolved or suspended before use meet the requirements of the Uniformity of Dosage Units <6.02>.

(16) Unless otherwise specified, the written, printed, or graphic matter in the package, the container, or the wrapper must include the following information:

(i) Names of employed vehicles and added substance(s), unless the vehicle is water for injection, or sodium chloride solution in concentrations not exceeding 0.9 w/v%, or unless the vehicle contains acids or alkalis in order to adjust the pH of the injections.

(ii) In the case that dissolving vehicles are attached to the preparations, the presence of the vehicles and their names, quantities, compositions or ratios of the vehicles on the outer containers or outer wrappers.

(iii) Names and quantities of added stabilizers, preservatives, and diluents. In the case where nitrogen or carbon dioxide is enclosed in the container to replace the inside air, the statement of this replacement is not necessary.

(17) For ampules or other containers of 2 mL or less, the designations "injection", "for injection" and "aqueous suspension for injection" may be replaced by "inj.", "for inj." and "aq. susp. for inj.", respectively.

For ampules or other containers of more than 2 mL and not exceeding 10 mL, made of glass or similar materials, the designations "injection", "for injection" and "aqueous suspension for injection" may be replaced by "inj.", "for inj." and "aq. susp. for inj.", respectively, when information is printed directly on the surface of ampules or containers.

(18) Hermetic containers are used for preservation. Plastic containers for aqueous injections may be used when specified in an individual monograph.

12. Lemonades

(1) Lemonades are sweet, sour, and usually clear liquid preparations intended for oral use.

(2) Unless otherwise specified, Lemonades are usually prepared by dissolving hydrochloric acid, citric acid, L-tartaric acid, or lactic acid in simple syrup and purified water, and filtering if necessary.

Prepare Lemonades before use.

- (3) Tight containers are used for preservation.

13. Liniments

(1) Liniments are usually liquid or semisolid preparations intended for external application to the skin by inunction.

(2) Unless otherwise specified, Liniments are usually prepared by adding drugs to water, ethanol, fatty oils, glycerin, soap, emulsifying agents, suspending agents, other suitable excipients or their mixtures, and kneading the mixture until homogeneity is attained.

(3) Liniments which have separated out one or more of their components during storage are rehomogenized before use unless the substances have deteriorated.

- (4) Tight containers are used for preservation.

14. Liquids and Solutions

(1) Liquids and Solutions are liquid preparations intended for oral or external use. They are not identical with any other preparations under General Rules for Preparations.

(2) Liquids and Solutions are usually prepared directly with drug substance(s) or by dissolving drug substance(s) in a solvent.

- (3) Tight containers are used for preservation.

15. Lotions

(1) Lotions are external preparations applied to the skin by inunction, which are usually prepared by dissolving drug substance(s) in an aqueous vehicle or emulsifying or dispersing them homogeneously.

(2) Unless otherwise specified, Lotions are usually prepared by adding drug substance(s) with solvents, emulsifying agents, suspending agents, etc. to an aqueous vehicle and mixing to complete uniformity by a suitable method.

Prepare before use in the case of Lotions which are apt to deteriorate.

(3) Lotions which have separated out one or more of their components during storage are rehomogenized before use unless the substances have deteriorated.

- (4) Tight containers are used for preservation.

16. Ointments

(1) Ointments are usually homogeneous, semisolid preparations for external application, of such consistency that they may be applied to the skin by inunction.

(2) Unless otherwise specified, Ointments are usually prepared by kneading and mixing homogeneously drug substance(s) with fats, fatty oils, lanolin, petrolatum, paraffin, waxes, resins, plastics, glycols, higher alcohols, glycerin, water, emulsifying agents, suspending agents, or other suitable excipients, or with above excipients emulsified in a suitable way as bases.

Prepare before use in the case of Ointments which are apt to deteriorate.

Ointments which are prepared with emulsified bases may be described as Cream.

- (3) Ointments are free from rancid odor.

- (4) Tight containers are used for preservation.

17. Ophthalmic Ointments

(1) Ophthalmic Ointments are aseptic ointments intended for the application to the conjunctiva.

(2) Ophthalmic Ointments are usually prepared by the following method. Solution of drug substance(s) or finely powdered drug substance(s) are thoroughly mixed with petrolatum or other suitable materials as a base, and are distributed into collapsible tubes or other tight containers. Sufficient care should be taken to prevent any kinds of contamination, and to proceed as fast as possible in the manufacturing of products.

(3) The particle size of drug substance(s) in Ophthalmic Ointments is usually not larger than 75 μm .

(4) Unless otherwise specified, Ophthalmic Ointments meet the requirements of the Sterility Test <4.06>, and unless otherwise specified, carry out the test by the Membrane filtration method.

(5) Unless otherwise specified, Ophthalmic Ointments meet the requirements of the Test of Metal Particles in Ophthalmic Ointments <6.01>.

The requirement is met if a total of not more than 50 metal particles, each measuring 50 μm or more in any dimension, is found in the 10 samples, and if not more than one sample is found to contain more than 8 such particles. If Ophthalmic Ointments fail the foregoing test, repeat the test on 20 additional samples of Ophthalmic Ointments. The requirement is met if a total of not more than 150 metal particles, each measuring 50 μm or more in any dimension, is found in the 30 samples, and if not more than three samples are

found to contain more than 8 such particles each.

- (6) Tight containers are used for preservation.

18. Ophthalmic Solutions

(1) Ophthalmic Solutions are aseptic preparations intended for application to the conjunctiva. They are solutions or suspensions of the drug substance(s), or preparations which contain drug substance(s) to be dissolved or suspended before use.

(2) Unless otherwise specified, Ophthalmic Solutions are prepared either by dissolving or suspending drug substance(s) in a prescribed volume of a solvent, or by placing drug substance(s) in tight containers. Every caution is required to avoid contamination in preparing Ophthalmic Solutions. The entire process of preparing Ophthalmic Solutions should be completed as rapidly as possible. The concentration of Ophthalmic Solutions expressed as % of a drug substance indicates w/v%.

Preparations to be dissolved or suspended before use and designated as "for ophthalmic solutions" may be accompanied by a suitable solvent.

(3) Solvents used in the preparation of Ophthalmic Solutions or attached to Ophthalmic Solutions must be harmless in the amounts usually administered and must not interfere with therapeutic efficacy, or with testing.

Solvents for Ophthalmic Solutions are classified into the following two major groups. They should meet the following requirements.

(i) Aqueous vehicles: The usual vehicle for aqueous ophthalmic solutions is purified water or suitable aqueous solutions. Solvents constituted to Ophthalmic Solutions are sterilized, purified water or suitable sterilized aqueous solutions.

(ii) Non-aqueous vehicles: The vehicles for non-aqueous ophthalmic solutions are usually vegetable oils. Also, suitable organic solvents may be used as non-aqueous solvents for some preparations.

(4) The usual particle size observed in suspensions for Ophthalmic Solutions is not larger than 75 μm .

(5) Unless otherwise specified, no coloring agent may be added solely for the purpose of coloring the preparations.

(6) Unless otherwise specified, sodium chloride or other suitable excipients may be added to aqueous preparations to render them isotonic with lachrymal liquid. Acids or alkalis or other suitable excipients, may be added to aqueous preparations to adjust the pH.

(7) Unless otherwise specified, Ophthalmic Solutions and solvents attached to Ophthalmic Solutions

meet the requirements of the Sterility Test <4.06>.

(8) Ophthalmic Solutions prepared as aqueous solution and aqueous vehicles attached to Ophthalmic Solutions to be prepared before use should be clear and free from foreign insoluble matter when inspected with the unaided eye at a position of luminous intensity of 3000 to 5000 luxes under an incandescent electric bulb. The containers of Ophthalmic Solutions should have a transparency which does not interfere with the test for foreign matter.

(9) Unless otherwise specified, Ophthalmic Solutions meet the Insoluble Particulate Matter Test for Ophthalmic Solutions <6.08>. The limit of the particulates is not more than 1 particle per mL equal to or greater than 300 μm .

- (10) Tight containers are used for preservation.

19. Pills

(1) Pills are spherical masses.

(2) Pills are usually prepared by mixing drug substance(s) uniformly with diluents, binders, disintegrators or other suitable excipients, and rolling into spherical form by a suitable method.

(3) Unless otherwise specified, Pills comply with the Dissolution Test <6.10> or the Disintegration Test <6.09>.

(4) Well-closed or tight containers are used for preservation.

20. Plasters and Pressure Sensitive Adhesive Tapes

(1) Plasters and Pressure Sensitive Adhesive Tapes are usually used as topical drugs of external use by spreading or sealing a mixture of drug substance(s), bases and excipients on a cloth or on/in a plastic film, and adhering to the skin in order to deliver the drug substance(s) to the disease sites located to the skin or nearby skin.

(2) Unless otherwise specified, Plasters and Pressure Sensitive Adhesive Tapes are usually prepared by mixing bases such as water soluble or insoluble, natural or artificial high-molecular-mass compound, or their mixture uniformly with drug substance(s) and kneading or sealing on a cloth or film into a suitable shape.

Unless otherwise specified, Plasters and Pressure Sensitive Adhesive Tapes prepared from fats, fatty oils, salts of fatty acids, waxes, resins, plastics, purified lanolin, rubber, or a mixture of the above substances, or prepared by mixing the drug substance(s) with the

above bases uniformly and as a solid at the ordinary temperature, may be described as plasters.

(3) Well-closed containers are used for preservation.

21. Powders

(1) Powders are preparations in powdered or finely granulated form.

(2) Powders are usually prepared by uniformly mixing drug substance(s) with or without diluents, binders, disintegrators or other suitable excipients by a suitable method to produce a pulverized or finely granulated form.

(3) When the Particle Size Distribution Test <6.03> is performed with Powders, all the powders pass through a No. 18 (850 μm) sieve and not more than 5% of total powders remain on a No. 30 (500 μm) sieve. Powders with not more than 10% of total passing through a No. 200 (75 μm) sieve may be described as Fine Granules.

(4) Unless otherwise specified, Powders for single-dose use meet the requirements of the Uniformity of Dosage Units <6.02>.

(5) Well-closed or tight containers are used for preservation.

22. Spirits

(1) Spirits are usually alcoholic or hydro-alcoholic solutions of volatile drug substance(s).

(2) Unless otherwise specified, Spirits are usually prepared by dissolving drug substance(s) in ethanol or in a mixture of ethanol and water.

(3) Tight containers are used for preservation, removing from fire.

23. Suppositories

(1) Suppositories are solid preparations intended for insertion into the rectal or vaginal cavity. Suppositories are usually prepared by molding bases into a suitable shape.

Suppositories melt or soften at body temperature or dissolve slowly in the secretions.

(2) Unless otherwise specified, Suppositories are usually prepared by mixing drug substance(s) with fat-type bases, watermiscible bases or other suitable materials, and, if necessary, with emulsifying agents, suspending agents, etc. into a homogeneous mass, and

molding it into a suitable shape or coating it with a suitable coating agent, or prepared as a liquid form-fill-seal.

(3) Rectal suppositories are usually conical or spindle-shaped, and Vaginal suppositories are globular or oval.

(4) Unless otherwise specified, Suppositories meet the requirements of the Uniformity of Dosage Units <6.02>.

(5) Well-closed or tight containers are used for preservation.

24. Suspensions and Emulsions

(1) Suspensions and Emulsions are usually liquid preparations of finely divided drug substance(s) suspended or emulsified uniformly in liquid vehicles, respectively.

(2) Suspensions and Emulsions are usually prepared by the following method.

Suspensions: Suspensions are prepared by adding suspending agents or other suitable excipients and purified water or oil to drug substance(s), and suspending to complete uniformity by a suitable method.

Emulsions: Emulsions are prepared by adding emulsifying agents and purified water to drug substance(s), and emulsifying to complete uniformity by a suitable method.

If necessary, preservatives, stabilizers, etc., may be added.

Prepare before use in the case of Suspensions or Emulsions which are apt to deteriorate.

(3) Mix uniformly before use, if necessary.

(4) Tight containers are used for preservation.

25. Syrups

(1) Syrups are oral liquid preparations. Syrups are solutions of sucrose, or viscous liquids or suspensions of drug substance(s) containing sucrose, other sugars or sweetening agents.

Syrups include the preparations which are dissolved or suspended before use depending on the properties of the drug substance(s).

(2) Unless otherwise specified, Syrups are usually prepared by dissolving, mixing, suspending or emulsifying drug substance(s) in solutions of sucrose, other sugars or sweetening agents, or in simple syrup. If necessary, the mixtures are boiled and filtered while hot.

(3) Unless otherwise specified, Syrups which are

dissolved or suspended before use and are for single-dose use (divided dosage forms) meet the requirements of the Uniformity of Dosage Units <6.02>.

(4) Tight containers are used for preservation.

26. Tablets

(1) Tablets are prepared by compressing drug substance(s) directly, or by forming or molding drug substance(s) dampened with a solvent into a desired shape and size. Sugar- and film-coated tablets can be prepared by coating core tablets using suitable coating agents containing sugars, sugar alcohols and related substances and by coating with thin films using suitable film coating agents, respectively. Enteric coated and extended release tablets can be prepared by suitable methods.

(2) Tablets are usually prepared by the following procedures:

(i) Drug substance(s) are first rendered granular in a suitable method with or without uniform admixture with a diluent, binder, disintegrator, and other suitable excipients. The resultant granules are provided with additives such as a lubricant, and compressed into a desired shape and size.

(ii) Tablets may also be prepared either by direct compression of drug substance(s) with or without a diluent, binder, disintegrator, and other suitable excipients, or by compression after drug substance(s) with or without suitable excipients have been added to previously prepared inactive granules.

(iii) Tablets may also be prepared by drying the admixture by a suitable method after forming or molding drug substance(s), uniformly mixed with a diluent, binder and other suitable excipients and dampened with a solvent, into a desired shape and size.

(iv) Multilayer tablets can be prepared by compressing different layers of particles or granules in composition. Press-coated tablets can be prepared by covering inner core tablets with different layers in composition by a suitable method.

(3) Unless otherwise specified, Tablets meet the requirements of the Dissolution Test <6.10> or the Disintegration Test <6.09>.

(4) Unless otherwise specified, Tablets meet the requirements of the Uniformity of Dosage Units <6.02>. The requirements for coated tablets are provided in each monograph.

(5) Well-closed or tight containers are used for preservation.

27. Tinctures

(1) Tinctures are liquid preparations, and usually prepared by extracting crude drug substance(s) with ethanol or with a mixture of ethanol and purified water.

(2) Unless otherwise specified, Tinctures are usually prepared from coarse powder or fine cuttings of crude drug substance(s) either by maceration or by percolation as described below.

Maceration: Place crude drugs in a suitable container, and add about three-fourths of the total volume of a solvent to be used. Stopper, and allow the container to stand at ordinary temperature with occasional stirring for about 5 days or until the soluble constituents have satisfactorily dissolved. Filter the liquid through cloth. Wash the residue with several portions of the solvent, and press. Combine the filtrate and washings, and add sufficient solvent to make up the volume. Allow the mixture to stand for about 2 days, and obtain a clear liquid by decantation or filtration.

Percolation: Pour the solvent in small portions on crude drugs placed in a container, and mix well to moisten the crude drugs. Stopper the container, and allow it to stand for about 2 hours at room temperature. Pack the contents as tightly as possible in a suitable percolator, open the lower opening, and slowly pour sufficient solvent to cover the crude drugs. When the percolate begins to drip, close the opening, and allow the mixture to stand for 2 to 3 days at room temperature. Open the opening, and allow the percolate to drip at a rate of 1 to 3 mL per minute. Add an appropriate quantity of the solvent, and continue to percolate until the desired volume has passed. Mix thoroughly, allow standing for 2 days, and obtain a clear liquid by decantation or filtration. The time of standing and the flow rate may be varied depending on the kind and amount of crude drugs to be percolated.

Tinctures prepared by either of the above methods for which the content of the drug substance is specified are prepared by assaying the drug substance using a portion of the sample and adjusting, if necessary, with the percolate or with the solvent to the specified content.

(3) Tight containers are used for preservation, removing from fire.

28. Transdermal Systems

(1) Transdermal Systems are preparations applied to the skin that are designed to deliver drug substance(s) through the skin to the systemic blood circulation.

Transdermal Systems include semisolid mixtures of drug substance(s) and excipients which are used by spreading a suitable amount of the mixture on the backing layer.

(2) Unless otherwise specified, Transdermal Systems are usually prepared by spreading the mixtures of emulsified or suspended drug substance(s) and soluble or insoluble high molecular weight of natural or synthetic bases or their mixtures on the liner or backing sheet. If necessary, adhesives agents, solvents or skin permeation enhancers etc., may be added. The transdermal systems are also prepared by filling the mixture of drug substance(s) and bases or excipients in a reservoir made of a backing layer and a membrane which controls the release of drug substance(s).

(3) Transdermal Systems meet the requirements of release tests specified.

(4) Well-closed or tight containers are used for preservation.

29. Troches

(1) Troches are usually preparations of suitable shape to dissolve or disintegrate slowly in the mouth, and are intended for application to the mouth or the throat.

(2) Troches are usually prepared by the following procedures:

(i) Drug substance(s) are first rendered granular by a suitable method with or without uniform admixing with a diluent, binder, and other suitable excipients. The resultant granules are provided with additives such as a lubricant, and compressed into a desired shape and size.

(ii) Troches may also be prepared either by direct compression of drug substance(s) with or without a diluent, binder or other suitable excipients, or by compression of drug substance(s) with or without suitable excipients after they have been uniformly mixed with previously prepared inactive granules.

(iii) Troches are also prepared by mixing drug substance(s) with a diluent such as sucrose, binder, moistening agent, other suitable excipients, etc., to make a homogeneous paste, spreading the paste, stamping out or cutting into a suitable shape and drying.

(3) Unless otherwise specified, Troches meet the requirements of the Uniformity of Dosage Units <6.02>.

(4) Well-closed or tight containers are used for preservation.

GENERAL TESTS, PROCESSES AND APPARATUS

General Tests, Processes and Apparatus includes common methods for tests, useful test methods for quality recognition of drugs and other articles related to them. Unless otherwise specified, the procedures for acid-neutralizing capacity determination of gastrointestinal medicines, alcohol number determination, ammonium determination, arsenic determination, atomic absorption spectrophotometry, test for bacterial endotoxins, boiling point determination, distilling range determination, chloride determination, conductivity measurement, congealing point determination, determination of bulk and tapped densities, digestion test, disintegration test, dissolution test, endpoint detection in titrimetry, test of extractable volume for injection, flame coloration, fluorometry, foreign insoluble matter test for injections, gas chromatography, heavy metals determination, test for glass containers for injections, infrared spectrophotometry, insoluble particulate matter test for injections, insoluble particulate matter test for ophthalmic solutions, iron determination, liquid chromatography, loss on drying determination, loss on ignition determination, melting point determination, test for metal particles in ophthalmic ointments, methanol determination, microbial assay for antibiotics, test for microbial limit, test for microbial limit for crude drugs, mineral oil determination, nitrogen determination, nuclear magnetic resonance spectroscopy, optical rotation determination, osmolarity determination, oxygen flask combustion method, particle size distribution test for preparations, pH determination, test for plastic containers, powder particle density determination, powder particle size determination, test for pyrogen, qualitative test, test for readily carbonizable substances, refractive index determination, residual solvents test, residue on ignition determination, test for rubber closure for aqueous infusions, specific gravity and density determination, specific surface area determination, test for sterility, sulfate determination, thermal analysis, thin-layer chromatography, test for total organic carbon, ultraviolet-visible spectrophotometry, uniformity of dosage units (test for content uniformity, mass variation test), viscosity determination, vitamin A assay, water determination, and X-ray powder diffraction are performed as directed in the corresponding articles under the General Tests, Processes and Apparatus. The tests for melting point of fats, congealing point of fatty acids, specific gravity, acid value, saponification value, ester value, hydroxyl value, unsaponifiable matter and iodine value of fats and fatty oils are performed as directed in the corresponding items under Fats and Fatty Oils Test, and sampling, preparation of sample for analysis, microscopic examination, purity test, loss on drying, total ash, acid-insoluble ash, extract content, and essential oil content of crude drugs are performed as directed in the corresponding items under Crude Drugs Test.

The number of each test method is a category number given individually. The number in brackets (< >) appeared in monograph indicates the number corresponding to the

general test method.

1. Chemical Methods

1.01 Alcohol Number Determination

Alcohol Number Determination represents the number of milliliters of ethanol at 15°C obtained from 10 mL of tincture or other preparations containing ethanol by the following procedures.

Method 1 Distilling method

This is a method to determine the Alcohol Number by reading the number of milliliters of ethanol distillate at 15°C obtained from 10 mL of a sample measured at 15°C by the following procedures.

(1) Apparatus

Use hard glass apparatus as illustrated in Fig. 1.01-1. Ground glass may be used for the joints.

(2) Reagent

Alkaline phenolphthalein solution: To 1 g of phenolphthalein add 7 mL of sodium hydroxide TS and water to make 100 mL.

(3) Procedure

Transfer 10 mL of the sample preparation, accurately measured at $15 \pm 2^\circ\text{C}$, to the distilling flask A, add 5 mL of water and boiling chips. Distil ethanol carefully into the glass-stoppered, volumetric cylinder D.

By reference to Table 1.01-1, a suitable volume of distillate (mL) should be collected, according to the content of ethanol in the sample preparation.

Prevent bumping during distillation by rendering the sample strongly acidic with phosphoric acid or sulfuric acid, or by adding a small amount of paraffin, beeswax or silicone resin before starting the distillation.

When the samples contain the following substances, carry out pretreatment as follows before distillation.

(i) Glycerin: Add sufficient water to the sample so that the residue in the distilling flask, after distillation, contains at least 50% of water.

(ii) Iodine: Decolorize the sample with zinc powder.

(iii) Volatile substances: Preparations containing appreciable proportions of essential oil, chloroform, diethyl ether or camphor require treatment as follows. Mix 10 mL of the sample, accurately measured, with 10 mL of saturated sodium chloride solution in a separator, add 10 mL of petroleum benzine, and shake. Collect the separated aqueous layer. The petroleum benzine layer was extracted with two 5 mL portions of saturated sodium chloride solution. Combine the aqueous layers, and distill. According to the ethanol content in the sample, collect a volume of distillate 2 to 3 mL

Table 1.01-1

Ethanol content in the sample (vol%)	Distillate to be collected (mL)
more than 80	13
80 - 70	12
70 - 60	11
60 - 50	10
50 - 40	9
40 - 30	8
less than 30	7

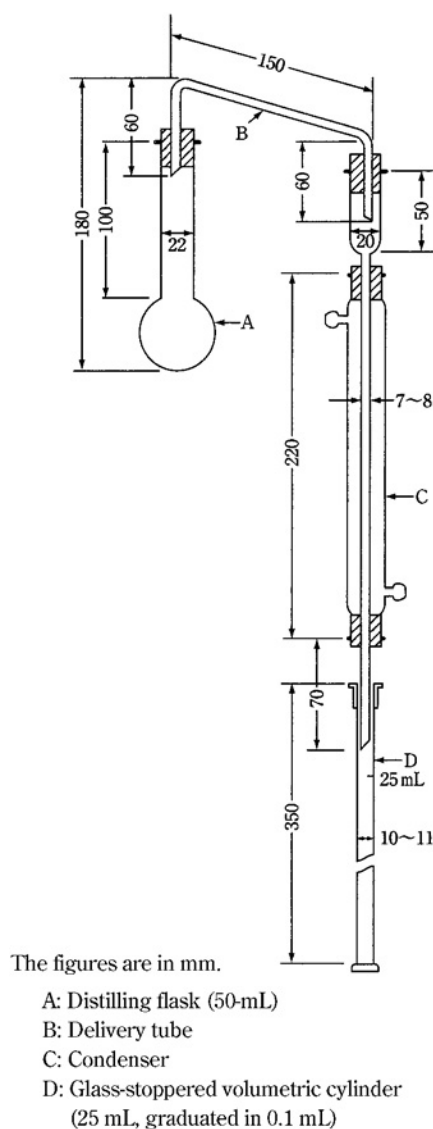


Fig. 1.01-1

more than that shown in the above Table.

(iv) Other substances: Render preparations containing free ammonia slightly acidic with dilute sulfuric acid. If volatile acids are present, render the preparation slightly alkaline with sodium hydroxide TS, and if the preparations contain soap along with volatile substances, decompose the soap with an excess of dilute sulfuric acid before the extraction with petroleum benzin in the treatment described in (iii).

To the distillate add 4 to 6 g of potassium carbonate and 1

to 2 drops of alkaline phenolphthalein solution, and shake vigorously. If the aqueous layer shows no white turbidity, agitate the distillate with additional potassium carbonate. After allowing to stand in water at $15 \pm 2^\circ\text{C}$ for 30 minutes, read the volume of the upper reddish ethanol layer in mL, and regard it as the Alcohol Number. If there is no clear boundary surface between these two layers, shake vigorously after addition of a few drops of water, then observe in the same manner.

Method 2 Gas chromatography

This is a method to determine the alcohol number by determining ethanol ($\text{C}_2\text{H}_5\text{OH}$) content (vol%) from a sample measured at 15°C by the following procedures.

(1) Reagent

Ethanol for alcohol number: Ethanol (99.5) with determined ethanol ($\text{C}_2\text{H}_5\text{OH}$) content. The relation between specific gravity d_{15}^{15} of ethanol and content of ethanol ($\text{C}_2\text{H}_5\text{OH}$) is 0.797 : 99.46 vol%, 0.796 : 99.66 vol%, and 0.795 : 99.86 vol%.

(2) Preparation of sample solution and standard solution

Sample solution: Measure accurately a volume of sample at $15 \pm 2^\circ\text{C}$ equivalent to about 5 mL of ethanol ($\text{C}_2\text{H}_5\text{OH}$), and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

Standard solution: Measure accurately 5 mL of ethanol for alcohol number at the same temperature as the sample, and add water to make exactly 50 mL. Measure accurately 25 mL of this solution, add exactly 10 mL of the internal standard solution, and add water to make 100 mL.

(3) Procedure

Place 25 mL each of the sample solution and the standard solution in a 100-mL, narrow-mouthed, cylindrical glass bottle sealed tightly with a rubber closure and aluminum band, immerse the bottle up to the neck in water, allowed to stand at room temperature for more than 1 hour in a room with little change in temperature, shake gently so as not to splash the solution on the closure, and allow to stand for 30 minutes. Perform the test with 1 mL each of the gas in the bottle with a syringe according to the Gas Chromatography <2.02> under the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of ethanol to that of the internal standard.

$$\text{Alcohol number} = \frac{Q_T}{Q_S} \times \frac{5 \text{ (mL)}}{\text{a volume (mL) of sample}} \times \frac{\text{ethanol (C}_2\text{H}_5\text{OH) content (vol\%) of ethanol for alcohol number}}{9.406}$$

Internal standard solution—A solution of acetonitrile (3 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 1.5 m in length, packed with 150- to 180- μm porous ethylvinylbenzene-divinylbenzene copolymer (mean pore size: 0.0075 μm , 500 - 600 m^2/g) for gas chromatography.

Column temperature: A constant temperature between 105°C and 115°C .

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: Proceed with 1 mL of the gas obtained from the standard solution in the bottle under the above operating conditions, and calculate the resolution. Use a column giving elution of ethanol and the internal standard in this order with the resolution between these peaks being not less than 2.0.

1.02 Ammonium Limit Test

Ammonium Limit Test is a limit test for ammonium contained in drugs.

In each monograph, the permissible limit for ammonium (as NH_4^+) is described in terms of percentage (%) in parentheses.

Apparatus

Use a distilling apparatus for ammonium limit test as illustrated in Fig. 1.02-1. For the distillation under reduced pressure, use the apparatus shown in Fig. 1.02-2. Either apparatus are composed of hard glass, and ground-glass joints may be used. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Procedure

(1) Preparation of test solution and control solution—Unless otherwise specified, test solutions and control solution are prepared as directed in the following.

Place an amount of the sample, directed in the monograph, in the distilling flask A. Add 140 mL of water and 2 g of magnesium oxide, and connect the distillation apparatus. To the receiver (measuring cylinder) F add 20 mL of boric acid solution (1 in 200) as an absorbing solution, and immerse the lower end of the condenser. Adjust the heating to

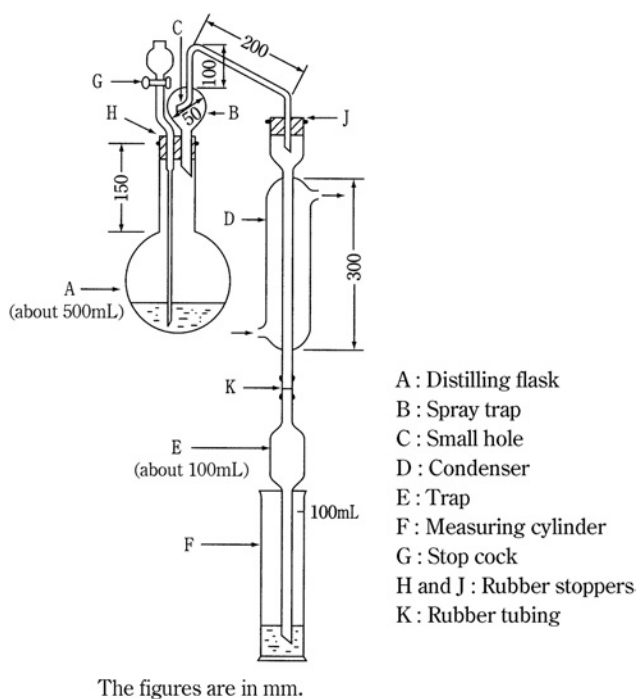
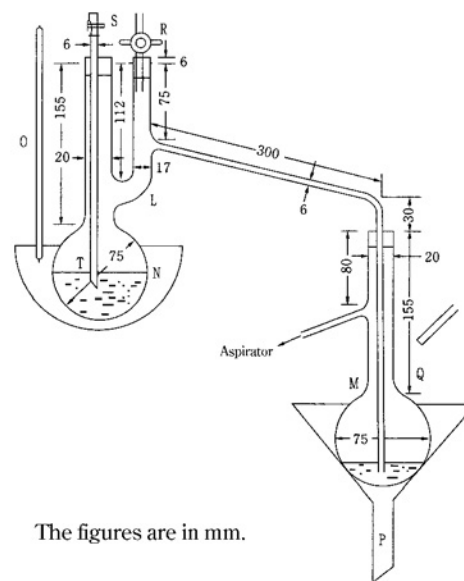


Fig. 1.02-1 Distilling apparatus for ammonium limit test



- | | |
|---------------------------------------|--------------------------------|
| L: Vacuum distillation flask (200-mL) | Q: Cooling water |
| M: Receiver (a 200-mL flask) | R: Glass cock |
| N: Water bath | S: Rubber tube with screw cock |
| O: Thermometer | T: Glass tube for anti-bumping |
| P: Funnel | |

Fig. 1.02-2 Vacuum distilling apparatus for ammonium limit test

give a rate of 5 to 7 mL per minute of distillate, and distill until the distillate measures 60 mL. Remove the receiver from the lower end of the condenser, rinsing the end part with a small quantity of water, add sufficient water to make 100 mL and designate it as the test solution.

For the distillation under reduced pressure, take the amount of sample specified in the monograph to the vacuum distillation flask L, add 70 mL of water and 1 g of magnesium oxide, and connect to the apparatus (Fig. 1.02-2). To the receiver M add 20 mL of a solution of boric acid (1 in 200) as absorbing liquid, put the end of the branch tube of the distillation flask L in the absorbing liquid, and keep at 60°C using a water bath or alternative equipment. Adjust the reduced pressure to get the distillate at a rate of 1 to 2 mL per minute, and continue the distillation until to get 30 mL of the distillate. Cool the receiver M with running water during the distillation. Get off the end of the branch tube from surface of the absorbing liquid, rinse in the end with a small amount of water, then add water to the liquid to make 100 mL, and perform the test using this solution as the test solution.

Place a volume of Standard Ammonium Solution, directed in the monograph, in the distilling flask A or the vacuum distillation flask L, proceed as for the preparation of the test solution, and designate it as the control solution.

(2) Test of the test solution and the control solution—Unless otherwise specified, proceed as directed in the following.

Place 30 mL each of the test solution and the control solution in Nessler tubes, add 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS to each solution, and mix. Then add 4 mL of sodium hypochlorite-sodium hydroxide TS and water to make 50 mL, mix, and allow to stand for 60

minutes. Compare the color of both solutions against a white background by viewing downward or transversely: the color developed in the test solution is not more intense than that of the control solution.

1.03 Chloride Limit Test

Chloride Limit Test is a limit test for chloride contained in drugs.

In each monograph, the permissible limit for chloride (as Cl) is described in terms of percentage (%) in parentheses.

Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, and dissolve it in a proper volume of water to make 40 mL. Add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.01 mol/L hydrochloric acid VS, directed in the monograph, to another Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions by using the same procedure.

Add 1 mL of silver nitrate TS to the test solution and to the control solution, mix well, and allow to stand for 5 minutes protecting from direct sunlight. Compare the opalescence developed in both solutions against a black background by viewing downward or transversely.

The opalescence developed in the test solution is not more than that of the control solution.

1.04 Flame Coloration Test

Flame Coloration Test is a method to detect an element, by means of the property that the element changes the colorless flame of a Bunsen burner to its characteristic color.

(1) Salt of metal—The platinum wire used for this test is about 0.8 mm in diameter, and the end part of it is straight. In the case of a solid sample, make the sample into a gruel by adding a small quantity of hydrochloric acid, apply a little of the gruel to the 5-mm end of the platinum wire, and test by putting the end part in a colorless flame, keeping the platinum wire horizontal. In the case of a liquid sample, immerse the end of the platinum wire into the sample to about 5 mm in length, remove from the sample gently, and perform the test in the same manner as for the solid sample.

(2) Halide—Cut a copper net, 0.25 mm in opening and 0.174 mm in wire diameter, into a strip 1.5 cm in width and 5 cm in length, and wind in round one end of a copper wire. Heat the copper net strongly in the colorless flame of Bunsen burner until the flame no longer shows a green or blue color, and then cool it. Repeat this procedure several times, and coat the net completely with cupric oxide. After cooling, unless otherwise specified, apply about 1 mg of the sample to the copper net, ignite, and burn it. Repeat this procedure three times, and then test by putting the copper net in the colorless flame.

The description, "Flame coloration persists", in a monograph, indicates that the reaction persists for 4 seconds.

1.05 Mineral Oil Test

Mineral Oil Test is a method to test mineral oil in nonaqueous solvents for injections and for eye drops.

Procedure

Pour 10 mL of the sample into a 100-mL flask, and add 15 mL of sodium hydroxide solution (1 in 6) and 30 mL of ethanol (95). Put a short-stemmed, small funnel on the neck of the flask, and heat on a water bath to make clear, with frequent shaking. Then transfer the solution to a shallow porcelain dish, evaporate the ethanol on a water bath, add 100 mL of water to the residue, and heat on a water bath: no turbidity is produced in the solution.

1.06 Oxygen Flask Combustion Method

Oxygen Flask Combustion Method is a method for the identification or the determination of halogens or sulfur produced by combusting organic compounds, which contain chlorine, bromine, iodine, fluorine or sulfur, in a flask filled with oxygen.

Apparatus

Use the apparatus shown in Fig. 1.06-1.

Preparation of test solution and blank solution

Unless otherwise specified, prepare them by the following method.

(1) Preparation of sample

(i) For solid samples: Place the quantity of the sample specified in the monograph on the center of the filter illustrated in the figure, weigh accurately, wrap the sample carefully along the dotted line without scattering, and place the parcel in a platinum basket or cylinder B, leaving its fuse-strip on the outside.

(ii) For liquid samples: Roll a suitable amount of absorbent cotton with filter paper, 50 mm in length and 5 mm in width, so that the end part of the paper is left to a length of about 20 mm as a fuse-strip, and place the parcel in a platinum basket or cylinder B. Place the sample in a suitable glass tube, weigh accurately, and moisten the cotton with the quantity of the sample specified in the monograph, bringing the edge of the sample in contact with the cotton.

(2) Method of combustion

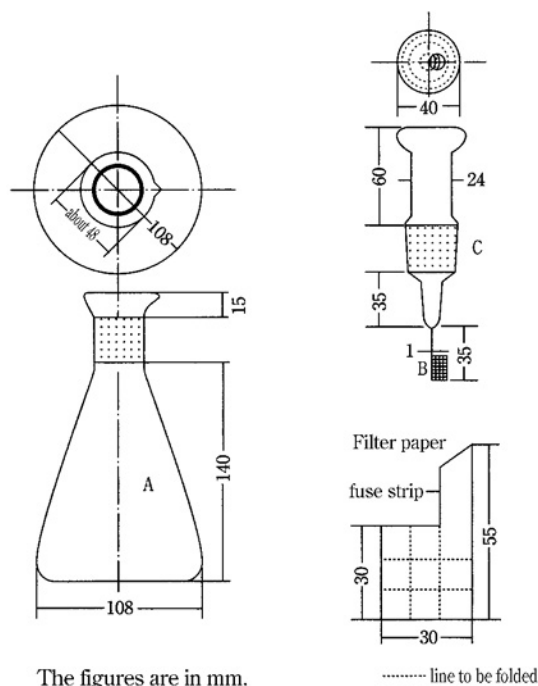
Place the absorbing liquid specified in the monograph in flask A, fill it with oxygen, moisten the ground part of the stopper C with water, then ignite the fuse-strip, immediately transfer it to the flask, and keep the flask airtight until the combustion is completed. Shake the flask occasionally until the white smoke in A vanishes completely, allow to stand for 15 to 30 minutes, and designate the resulting solution as the test solution. Prepare the blank solution in the same manner, without sample.

Procedure of determination

Unless otherwise specified in the monograph, perform the test as follows.

(1) Chlorine and bromine

Apply a small amount of water to the upper part of A, pull



The figures are in mm.

..... line to be folded

- A: Colorless, thick-walled (about 2 mm), 500-mL hard glass flask, the upper part of which is made like a saucer. A flask made of quartz should be used for the determination of fluorine.
- B: Platinum basket or cylinder made of platinum woven gauge. (It is hung at the end of the stopper C with platinum wire).
- C: Ground stopper made of hard glass. A stopper made of quartz should be used for the determination of fluorine.

Fig. 1.06-1

out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 15 mL of 2-propanol, and combine the washings with the test solution. To this solution add 1 drop of bromophenol blue TS, add dilute nitric acid dropwise until a yellow color develops, then add 25 mL of 2-propanol, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the potentiometric titration under the Electrometric titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS
= 0.1773 mg of Cl

Each mL of 0.005 mol/L silver nitrate VS
= 0.3995 mg of Br

(2) Iodine

Apply a small amount of water to the upper part of A, pull out C carefully, add 2 drops of hydrazine hydrate to the test solution, put C on A, and decolorize the solution by vigorous shaking. Transfer the content of A to a beaker, wash C, B and the inner side of A with 25 mL of 2-propanol, and transfer the washings to the above beaker. To this solution add 1 drop of bromophenol blue TS, then add dilute nitric acid dropwise until a yellow color develops, and titrate <2.50> with 0.005 mol/L silver nitrate VS according to the Potentiometric titration under Electrometric Titration. Perform the test with the blank solution in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L silver nitrate VS
= 0.6345 mg of I

(3) Fluorine

Apply a small amount of water to the upper part of A, pull out C carefully, transfer the test solution and the blank solution to 50 mL volumetric flasks separately, wash C, B and the inner side of A with water, add the washings and water to make 50 mL, and use these solutions as the test solution and the correction solution. Pipet the test solution (V mL) equivalent to about 30 ng of fluorine, V mL of the correction solution and 5 mL of standard fluorine solution, transfer to 50-mL volumetric flasks separately, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a blank prepared with 5 mL of water in the same manner. Determine the absorbances, A_T , A_C and A_S , of the subsequent solutions of the test solution, the correction solution and the standard solution at 600 nm.

Amount (mg) of fluorine (F) in the test solution
= amount (mg) of fluorine in 5 mL of

$$\text{the standard solution} \times \frac{A_T - A_C}{A_S} \times \frac{50}{V}$$

Standard Fluorine Solution: Dry sodium fluoride (standard reagent) in a platinum crucible between 500°C and 550°C for 1 hour, cool it in a desiccator (silica gel), weigh accurately about 66.3 mg of it, and dissolve in water to make exactly 500 mL. Pipet 10 mL of this solution, and dilute with sufficient water to make exactly 100 mL.

(4) Sulfur

Apply a small amount of water to the upper part of A, pull out C carefully, and wash C, B and the inner side of A with 15 mL of methanol. To this solution add 40 mL of methanol, then add exactly 25 mL of 0.005 mol/L barium perchlorate VS, allow to stand for 10 minutes, add 0.15 mL of arsenazo III TS with a measuring pipet, and titrate <2.50> with 0.005 mol/L sulfuric acid VS. Perform the test with the blank solution in the same manner.

Each mL of 0.005 mol/L barium perchlorate VS
= 0.1604 mg of S

1.07 Heavy Metals Limit Test

Heavy Metals Limit Test is a limit test of the quantity of heavy metals contained as impurities in drugs. The heavy metals are the metallic inclusions that are darkened with sodium sulfide TS in acidic solution, as their quantity is expressed in terms of the quantity of lead (Pb).

In each monograph, the permissible limit for heavy metals (as Pb) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as directed in the following:

(1) Method 1

Place an amount of the sample, directed in the monograph, in a Nessler tube. Dissolve in water to make 40 mL.

Add 2 mL of dilute acetic acid and water to make 50 mL, and designate it as the test solution.

The control solution is prepared by placing the volume of Standard Lead Solution directed in the monograph in a Nessler tube, and adding 2 mL of dilute acetic acid and water to make 50 mL.

(2) Method 2

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, cover loosely with a lid, and carbonize by gentle ignition. After cooling, add 2 mL of nitric acid and 5 drops of sulfuric acid, heat cautiously until white fumes are no longer evolved, and incinerate by ignition between 500°C and 600°C. Cool, add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Then add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate a mixture of 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid on a water bath, further evaporate to dryness on a sand bath, and moisten the residue with 3 drops of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(3) Method 3

Place an amount of the sample, directed in the monograph, in a quartz or porcelain crucible, heat cautiously, gently at first, and then increase the heat until incineration is completed. After cooling, add 1 mL of aqua regia, evaporate to dryness on a water bath, moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and warm for 2 minutes. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until the solution develops a pale red color, add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and washings to a Nessler tube, and add water to make 50 mL. Designate it as the test solution.

The control solution is prepared as follows: Evaporate 1 mL of aqua regia to dryness on a water bath. Hereinafter, proceed as directed for the test solution, and add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

(4) Method 4

Place an amount of the sample, directed in the monograph, in a platinum or porcelain crucible, mix with 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and carbonize by gradual heating. Cool, add 1 mL of sulfuric acid, heat carefully, and incinerate by ignition between 500°C and 600°C. If a carbonized substance remains, moisten with a small amount of sulfuric acid, and incinerate by ignition. Cool, dissolve the residue in 3 mL of hydrochloric acid, evaporate on a water bath to dryness, wet the residue with 3 drops of hydrochloric acid, add 10 mL of water, and dissolve by warming. Add 1 drop of phenolphthalein TS, add ammonia TS dropwise until a pale red color develops, then add 2 mL of dilute acetic acid, filter if necessary, wash with 10 mL of water, transfer the filtrate and the washing to a Nessler tube, add water to make

50 mL, and use this solution as the test solution.

The control solution is prepared as follows: Take 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. Cool, add 1 mL of sulfuric acid, heat carefully, and ignite between 500°C and 600°C. Cool, and add 3 mL of hydrochloric acid. Hereinafter, proceed as directed in the test solution, then add the volume of Standard Lead Solution directed in the monograph and water to make 50 mL.

Procedure

Add 1 drop of sodium sulfide TS to each of the test solution and the control solution, mix thoroughly, and allow to stand for 5 minutes. Then compare the colors of both solutions by viewing the tubes downward or transversely against a white background. The test solution has no more color than the control solution.

1.08 Nitrogen Determination (Semimicro-Kjeldahl Method)

Nitrogen Determination is a method to determine ammonia in ammonium sulfate obtained by decomposition of organic substances containing nitrogen with sulfuric acid.

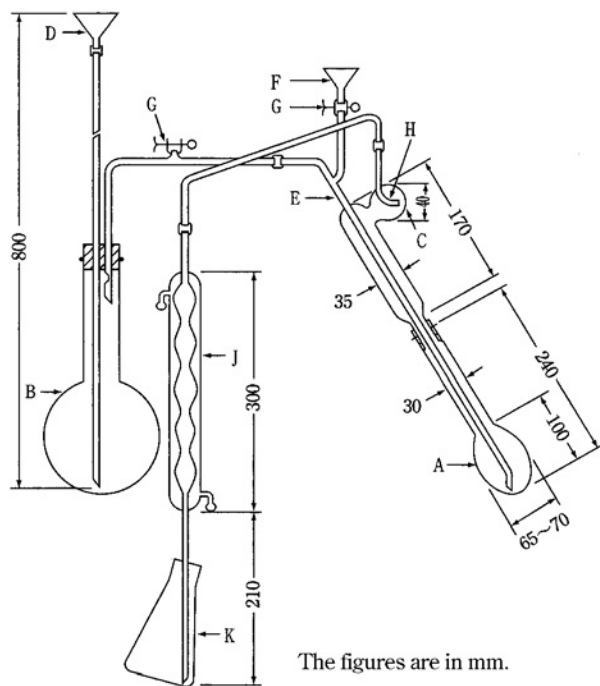
Apparatus

Use the apparatus illustrated in Fig. 1.08-1. It is thoroughly constructed of hard glass, and ground glass surfaces may be used for joints. All rubber parts used in the apparatus should be boiled for 10 to 30 minutes in sodium hydroxide TS and for 30 to 60 minutes in water, and finally washed thoroughly with water before use.

Procedure

Unless otherwise specified, proceed by the following method. Weigh accurately or pipet a quantity of the sample corresponding to 2 to 3 mg of nitrogen (N:14.01), and place in the Kjeldahl flask A. Add 1 g of a powdered mixture of 10 g of potassium sulfate and 1 g of copper (II) sulfate pentahydrate. Wash down any adhering sample from the neck of the flask with a small quantity of water. Add 7 mL of sulfuric acid, allowing it to flow down the inside wall of the flask.

Then, while shaking the flask, add cautiously 1 mL of hydrogen peroxide (30) drop by drop along the inside wall of the flask. Heat the flask gradually, then heat so strong that the vapor of sulfuric acid is condensed at the neck of the flask, until the solution changes through a blue and clear to a vivid green and clear, and the inside wall of the flask is free from a carbonaceous material. If necessary, add a small quantity of hydrogen peroxide (30) after cooling, and heat again. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus (Fig. 1.08-1) washed beforehand by passing steam through it. To the absorption flask K add 15 mL of boric acid solution (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube J. Add 30 mL of sodium hydroxide solution (2 in 5) through the funnel F, rinse cautiously the funnel with 10 mL of water, immediately close the clamp attached to the rubber tubing G, then begin the distillation with steam, and continue until the distillate measures 80 to 100 mL. Remove the absorption flask from the lower end of the condenser tube J,



- A: Kjeldahl flask
 B: Steam generator, containing water, to which 2 to 3 drops of sulfuric acid and fragments of boiling chips for preventing bumping have been added
 C: Spray trap
 D: Water supply funnel
 E: Steam tube
 F: Funnel for addition of alkali solution to flask A
 G: Rubber tubing with a clamp
 H: A small hole having a diameter approximately equal to that of the delivery tube
 J: Condenser, the lower end of which is beveled
 K: Absorption flask

Fig. 1.08-1

rinsing the end part with a small quantity of water, and titrate <2.50> the distillate with 0.005 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.005 mol/L sulfuric acid VS
 = 0.1401 mg of N

1.09 Qualitative Tests

Qualitative Tests are applied to the identification of drugs and are done generally with quantities of 2 to 5 mL of the test solution.

Acetate

(1) When warmed with diluted sulfuric acid (1 in 2), acetates evolve the odor of acetic acid.

(2) When an acetate is warmed with sulfuric acid and a small quantity of ethanol (95), the odor of ethyl acetate is evolved.

(3) Neutral solutions of acetates produce a red-brown color with iron (III) chloride TS, and a red-brown precipitate when boiled. The precipitate dissolves and the color of the solution changes to yellow upon addition of hydrochloric acid.

Aluminum salt

(1) Solutions of aluminum salts, when treated with ammonium chloride TS and ammonia TS, yield a gelatinous, white precipitate which does not dissolve in an excess of ammonia TS.

(2) Solutions of aluminum salts, when treated with sodium hydroxide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(3) Solutions of aluminum salts, when treated with sodium sulfide TS, yield a gelatinous, white precipitate which dissolves in an excess of the reagent.

(4) Add ammonia TS to solutions of aluminum salts until a gelatinous, white precipitate is produced. The color of the precipitate changes to red upon addition of 5 drops of alizarin red S TS.

Ammonium salt

When heated with an excess of sodium hydroxide TS, ammonium salts evolve the odor of ammonia. This gas changes moistened red litmus paper to blue.

Antimony salt, primary

(1) When primary antimony salts are dissolved in a slight excess of hydrochloric acid for the test and then diluted with water, a white turbidity is produced. The mixture produces an orange precipitate upon addition of 1 to 2 drops of sodium sulfide TS. When the precipitate is separated, and sodium sulfide TS is added to one portion of the precipitate and sodium hydroxide TS is added to another portion, it dissolves in either of these reagents.

(2) Add water to acidic solutions of primary antimony salts in hydrochloric acid until a small quantity of precipitate is produced, and then add sodium thiosulfate TS: the precipitate dissolves. A red precipitate is reproduced when the solution is heated.

Aromatic amines, primary

Acidic solutions of primary aromatic amines, when cooled in ice, mixed with 3 drops of sodium nitrite TS under agitation, allowed to stand for 2 minutes, mixed well with 1 mL of ammonium amidosulfate TS, allowed to stand for 1 minute, and then mixed with 1 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS, exhibit a red-purple color.

Arsenate

(1) Neutral solutions of arsenates produce no precipitate with 1 to 2 drops of sodium sulfide TS, but produce a yellow precipitate with hydrochloric acid subsequently added. The separated precipitate dissolves in ammonium carbonate TS.

(2) Neutral solutions of arsenates produce a dark red-brown precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia TS is added to another portion, the precipitate dissolves in either of these reagents.

(3) Neutral or ammonia alkaline solutions of arsenates produce with magnesia TS a white, crystalline precipitate, which dissolves by addition of dilute hydrochloric acid.

Arsenite

(1) Acidic solutions of arsenites in hydrochloric acid

produce a yellow precipitate with 1 to 2 drops of sodium sulfide TS. When hydrochloric acid is added to one portion of the separated precipitate, it does not dissolve. When ammonium carbonate TS is added to another portion, the precipitate dissolves.

(2) Slightly alkaline solutions of arsenites produce a yellowish white precipitate with silver nitrate TS. When ammonia TS is added to one portion of the suspension, and dilute nitric acid is added to another portion, the precipitate dissolves in either of these reagents.

(3) Slightly alkaline solutions of arsenites produce a green precipitate with copper (II) sulfate TS. When the separated precipitate is boiled with sodium hydroxide TS, it changes to red-brown.

Barium salt

(1) When the Flame Coloration Test (1) <1.04> is applied to barium salts, a persistent yellow-green color develops.

(2) Solutions of barium salts produce with dilute sulfuric acid a white precipitate, which does not dissolve upon addition of dilute nitric acid.

(3) Acidic solutions of barium salts in acetic acid produce a yellow precipitate with potassium chromate TS. The precipitate dissolves by addition of dilute nitric acid.

Benzoate

(1) Concentrated solutions of benzoates produce a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed with cold water and dried, melts between 120°C and 124°C <2.60>.

(2) Neutral solutions of benzoates produce a pale yellow-red precipitate upon dropwise addition of iron (III) chloride TS. The precipitate changes to white on subsequent addition of dilute hydrochloric acid.

Bicarbonate

(1) Bicarbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with carbonates).

(2) Solutions of bicarbonates produce no precipitate with magnesium sulfate TS, but produce a white precipitate when boiled subsequently.

(3) A cold solution of bicarbonates remains unchanged or exhibits only a slightly red color upon addition of 1 drop of phenolphthalein TS (discrimination from carbonates).

Bismuth salt

(1) Bismuth salts, dissolved in a slight excess of hydrochloric acid, yield a white turbidity upon dilution with water. A dark brown precipitate is produced with 1 to 2 drops of sodium sulfide TS subsequently added.

(2) Acidic solutions of bismuth salts in hydrochloric acid exhibit a yellow color upon addition of thiourea TS.

(3) Solution of bismuth salts in dilute nitric acid or in dilute sulfuric acid yield with potassium iodide TS a black precipitate, which dissolves in an excess of the reagent to give an orange-colored solution.

Borate

(1) When ignite a mixture of a borate with sulfuric acid and methanol, it burns with a green flame.

(2) Turmeric paper, when moistened with acidic solutions of borates in hydrochloric acid and dried by warming, exhibits a red color, which changes to blue with ammonia TS

added dropwise.

Bromate

(1) Acidic solutions of bromates in nitric acid yield with 2 to 3 drops of silver nitrate TS a white, crystalline precipitate, which dissolves upon heating. When 1 drop of sodium nitrite TS is added to this solution, a pale yellow precipitate is produced.

(2) Acidic solutions of bromates in nitric acid exhibit a yellow to red-brown color upon addition of 5 to 6 drops of sodium nitrite TS. When 1 mL of chloroform is added to the mixture and shaken, the chloroform layer exhibits a yellow to red-brown color.

Bromide

(1) Solutions of bromides yield a pale yellow precipitate with silver nitrate TS. Upon addition of dilute nitric acid to a portion of the separated precipitate, it does not dissolve. When ammonia solution (28) is added to another portion and shaken, the separated solution yields a white turbidity upon acidifying with dilute nitric acid.

(2) Solutions of bromides exhibit a yellow-brown color with chlorine TS. The mixture is separated into 2 portions. When one portion is shaken with chloroform, the chloroform layer exhibits a yellow-brown to red-brown color. When phenol is added to the other portion, a white precipitate is produced.

Calcium salt

(1) When the Flame Coloration Test (1) <1.04> is applied to calcium salts, a yellow-red color develops.

(2) Solutions of calcium salts yield a white precipitate with ammonium carbonate TS.

(3) Solutions of calcium salts yield a white precipitate with ammonium oxalate TS. The separated precipitate does not dissolve in dilute acetic acid, but dissolves in dilute hydrochloric acid.

(4) Neutral solutions of calcium salts produce no precipitate, when mixed with 10 drops of potassium chromate TS and heated (discrimination from strontium salts).

Carbonate

(1) Carbonates effervesce upon addition of dilute hydrochloric acid, generating a gas, which produces a white precipitate immediately, when passed into calcium hydroxide TS (common with bicarbonates).

(2) Solutions of carbonates yield with magnesium sulfate TS a white precipitate, which dissolves by addition of dilute acetic acid.

(3) Cold solutions of carbonates exhibit a red color with 1 drop of phenolphthalein TS (discrimination from bicarbonates).

Ceric salt

(1) When a cerous salt is mixed with 2.5 times its mass of lead (IV) oxide, nitric acid is added and the solution is boiled, it exhibits a yellow color.

(2) Solutions of cerous salts yield a yellow to red-brown precipitate upon addition of hydrogen peroxide TS and ammonia TS.

Chlorate

(1) Solutions of chlorates yield no precipitate with silver nitrate TS. When 2 to 3 drops of sodium nitrite TS and dilute nitric acid are added to the mixture, a white precipitate is produced gradually, which dissolves by addition of ammonia

TS.

(2) When indigocarmine TS is added dropwise to neutral solutions of chlorates until a pale blue color appears, and the mixture is acidified with dilute sulfuric acid, the blue color vanishes promptly upon subsequent dropwise addition of sodium hydrogensulfite TS.

Chloride

(1) Solution of chlorides evolve an odor of chlorine, when mixed with sulfuric acid and potassium permanganate, and heated. The gas evolved turns moistened potassium iodide starch paper blue.

(2) Solutions of chlorides yield a white precipitate with silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

Chromate

(1) Solutions of chromates exhibit a yellow color.

(2) Solutions of chromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to a portion of the suspension, the precipitate does not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of chromates in sulfuric acid are mixed with an equal volume of ethyl acetate and 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

Citrate

(1) When 20 mL of a mixture of pyridine and acetic anhydride (3:1) is added to 1 or 2 drops of a solution of citrate, and the solution is allowed to stand for 2 to 3 minutes, a red-brown color develops.

(2) Neutral solutions of citrates, when mixed with an equal volume of dilute sulfuric acid and two-thirds volume of potassium permanganate TS, heated until the color of permanganate is discharged, and then treated dropwise with bromine TS to one-tenth of total volume, yield a white precipitate.

(3) Neutral solutions of citrates, when boiled with an excess of calcium chloride TS, yield a white crystalline precipitate. When sodium hydroxide TS is added to a portion of the separated precipitate, it does not dissolve. When dilute hydrochloric acid is added to another portion, the precipitate dissolves.

Cupric salt

(1) When a well polished iron plate is immersed in acidic solutions of cupric salts in hydrochloric acid, a red metallic film appears on its surface.

(2) Solutions of cupric salts produce a pale blue precipitate with a small quantity of ammonia TS. The precipitate dissolves in an excess of the reagent, yielding a deep blue-colored solution.

(3) Solutions of cupric salts yield a red-brown precipitate with potassium hexacyanoferrate (II) TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves, yielding a deep blue-colored solution.

(4) Solutions of cupric salts produce a black precipitate with sodium sulfide TS. When dilute hydrochloric acid, dilute sulfuric acid or sodium hydroxide TS is added to a portion of

the separated precipitate, it does not dissolve. When hot dilute nitric acid is added to another portion, the precipitate dissolves.

Cyanide

(1) Solutions of cyanides yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of cyanides yield a blue precipitate, when mixed by shaking with 2 to 3 drops of iron (II) sulfate TS, 2 to 3 drops of dilute iron (III) chloride TS and 1 mL of sodium hydroxide TS, and then acidified with dilute sulfuric acid.

Dichromate

(1) Solutions of dichromates exhibit a yellow-red color.

(2) Solutions of dichromates produce a yellow precipitate with lead (II) acetate TS. When acetic acid (31) is added to one portion of the suspension, the precipitate does not dissolve. When dilute nitric acid is added to another portion, the precipitate dissolves.

(3) When acidic solutions of dichromates in sulfuric acid are mixed with an equal volume of ethyl acetate and with 1 to 2 drops of hydrogen peroxide TS, shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

Ferric salt

(1) Slightly acidic solutions of ferric salts yield with potassium hexacyanoferrate (II) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferric salts yield with sodium hydroxide TS a gelatinous, red-brown precipitate, which changes to black upon addition of sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid, yielding a white turbidity.

(3) Slightly acidic solutions of ferric salts exhibit a purple color with 5-sulfosalicylic acid TS.

Ferricyanide

(1) Solutions of ferricyanides exhibit a yellow color.

(2) Solutions of ferricyanides yield with iron (II) sulfate TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

Ferrocyanide

(1) Solutions of ferrocyanides yield with iron (III) chloride TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrocyanides yield with copper (II) sulfate TS a red-brown precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

Ferrous salt

(1) Slightly acidic solutions of ferrous salts yield with potassium hexacyanoferrate (III) TS a blue precipitate, which does not dissolve in dilute hydrochloric acid subsequently added.

(2) Solutions of ferrous salts yield with sodium hydroxide TS a greenish gray, gelatinous precipitate, which changes to black with sodium sulfide TS. The separated precipitate dissolves in dilute hydrochloric acid.

(3) Neutral or slightly acidic solutions of ferrous salts exhibit an intense red color upon dropwise addition of a solution of 1,10-phenanthroline monohydrate in ethanol (95) (1

in 50).

Fluoride

(1) When solutions of fluorides are heated with chromic acid-sulfuric acid TS, the inside of the test tube is not moistened uniformly.

(2) Neutral or slightly acidic solutions of fluorides exhibit a blue-purple color after standing with 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1).

Glycerophosphate

(1) Solutions of glycerophosphates remain unaffected by addition of calcium chloride TS, but yield a precipitate when boiled.

(2) Solutions of glycerophosphates yield no precipitate with hexaammonium heptamolybdate TS in the cold, but yield a yellow precipitate when boiled for a long time.

(3) When glycerophosphates are mixed with an equal mass of powdered potassium hydrogen sulfate and heated gently over a free flame, the pungent odor of acrolein is evolved.

Iodide

(1) Solutions of iodides yield a yellow precipitate with silver nitrate TS. When dilute nitric acid is added to one portion of the suspension, and ammonia solution (28) to another portion, the precipitates do not dissolve in either of these reagents.

(2) Acidic solutions of iodides exhibit a yellow-brown color with 1 to 2 drops of sodium nitrite TS and then yield a black-purple precipitate. The solutions exhibit a deep blue color with starch TS subsequently added.

Lactate

Acidic solutions of lactates in sulfuric acid, when heated with potassium permanganate TS, evolve the odor of acetaldehyde.

Lead salt

(1) Solutions of lead salts yield a white precipitate with dilute sulfuric acid. When dilute nitric acid is added to a portion of the separated precipitate, it does not dissolve. When sodium hydroxide TS is added to another portion and warmed, or when ammonium acetate TS is added to another portion, the precipitate dissolves.

(2) Solutions of lead salts yield with sodium hydroxide TS a white precipitate, which dissolves in an excess of sodium hydroxide TS, and yields a black precipitate upon subsequent addition of sodium sulfide TS.

(3) Acidic solutions of lead salts in dilute acetic acid yield with potassium chromate TS a yellow precipitate, which does not dissolve in ammonia TS but dissolves in sodium hydroxide TS subsequently added.

Lithium salt

(1) When the Flame Coloration Test (1) <1.04> is applied to lithium salts, a persistent red color develops.

(2) Solutions of lithium salts yield with disodium hydrogenphosphate TS a white precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

(3) Solutions of lithium salts yield no precipitate with dilute sulfuric acid (discrimination from strontium salts).

Magnesium salt

(1) Solutions of magnesium salts yield upon warming

with ammonium carbonate TS a white precipitate, which dissolves in ammonium chloride TS. A white, crystalline precipitate is reproduced by subsequent addition of disodium hydrogenphosphate TS.

(2) Solutions of magnesium salts yield with sodium hydroxide TS a white, gelatinous precipitate. When iodine TS is added to one portion of the suspension, the precipitate develops a dark-brown color. When excess sodium hydroxide TS is added to another portion, the precipitate does not dissolve.

Manganese salt

(1) Solutions of manganese salts yield a white precipitate with ammonia TS. When silver nitrate TS is added to a portion of the suspension, the precipitate changes to black. When another portion is allowed to stand, the upper part of the precipitate exhibits a brownish color.

(2) Acidic solutions of manganese salts in dilute nitric acid exhibit a purple-red color with a small quantity of powdered bismuth sodium trioxide.

Mercuric salt

(1) A copper plate is immersed in solutions of mercuric salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercurous salts).

(2) Solutions of mercuric salts yield with a small quantity of sodium sulfide TS a black precipitate, which dissolves in an excess of the reagent. The black precipitate is reproduced by subsequent addition of ammonium chloride TS.

(3) When potassium iodide TS is added dropwise to neutral solutions of mercuric salts, a red precipitate is produced. The precipitate dissolves in an excess of the reagent.

(4) Acidic solutions of mercuric salts in hydrochloric acid yield with a small quantity of tin (II) chloride TS a white precipitate, which changes to grayish black upon addition of an excess of the reagent.

Mercurous salt

(1) A copper plate is immersed in solutions of mercurous salts, allowed to stand, taken out, and then washed with water. The plate becomes bright and silvery white in appearance, when rubbed with paper or cloth (common with mercuric salts).

(2) Mercurous salts or their solutions exhibit a black color with sodium hydroxide TS.

(3) Solutions of mercurous salts yield a white precipitate with dilute hydrochloric acid. The separated precipitate changes to black upon addition of ammonia TS.

(4) Solutions of mercurous salts yield with potassium iodide TS a yellow precipitate, which changes to green, when allowed to stand, and changes again to black upon subsequent addition of an excess of the reagent.

Nitrate

(1) When a solution of nitrates is mixed with an equal volume of sulfuric acid, the mixture is cooled, and iron (II) sulfate TS is superimposed, a dark brown ring is produced at the junction of the two liquids.

(2) Solutions of nitrates exhibit a blue color with diphenylamine TS.

(3) When potassium permanganate TS is added to acidic solutions of nitrates in sulfuric acid, the red-purple color of the reagent does not fade (discrimination from nitrites).

Nitrite

(1) Solutions of nitrites, when acidified with dilute sulfuric acid, evolve a yellow-brown gas with a characteristic odor. The solutions exhibit a dark brown color upon addition of a small quantity of iron (II) sulfate crystals.

(2) Solutions of nitrites, when 2 to 3 drops of potassium iodide TS and dilute sulfuric acid are added dropwise, exhibit a yellow-brown color, and then yield a black-purple precipitate. When the mixture is shaken with 2 mL of chloroform, the chloroform layer exhibits a purple color.

(3) Solutions of nitrites, when mixed with thiourea TS and acidified with dilute sulfuric acid, and iron (III) chloride TS is added dropwise, exhibit a dark red color. When the mixture is shaken with 2 mL of diethyl ether, the diethyl ether layer exhibits a red color.

Oxalate

(1) When potassium permanganate TS is added dropwise to warm acidic solutions of oxalates in sulfuric acid, the reagent is decolorized.

(2) Solutions of oxalates yield a white precipitate with calcium chloride TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

Permanganate

(1) Solutions of permanganates exhibit a red-purple color.

(2) When an excess of hydrogen peroxide TS is added to acidic solutions of permanganates in sulfuric acid, the solutions effervesce and decolorize permanganates.

(3) Acidic solutions of permanganates in sulfuric acid are decolorized, when an excess of oxalic acid TS is added and heated.

Peroxide

(1) Solutions of peroxides are mixed with an equal volume of ethyl acetate and 1 to 2 drops of potassium dichromate TS, and then acidified with dilute sulfuric acid. When the mixture is shaken immediately and allowed to stand, the ethyl acetate layer exhibits a blue color.

(2) Acidic solutions of peroxides in sulfuric acid decolorize dropwise added potassium permanganate TS, and effervesce to evolve a gas.

Phosphate (Orthophosphate)

(1) Neutral solutions of phosphates yield with silver nitrate TS a yellow precipitate, which dissolves upon addition of dilute nitric acid or ammonia TS.

(2) Neutral solutions or acidic solutions in dilute nitric acid of phosphates yield a yellow precipitate with hexaammonium heptamolybdate TS on warming. The precipitate dissolves upon subsequent addition of sodium hydroxide TS or ammonia TS.

(3) Neutral or ammonia-alkaline solutions of phosphates yield with magnesia TS a white, crystalline precipitate, which dissolves upon subsequent addition of dilute hydrochloric acid.

Potassium salt

(1) When the Flame Coloration Test (1) <1.04> is applied to potassium salts, a pale purple color develops. When it gives a yellow color, a red-purple color can be seen through cobalt glass.

(2) Neutral solutions of potassium salts yield a white,

crystalline precipitate with sodium hydrogen tartrate TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod. The separated precipitate dissolves upon addition of any of ammonia TS, sodium hydroxide TS or sodium carbonate TS.

(3) Acidic solutions of potassium salts in acetic acid (31) yield a yellow precipitate with sodium hexanitrocobaltate (III) TS.

(4) Potassium salts do not evolve the odor of ammonia, when an excess of sodium hydroxide TS is added and warmed (discrimination from ammonium salts).

Salicylate

(1) Salicylates evolve the odor of phenol, when an excess of soda-lime is added and heated.

(2) Concentrated solutions of salicylates yield a white, crystalline precipitate with dilute hydrochloric acid. The separated precipitate, washed well with cold water and dried, melts <2.60> at about 159°C.

(3) Neutral solutions of salicylates exhibit with 5 to 6 drops of dilute iron (III) chloride TS a red color, which changes to purple and then fades when dilute hydrochloric acid is added dropwise.

Silver salt

(1) Solutions of silver salts yield a white precipitate with dilute hydrochloric acid. When dilute nitric acid is added subsequently to a portion of the suspension, the precipitate does not dissolve. When an excess of ammonia TS is added to another portion, the precipitate dissolves.

(2) Solutions of silver salts yield with potassium chromate TS a red precipitate, which dissolves upon addition of dilute nitric acid.

(3) Solutions of silver salts yield a brownish gray precipitate with ammonia TS added dropwise. When ammonia TS is added dropwise until the precipitate dissolves, then 1 to 2 drops of formaldehyde solution are added and warmed, a mirror of metallic silver is deposited on the inside wall of the container.

Sodium salt

(1) When the Flame Coloration Test (1) <1.04> is applied to sodium salts, a yellow color develops.

(2) Concentrated, neutral or slightly alkaline solutions of sodium salts yield a white, crystalline precipitate with potassium hexahydroxoantimonate (V) TS. The formation of the precipitate is accelerated by rubbing the inside wall of the test tube with a glass rod.

Stannic salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannic salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannous salts).

(2) When granular zinc is immersed in acidic solutions of stannic salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannous salts).

(3) Add iron powder to acidic solutions of stannic salts in hydrochloric acid, allow to stand, and then filter. When iodine-starch TS is added dropwise to the filtrate, the color of the test solution disappears.

(4) Acidic solutions of stannic salts in hydrochloric acid, to which ammonia TS is added dropwise until a small quanti-

ty of precipitate is produced, yield a pale yellow precipitate with 2 to 3 drops of sodium sulfide TS. The separated precipitate dissolves upon addition of sodium sulfide TS and pale yellow precipitate is reproduced by subsequent addition of hydrochloric acid.

Stannous salt

(1) When the outside bottom of a test tube containing water is moistened with acidic solutions of stannous salts in hydrochloric acid and is placed in a nonluminous flame of a Bunsen burner, a blue flame mantle is seen around the bottom of the test tube (common with stannic salts).

(2) When granular zinc is immersed in acidic solutions of stannous salts in hydrochloric acid, a spongy, gray substance is deposited on the surface of the granules (common with stannic salts).

(3) When iodine-starch TS is added dropwise to solutions of stannous salts, the color of the test solution disappears.

(4) Acidic solutions of stannous salts in hydrochloric acid, to which ammonia TS is added dropwise until a small quantity of precipitate is produced, yield a dark brown precipitate with 2 to 3 drops of sodium sulfide TS. When sodium sulfide TS is added to a portion of the separated precipitate, it does not dissolve. When ammonium polysulfide TS is added to another portion, the precipitate dissolves.

Sulfate

(1) Solutions of sulfates yield with barium chloride TS a white precipitate, which does not dissolve upon addition of dilute nitric acid.

(2) Neutral solutions of sulfates yield with lead (II) acetate TS a white precipitate, which dissolves upon subsequent addition of ammonium acetate TS.

(3) When an equal volume of dilute hydrochloric acid is added, solutions of sulfates yield no white turbidity (discrimination from thiosulfates), and do not evolve the odor of sulfur dioxide (discrimination from sulfites).

Sulfide

Most kinds of sulfides evolve the odor of hydrogen sulfide with dilute hydrochloric acid. This gas blackens lead (II) acetate paper moistened with water.

Sulfite and Bisulfite

(1) When iodine TS is added dropwise to acidic solutions of sulfites or bisulfites in acetic acid (31), the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of sulfites or bisulfites evolve the odor of sulfur dioxide but yield no turbidity (discrimination from thiosulfates). The solutions yield immediately with 1 drop of sodium sulfide TS a white turbidity, which changes gradually to a pale yellow precipitate.

Tartrate

(1) Neutral tartrate solutions yield a white precipitate with silver nitrate TS. When nitric acid is added to a portion of the separated precipitate, it dissolves. When ammonia TS is added to another portion and warmed, the precipitate dissolves and metallic silver is deposited gradually on the inside wall of the test tube, forming a mirror.

(2) Solutions of tartrates exhibit a red-purple to purple color, when 2 drops of acetic acid (31), 1 drop of iron (II) sulfate TS, 2 to 3 drops of hydrogen peroxide TS and an excess of sodium hydroxide TS are added.

(3) When a solution, prepared by mixing 2 to 3 drops of a solution of resorcinol (1 in 50) and 2 to 3 drops of a solution of potassium bromide (1 in 10) with 5 mL of sulfuric acid, is added to 2 to 3 drops of solutions of tartrates, and then heated for 5 to 10 minutes on a water bath, a deep blue color is produced. The solution exhibits a red to red-orange color when poured to 3 mL of water after cooling.

Thiocyanate

(1) Solutions of thiocyanates yield a white precipitate with an excess of silver nitrate TS. When dilute nitric acid is added to a portion of the suspension, the precipitate does not dissolve. When ammonia solution (28) is added to another portion, the precipitate dissolves.

(2) Solutions of thiocyanates produce with iron (III) chloride TS a red color, which is not decolorized by addition of hydrochloric acid.

Thiosulfate

(1) When iodine TS is added dropwise to acidic solutions of thiosulfates in acetic acid (31), the color of the reagent fades.

(2) When an equal volume of dilute hydrochloric acid is added, solutions of thiosulfates evolve the odor of sulfur dioxide, and yield gradually a white turbidity, which changes to yellow on standing.

(3) Solutions of thiosulfates yield with an excess of silver nitrate TS a white precipitate, which changes to black on standing.

Zinc salt

(1) Neutral to alkaline solutions of zinc salts yield a whitish precipitate with ammonium sulfide TS or sodium sulfide TS. The separated precipitate does not dissolve in dilute acetic acid but dissolves upon subsequent addition of dilute hydrochloric acid.

(2) Solutions of zinc salts yield a white precipitate with potassium hexacyanoferrate (II) TS. When dilute hydrochloric acid is added to a portion of the suspension, the precipitate does not dissolve. When sodium hydroxide TS is added to another portion, the precipitate dissolves.

(3) Neutral to weakly acidic solutions of zinc salts yield a white precipitate, when 1 or 2 drops of pyridine and 1 mL of potassium thiocyanate TS are added.

1.10 Iron Limit Test

Iron Limit Test is a limit test for iron contained in drugs. The limit is expressed in term of iron (Fe).

In each monograph, the permissible limit for iron (as Fe) is described in terms of ppm in parentheses.

Preparation of test solutions and control solutions

Unless otherwise specified, test solutions and control solutions are prepared as follows:

(1) Method 1

Weigh the amount of sample specified in individual monograph, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, dissolve by warming if necessary, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 30 mL of acetic acid-sodium acetate buffer solution for

iron limit test, pH 4.5.

(2) Method 2

Weigh the amount of sample specified in individual monograph, add 10 mL of dilute hydrochloric acid, and dissolve by warming if necessary. Dissolve 0.5 g of L-tartaric acid, and add one drop of phenolphthalein TS. Add ammonia TS dropwise until the solution develops a pale red color. Add 20 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: To the amount of Standard Iron Solution specified in individual monograph add 10 mL of dilute hydrochloric acid, and proceed as directed for the test solution.

(3) Method 3

Place the amount of sample specified in individual monograph in a crucible, moisten with a small amount of sulfuric acid, heat cautiously and gently at first, and then incinerate by ignition. After cooling, add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and to the residue add 0.5 mL of diluted hydrochloric acid (2 in 3) and 10 mL of water. After dissolving by warming, add 30 mL of acetic acid-sodium acetate buffer solution for iron limit test, pH 4.5, and designate this solution as the test solution.

Prepare the control solution as follows: Transfer the amount of Standard Iron Solution specified in individual monograph to a crucible, and add 1 mL of diluted hydrochloric acid (2 in 3) and 0.5 mL of diluted nitric acid (1 in 3), evaporate on a water bath to dryness, and proceed as directed for the test solution.

In this procedure, use a quartz or porcelain crucible, which is immersed in boiling dilute hydrochloric acid for 1 hour and washed thoroughly with water and dried.

Procedure

Unless otherwise specified, proceed as follows:

(1) Method A

Transfer the test solution and the control solution to separate Nessler tubes, to each add 2 mL of a solution of L-ascorbic acid (1 in 100), mix well, and allow to stand for 30 minutes. Add 1 mL of a solution of α , α' -dipyridyl in ethanol (95) (1 in 200), add water to make 50 mL, and allow to stand for 30 minutes. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

(2) Method B

Dissolve 0.2 g of L-ascorbic acid in the test solution and the control solution, and allow to stand for 30 minutes. Add 1 mL of a solution of α , α' -dipyridyl in ethanol (95) (1 in 200), and allow to stand for 30 minutes. Then add 2 mL of a solution of 2,4,6-trinitrophenol (3 in 1000) and 20 mL of 1,2-dichloroethane, shake vigorously, collect the 1,2-dichloroethane layer, and filter through a pledget of absorbent cotton in a funnel on which 5 g of anhydrous sodium sulfate is placed if necessary. Then compare the colors developed in both solutions against a white background. The test solution has no more color than the control solution.

1.11 Arsenic Limit Test

Arsenic Limit Test is a limit test for arsenic contained in

drugs. The limit is expressed in terms of arsenic (III) trioxide (As_2O_3).

In each monograph, the permissible limit for arsenic (as As_2O_3) is described in terms of ppm in parentheses.

Apparatus

Use the apparatus illustrated in Fig. 1.11-1.

Place glass wool F in the exit tube B up to about 30 mm in height, moisten the glass wool uniformly with a mixture of an equal volume of lead (II) acetate TS and water, and apply gentle suction to the lower end to remove the excess of the mixture. Insert the tube vertically into the center of the rubber stopper H, and attach the tube to the generator bottle A so that the small perforation E in the lower end of B extends slightly below. At the upper end of B, attach the rubber stopper J to hold the tube C vertically. Make the lower end of the exit tube of C level with that of the rubber stopper J.

Preparation of the test solution

Unless otherwise specified, proceed as directed in the following.

(1) Method 1

Weigh the amount of the sample directed in the monograph, add 5 mL of water, dissolve by heating if necessary, and designate the solution as the test solution.

(2) Method 2

Weigh the amount of the sample directed in the monograph, add 5 mL of water, and add 1 mL of sulfuric acid except in the cases that the samples are inorganic acids. Add 10 mL of sulfurous acid solution, transfer to a small beaker, and evaporate the mixture on a water bath until it is free from sulfurous acid and is reduced to about 2 mL in volume. Dilute with water to make 5 mL, and designate it as the test solution.

(3) Method 3

Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), ignite the ethanol, and heat gradually to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

(4) Method 4

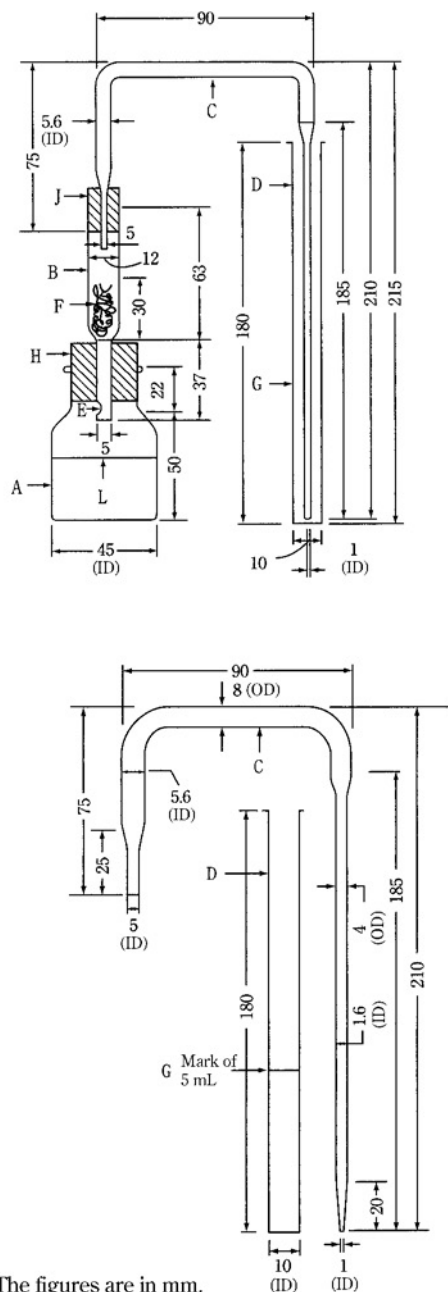
Weigh the amount of the sample directed in the monograph, and place it in a crucible of platinum, quartz or porcelain. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, heat gradually, and ignite to incinerate. If carbonized material still remains by this procedure, moisten with a small quantity of nitric acid, and ignite again to incinerate in the same manner. After cooling, add 3 mL of hydrochloric acid, heat on a water bath to dissolve the residue, and designate it as the test solution.

(5) Method 5

Weigh the amount of the sample directed in the monograph, add 10 mL of *N,N*-dimethylformamide, dissolve by heating if necessary, and designate the solution as the test solution.

Test solutions

Absorbing solution for hydrogen arsenide: Dissolve 0.50 g



- A: Generator bottle (capacity up to the shoulder: approximately 70 mL)
 B: Exit tube
 C: Glass tube (inside diameter: 5.6 mm, the tip of the part to be inserted in the absorber tube D is drawn out to 1 mm in diameter)
 D: Absorber tube (inside diameter: 10 mm)
 E: Small perforation
 F: Glass wool (about 0.2 g)
 G: Mark of 5 mL
 H and J: Rubber stoppers
 L: Mark of 40 mL

Fig. 1.11-1 Arsenic limit test apparatus

of silver *N,N*-diethyldithiocarbamate in pyridine to make 100 mL. Preserve this solution in a glass-stoppered bottle protected from light, in a cold place.

Standard Arsenic Stock Solution: Weigh accurately 0.100 g of finely powdered arsenic (III) trioxide dried at 105°C for 4 hours, and add 5 mL of sodium hydroxide solution (1 in 5) to dissolve. Add dilute sulfuric acid to neutralize, add further 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL.

Standard Arsenic Solution: Pipet 10 mL of Standard Arsenic Stock Solution, add 10 mL of dilute sulfuric acid, and add freshly boiled and cooled water to make exactly 1000 mL. Each mL of the solution contains 1 μg of arsenic (III) trioxide (As₂O₃). Prepare Standard Arsenic Solution just before use and preserve in a glass-stoppered bottle.

Procedure

Unless otherwise specified, proceed using apparatus shown in Fig. 1.11-1. Carry out the preparation of the standard color at the same time.

Place the test solution in the generator bottle A and, if necessary, wash down the solution in the bottle with a small quantity of water. Add 1 drop of methyl orange TS, and after neutralizing with ammonia TS, ammonia solution (28) or dilute hydrochloric acid, add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, and allow to stand for 10 minutes. Then add water to make 40 mL, add 2 g of zinc for arsenic analysis, and immediately connect the rubber stopper H fitted with B and C with the generator bottle A. Transfer 5 mL of the absorbing solution for hydrogen arsenide to the absorber tube D, insert the tip of C to the bottom of the absorber tube D, then immerse the generator bottle A up to the shoulder in water maintained at 25°C, and allow to stand for 1 hour. Disconnect the absorber tube, add pyridine to make 5 mL, if necessary, and observe the color of the absorbing solution: the color produced is not more intense than the standard color.

Preparation of standard color: Measure accurately 2 mL of Standard Arsenic Solution in the generator bottle A. Add 5 mL of diluted hydrochloric acid (1 in 2) and 5 mL of potassium iodide TS, and allow to stand for 2 to 3 minutes. Add 5 mL of acidic tin (II) chloride TS, allow to stand at room temperature for 10 minutes, and then proceed as directed above. The color produced corresponds to 2 μg of arsenic (III) trioxide (As₂O₃) and is used as the standard.

Note: Apparatus, reagents and test solutions used in the test should contain little or no arsenic. If necessary, perform a blank determination.

1.12 Methanol Test

Methanol Test is a method to determine methanol adhering in ethanol.

Reagents

(1) **Standard Methanol Solution**—To 1.0 g of methanol, accurately measured, add water to make exactly 1000 mL. To 5 mL of this solution, exactly measured, add 2.5 mL of methanol-free ethanol and water to make exactly 50 mL.

(2) **Solution A**—To 75 mL of phosphoric acid add water to make 500 mL, then dissolve 15 g of potassium perman-

ganate in this solution.

(3) Solution B—Add sulfuric acid carefully to an equal volume of water, cool, and dissolve 25 g of oxalic acid dihydrate in 500 mL of this dilute sulfuric acid.

Procedure

Pipet 1 mL of the sample, and add water to make exactly 20 mL. Use this solution as the sample solution. Transfer 5 mL each of the sample solution and the Standard Methanol Solution, accurately measured, to test tubes, add 2 mL of Solution A to each solution, and allow to stand for 15 minutes. Decolorize these solutions by adding 2 mL of Solution B, and mix with 5 mL of fuchsin-sulfurous acid TS. Allow to stand for 30 minutes at ordinary temperature. The sample solution has no more color than the Standard Methanol Solution.

1.13 Fats and Fatty Oils Test

Fats and Fatty Oils Test is a method applied to fats, fatty oils, waxes, fatty acids, higher alcohols, and related substances.

Preparation of test sample

For a solid sample, melt with care, and, if necessary, filter the melted sample with a dry filter paper by warming. For a turbid liquid sample, heat at about 50°C. If it is still turbid, filter it with a dry filter paper while warm. In either case, mix the sample to make it homogeneous.

Melting point

Proceed by the method described in Method 2 of Melting Point Determination <2.60>.

Congealing point of fatty acids

(1) Preparation of fatty acids—Dissolve 25 g of potassium hydroxide in 100 g of glycerin. Transfer 75 g of this solution into a 1-L beaker, and heat at 150°C. Add 50 g of the sample to this solution, and heat at a temperature not higher than 150°C for 15 minutes under frequent stirring to saponify completely. Cool the solution to 100°C, dissolve by addition of 500 mL of hot water, and add slowly 50 mL of diluted sulfuric acid (1 in 4). Heat the solution under frequent stirring until the clear layer of fatty acid is separated distinctly. Separate the fatty acid layer, and wash the fatty acid with hot water until the washing shows no acidity to methyl orange TS. Transfer the fatty acid layer to a small beaker, and heat on a water bath until the fatty acid becomes clear owing to the separation of water. Filter the warm solution, and complete the evaporation of water by carefully heating the filtered solution to 130°C.

(2) Measurement of congealing point—Proceed by the method described in Congealing Point Determination <2.42>.

Specific gravity

(1) Liquid sample at ordinary temperature

Proceed by the method described in Determination of Specific Gravity and Density <2.56>.

(2) Solid sample at ordinary temperature

Unless otherwise specified, fill a pycnometer with water at 20°C. Weigh accurately the pycnometer, and, after discarding the water and drying, weigh accurately the empty pycnometer. Then, fill the pycnometer with the melted sample to about three-fourths of the depth, and allow to stand at a temperature a little higher than the melting temperature of

Table 1.13-1

Acid value	Amount (g) of sample
Less than 5	20
5 to 15	10
15 to 30	5
30 to 100	2.5
More than 100	1.0

the sample for 1 hour to drive off the air in the sample. After keeping at the specified temperature, weigh accurately the pycnometer. Fill up the pycnometer with water over the sample at 20°C, and weigh accurately again.

The other procedure is the same as described in Method 1 of Determination of Specific Gravity and Density <2.56>.

$$d = \frac{W_1 - W}{(W_2 - W) - (W_3 - W_1)}$$

W : Mass (g) of the empty pycnometer.

W_1 : Mass (g) of the pycnometer filled with the sample.

W_2 : Mass (g) of the pycnometer filled with water.

W_3 : Mass (g) of the pycnometer filled with the sample and water.

Acid value

The acid value is the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids in 1 g of sample.

Procedure: Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-1, according to the expected acid value of the sample, in a glass-stoppered, 250-mL flask, add 100 mL of a mixture of diethyl ether and ethanol (95) (1:1 or 2:1) as the solvent, and dissolve the sample by warming, if necessary. Then, add a few drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds. If the sample solutions is turbid at lower temperature, titration should be done while warm. To the solvent used add phenolphthalein TS as an indicator, and add 0.1 mol/L potassium hydroxide-ethanol VS before use, until the solvent remains light red for 30 seconds.

$$\text{Acid value} = \frac{\text{consumed volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS} \times 5.611}{\text{amount (g) of sample}}$$

Saponification value

The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters and to neutralize the free acids in 1 g of the sample.

Procedure: Unless otherwise specified, weigh accurately 1 to 2 g of the sample, transfer to a 200-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS. Attach a short reflux condenser or an air condenser 750 mm in length and 6 mm in diameter to the neck of the flask, and heat gently in a water bath for 1 hour with frequent shaking. Cool the solution, add 1 mL of phenolphthalein TS, and titrate <2.50> immediately the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS. If the sample solution is turbid at lower temperature, titration should be done while warm. Perform a blank determination.

$$\text{Saponification value} = \frac{(a-b) \times 28.05}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed in the blank determination.

b: Volume (mL) of 0.5 mol/L hydrochloric acid VS consumed for titration of the sample.

Ester value

The ester value is the number of milligrams of potassium hydroxide (KOH) required to saponify the esters in 1 g of sample.

Procedure: Unless otherwise specified, designate the difference between the saponification value and the acid value determined as the ester value.

Hydroxyl value

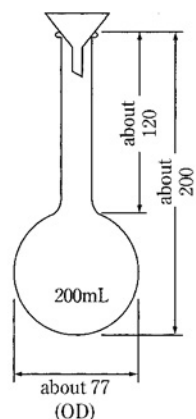
The hydroxyl value is the number of milligrams of potassium hydroxide (KOH) required to neutralize acetic acid combined with hydroxyl groups, when 1 g of the sample is acetylated by the following procedure.

Procedure: Place about 1 g of the sample, weighed accurately, in a 200-mL round-bottom flask (shown in Fig. 1.13-1), and add exactly 5 mL of pyridine-acetic anhydride TS. Place a small funnel on the neck of the flask, and heat by immersing the flask up to 1 cm from the bottom in an oil bath between 95°C and 100°C. Put a thick, round paper with a round hole on the joint of the neck of the flask to protect the neck from the heat of the oil bath. After heating for 1 hour, take the flask from the oil bath, and cool by standing. Add 1 mL of water to the flask, and shake to decompose acetic anhydride. Heat the flask in the oil bath for 10 minutes again. After cooling, wash the funnel and neck with 5 mL of neutralized ethanol down into the flask, and titrate <2.50> with 0.5 mol/L potassium hydroxide-ethanol VS (indicator: 1 mL of phenolphthalein TS). Perform a blank determination.

$$\text{Hydroxyl value} = \frac{(a-b) \times 28.05}{\text{amount (g) of sample}} + \text{acid value}$$

a: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed in the blank determination.

b: Volume (mL) of 0.5 mol/L potassium hydroxide-ethanol VS consumed for titration of the sample.



The figures are in mm.

Fig. 1.13-1 Hydroxyl value determination flask

Unsaponifiable matter

Unsaponifiable matter is calculated as the difference between the amount of materials, which are unsaponifiable by the procedure described below, soluble in diethyl ether and insoluble in water, and the amount of fatty acids expressed in terms of the amount of oleic acid. Its limit is expressed as a percentage in the monograph.

Procedure: Transfer about 5 g of the sample, accurately weighed, to a 250-mL flask. Add 50 mL of potassium hydroxide-ethanol TS, attach a reflux condenser to the flask, boil gently on a water bath for 1 hour with frequent shaking, and then transfer to the first separator. Wash the flask with 100 mL of warm water, and transfer the washing to the separator. Further, add 50 mL of water to the separator, and cool to room temperature. Wash the flask with 100 mL of diethyl ether, add the washing to the separator, extract by vigorous shaking for 1 minute, and allow to stand until both layers are separated clearly. Transfer the water layer to the second separator, add 50 mL of diethyl ether, shake, and allow to stand in the same manner. Transfer the water layer in the second separator to the third separator, add 50 mL of diethyl ether, and extract by shaking again in the same manner. Combine the diethyl ether extracts in the second and third separators into the first separator, wash each separator with a small amount of diethyl ether, and combine the washings into the first separator. Wash the combined extracts in the first separator with 30 mL portions of water successively, until the washing does not develop a light red color with 2 drops of phenolphthalein TS. Add a small amount of anhydrous sodium sulfate to the diethyl ether extracts, and allow to stand for 1 hour. Filter the diethyl ether extracts with dry filter paper, and collect the filtrates into a tared flask. Wash well the first separator with diethyl ether, and add the washing to the flask through the above filter paper. After evaporation of the filtrate and washing almost to dryness on a water bath, add 3 mL of acetone, and evaporate again to dryness on a water bath. Complete the drying between 70°C and 80°C under reduced pressure (about 2.67 kPa) for 30 minutes, allow to stand for cooling in a desiccator (reduced pressure, silica gel) for 30 minutes, and then weigh. After weighing, add 2 mL of diethyl ether and 10 mL of neutralized ethanol, and dissolve the residue by shaking well. Add a few drops of phenolphthalein TS, and titrate <2.50> the remaining fatty acids in the residue with 0.1 mol/L potassium hydroxide-ethanol VS until the solution develops a light red color which persists for 30 seconds.

$$\text{Unsaponifiable matter (\%)} = \frac{a - (b \times 0.0282)}{\text{amount (g) of sample}} \times 100$$

a: Amount (g) of the extracts.

b: Volume (mL) of 0.1 mol/L potassium hydroxide-ethanol VS consumed for titration.

Iodine value

The iodine value, when measured under the following conditions, is the number of grams of iodine (I), representing the corresponding amount of halogen, which combines with 100 g of sample.

Procedure: Unless otherwise specified, weigh accurately the amount of sample shown in Table 1.13-2, according to the expected iodine value of the sample, in a small glass container. In a 500-mL glass-stoppered flask place the container containing the sample, add 20 mL of cyclohexane to dissolve

the sample, then add exactly 25 mL of Wijs' TS, and mix well. Stopper the flask, and allow to stand, protecting against light, between 20°C and 30°C for 30 minutes (when the expected iodine value is more than 100, for 1 hour) with occasional shaking. Add 20 mL of potassium iodide solution (1 in 10) and 100 mL of water, and shake. Then, titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = \frac{(a - b) \times 1.269}{\text{amount (g) of sample}}$$

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination.

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed for titration of the sample.

Table 1.13-2

Iodine value	Amount (g) of sample
Less than 30	1.0
30 to 50	0.6
50 to 100	0.3
More than 100	0.2

1.14 Sulfate Limit Test

Sulfate Limit Test is a limit test for sulfate contained in drugs.

In each monograph, the permissible limit for sulfate (as SO₄) is described in terms of percentage (%) in parentheses.

Procedure

Unless otherwise specified, transfer the quantity of the sample, directed in the monograph, to a Nessler tube, dissolve it in sufficient water, and add water to make 40 mL. Add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the test solution. Transfer the volume of 0.005 mol/L sulfuric acid VS, directed in the monograph, to another Nessler tube, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and use this solution as the control solution. When the test solution is not clear, filter both solutions according to the same procedure.

Add 2 mL of barium chloride TS to the test solution and to the control solution, mix well, and allow to stand for 10 minutes. Compare the white turbidity produced in both solutions against a black background by viewing downward or transversely.

The turbidity produced in the test solution is not thicker than that of the control solution.

1.15 Readily Carbonizable Substances Test

Readily Carbonizable Substances Test is a method to examine the minute impurities contained in drugs, which are readily colored by addition of sulfuric acid.

Procedure

Before use, wash the Nessler tubes thoroughly with sulfuric acid for readily carbonizable substances. Unless otherwise specified, proceed as follows. When the sample is solid, place 5 mL of sulfuric acid for readily carbonizable substances in a Nessler tube, to which add a quantity of the finely powdered sample, little by little, as directed in the monograph, and dissolve it completely by stirring with a glass rod. When the sample is liquid, transfer a volume of the sample, as directed in the monograph, to a Nessler tube, add 5 mL of sulfuric acid for readily carbonizable substances, and mix by shaking. If the temperature of the content of the tube rises, cool the content; maintain it at the standard temperature, if the reaction may be affected by the temperature. Allow to stand for 15 minutes, and compare the color of the liquid with that of the matching fluid in the Nessler tube specified in the monograph, by viewing transversely against a white background.

2. Physical Methods

Chromatography

2.01 Liquid Chromatography

Liquid Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a liquid as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a liquid or soluble sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (*k*) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

The ratio *k* represents the mass distribution ratio (or the capacity factor) *k'* in liquid chromatography.

Since the relation given below exists among the ratio (*k*), the time for which the mobile phase is passed through the column (*t*₀: time measured from the time of injection of a compound with *k* = 0 to the time of elution at the peak maximum), and the retention time (*t*_R: time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k) t_0$$

Apparatus

Basically, the apparatus required for the liquid chromatographic procedure consists of a pumping system for the mobile phase, a sample injection port, a column, a detector and a recorder. A mobile phase component regulator, a thermostat for the column, a pumping system for reaction reagents and a chemical reaction chamber are also used, if necessary. The pumping system serves to deliver the mobile phase

and the reagents into the column and connecting tube at a constant flow rate. The sample injection port is used to deliver a quantity of the sample to the apparatus with high reproducibility. The column is a tube with a smooth interior, made of inert metal, etc., in which a packing material for liquid chromatography is uniformly packed. A column with a stationary phase chemically bound on the inside wall instead of the column packed with the packing material may be used. The detector is used to detect a property of the samples which is different from that of the mobile phase, and may be an ultraviolet or visible spectrophotometer, fluorometric detector, differential refractometer, electrochemical detector, chemiluminescence detector, electric conductivity detector, mass spectrophotometer, etc. The output signal is usually proportional to the concentration of samples at amounts of less than a few μg . The recorder is used to record the output signals of the detector. As required, a data processor may be used as the recorder to record or output the chromatogram, retention times or amounts of the components. The mobile phase component regulator is used to vary the ratio of the mobile phase components in a stepwise or gradient fashion.

Procedure

Fix the detector, column and mobile phase to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram. If the components to be analyzed have no readily detectable physical properties such as absorbance or fluorescence, the detection is achieved by changing the components to suitable derivatives. Usually, the derivatization is performed as a pre- or post-column labeling.

Identification and purity test

Identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sample with an authentic specimen.

In general, the purity of the sample is determined by comparing the sample solution with a standard solution which is prepared by diluting the sample solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on the relative sensitivity to the principal component.

Assay

(1) Internal standard method—In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all

other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the liquid chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the ratios of the peak area or peak height of the objective compound to those of the internal standard, which are obtained by repeating the injection of a fixed volume of the standard solution.

(2) Absolute calibration curve method—Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas or peak heights of the objective compound which are obtained by repeating the injection of a fixed volume of the standard solution.

Method for peak measuring

Generally, the following methods are used.

(1) Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on both sides of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing system.

(2) Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

Terminology

Reproducibility of test: Reproducibility of test is used as a method to ensure that the results obtained by a given procedure truly meet the requirements of the test described in the individual monograph. It is given as the relative standard deviation ($S_R(\%)$).

Symmetry factor: Symmetry factor shows the degree of symmetry of a peak in the chromatogram, and is defined as S in the following equation.

$$S = \frac{W_{0.05h}}{2f}$$

$W_{0.05h}$: Width of the peak at one-twentieth of the peak height,

f : Distance between the perpendicular from the peak maximum and the leading edge of the peak at one-twentieth of the peak height,

where $W_{0.05h}$ and f have the same unit.

Relative standard deviation: Generally, it is given as $S_R(\%)$ defined by the following equation.

$$S_R(\%) = \frac{100}{\bar{X}} \times \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n-1}}$$

x_i : Measured value

\bar{X} : Mean of measured values

n : Number of repeated measurements

Complete separation of peak: Complete separation of the peak means that the resolution between two peaks is not less than 1.5.

Separation factor: Separation factor shows the relation between the retention times of peaks in the chromatogram, and is defined as α in the following equation.

$$\alpha = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

t_{R1} , t_{R2} : Retention times of two compounds used for the resolution measurement ($t_{R1} < t_{R2}$).

t_0 : Time of passage of the mobile phase through the column (time measured from the time of injection of a compound with $k=0$ to the time of elution at the peak maximum).

The separation factor (α) is a characteristic of the thermodynamic difference in partition of two compounds. It is basically the ratio of their partition equilibrium coefficients or of their mass-distribution ratios, and is obtained from the chromatogram as the ratio of the retention times of the two compounds.

Resolution: Resolution shows the relation between the retention time and the peak width of peaks in the chromatogram, and is defined as R_S in the following equation.

$$R_S = 1.18 \times \frac{t_{R2} - t_{R1}}{W_{0.5h1} + W_{0.5h2}}$$

t_{R1} , t_{R2} : Retention times of two compounds used for measurement of the resolution ($t_{R1} < t_{R2}$),

$W_{0.5h1}$, $W_{0.5h2}$: Peak widths at half peak height,

where t_{R1} , t_{R2} , $W_{0.5h1}$ and $W_{0.5h2}$ have the same unit.

Number of theoretical plates: Number of theoretical plates is generally defined in terms of the following equation to indicate the extent of the band broadening of a compound in the column.

$$N = 5.54 \times \frac{t_R^2}{W_{0.5h}^2}$$

t_R : Retention time of compound,

$W_{0.5h}$: Width of the peak at half peak height,

where t_R and $W_{0.5h}$ have the same unit.

Note: Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the column packing material, column temperature, composition ratio of the mobile phase, composition of buffer solutions in the mobile phase, pH of the mobile phase, concentration of ion pair-forming agents in the mobile phase, ionic strength of the mobile phase, numbers of condition changes, timing of such changes, gradient program, composition and flow rate of derivative-producing reagents, reaction time and temperature of reaction chamber and flow rate of mobile phase may be modified within limits which allow the required elution order, resolution, symmetry factor, and relative standard deviation to be obtained.

2.02 Gas Chromatography

Gas Chromatography is a method to develop a mixture injected into a column prepared with a suitable stationary phase by passing a gas (carrier gas) as a mobile phase through the column, in order to separate the mixture into its components by making use of the difference of retention capacity against the stationary phase, and to determine the components. This method can be applied to a gaseous or vaporizable sample, and is used for identification, purity test, and quantitative determination.

A mixture injected into the column is distributed between the mobile phase and the stationary phase with a characteristic ratio (k) for each component.

$$k = \frac{\text{amount of compound in the stationary phase}}{\text{amount of compound in the mobile phase}}$$

Since the relation given below exists among the ratio (k), the time for which the mobile phase is passed through the column (t_0 : time measured from the time of injection of a compound with $k=0$ to the time of elution at the peak maximum), and the retention time (t_R : time measured from the time of injection of a compound to be determined to the time of elution at the peak maximum), the retention time for a compound on a column has a characteristic value under fixed chromatographic conditions.

$$t_R = (1 + k) t_0$$

Apparatus

Basically, the apparatus required for the gas chromatographic procedure consists of a carrier gas-introducing port and flow regulator, a sample injection port, a column, a column oven, a detector and a recorder. Gas introducing port and flow regulator for a combustion gas, a burning supporting gas and an accessory gas and sample injection port for headspace are also used, if necessary. The carrier gas-introducing port and flow regulator serves to deliver the carrier gas into the column at a constant flow rate, and usually consist of a pressure regulation valve, a flow rate regulation valve and a pressure gauge. The sample injection port is used to deliver a quantity of the sample to the flow line of carrier gas with high reproducibility. There are sample injection ports for packed column and for capillary column. There are both divided injection mode and non-divided injection mode to sample injection port for capillary column. The columns are usually classified as packed column or capillary column. The packed column is a tube made of inert metal, glass or synthetic resin, in which a packing material for gas chromatography is uniformly packed. The packed column with not more than 1 mm in inside diameter is also called a packed capillary column (micro packed column). A capillary column is a tube made of inert metal, glass, quartz or synthetic resin, whose inside wall is bound chemically with stationary phase for gas chromatography. The column oven has the setting capacity for a column with required length and the temperature regulation system for keeping the constant column temperature. The detector is used to detect a component separated on the column, and may be an alkaline thermal ionization detector, a flame photometry detector, mass spectrophotometer, hydrogen flame-ionization detector, an electron capture detector, a thermal conductivity detector, etc. The recorder is used to record the output signals of the detector.

Procedure

Unless otherwise specified, proceed by the following method. Fix the detector, column and carrier gas to the apparatus, and adjust the flow rate and the column temperature to the values described in the operating conditions specified in the individual monograph. Inject a volume of the sample solution or the standard solution specified in the individual monograph with the sample injector into the column system through the sample injection port. The separated components are detected by the detector, and recorded by the recorder as a chromatogram.

Identification and purity test

Identification of a component of a sample is performed by confirming agreement of the retention time of the sample with that of an authentic specimen, or by confirming that the peak shape of the sample is unchanged after mixing the sam-

ple with an authentic specimen.

In general, the purity of the sample is determined by comparing the sample solution with a standard solution which is prepared by diluting the sample solution to a concentration corresponding to the specified limit amount of the impurity, or by the peak area percentage method. Unless otherwise specified, if a sample is separated into isomers in the chromatogram, the isomer ratio is calculated by using the peak area percentage method.

The peak area percentage method is a method to calculate the proportion of the components from the ratio of the peak area of each component to the sum of the peak areas of every peak recorded in the chromatogram. In order to obtain accurate results in evaluating the proportion of the components, it is necessary to correct the area of each component based on its relative sensitivity to the principal component.

Assay

In general, perform the assay by using the internal standard method. The absolute calibration curve method is used when a suitable internal standard is not available. Perform the assay by using the standard addition method when the effect of the component other than the compound to be assayed on the quantitative determination is not negligible against a result of the determination.

(1) Internal standard method—In the internal standard method, choose a stable compound as an internal standard which shows a retention time close to that of the compound to be assayed, and whose peak is well separated from all other peaks in the chromatogram. Prepare several kinds of standard solutions containing a fixed amount of the internal standard and several graded amounts of the authentic specimen specified in the individual monograph. Based on the chromatogram obtained by injection of a fixed volume of individual standard solutions, calculate the ratio of peak area or peak height of the authentic specimen to that of the internal standard, and prepare a calibration curve by plotting these ratios on the ordinate against the amount of the authentic specimen or the ratio of the amount of the authentic specimen to that of the internal standard on the abscissa. The calibration curve is usually obtained as a straight line passing through the origin. Then, prepare a sample solution containing the internal standard in the same amount as in the standard solutions used for the preparation of the calibration curve according to the method specified in the individual monograph, perform the gas chromatography under the same operating conditions as for the preparation of the calibration curve, calculate the ratio of the peak area or peak height of the objective compound to that of the internal standard, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under fixed conditions to determine the amount of the objective compound. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the ratios of the peak area or peak height of the objective compound to those of the internal standard, which are obtained by repeating the injection of a fixed volume of the standard solution.

(2) Absolute calibration curve method—Prepare standard solutions with several graded amounts of the authentic specimen, and inject accurately a fixed volume of these standard solutions. With the chromatogram obtained, prepare a calibration curve by plotting the peak areas or peak heights on the ordinate against the amount of the authentic specimen on the abscissa. The calibration curve is generally obtained as a straight line passing through the origin. Then, prepare a sample solution according to the method specified in the individual monograph, perform the liquid chromatography under the same conditions as for the preparation of the calibration curve, measure the peak area or peak height of the objective compound, and read the amount of the compound from the calibration curve.

In an individual monograph, generally one of the standard solutions with a concentration within the linear range of the calibration curve and a sample solution with a concentration close to that of the standard solution are prepared, and the chromatography is performed with these solutions under a fixed condition to obtain the amount of the component. In this method, all procedures, such as the injection procedure, must be carried out under a strictly constant condition. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas or peak heights of the objective compound, which are obtained by repeating the injection of a fixed volume of the standard solution.

(3) Standard addition method—Pipet a fixed volume of more than 4 sample solutions, add exactly the standard solution so that stepwise increasing amounts of the object compound are contained in the solutions except 1 sample solution, diluted exactly each solution with and without standard solution to a definite volume, and use each solution as the sample solution. Based on the chromatogram obtained by exact injection of a fixed volume of individual sample solutions, measure the peak area of individual sample solutions. Calculate the concentration of standard objective compound added into each sample solution, plot the amounts (concentration) of added standard object compound on the abscissa and the peak area on the ordinate on the graph, extend the calibration curve obtained by linking the plots, and determine the amount of object compound to be assayed from the distance between the origin and the intersecting point of the calibration curve with the abscissa. Generally, the relative standard deviation (variation coefficient) is calculated to confirm the reproducibility of the peak areas of the objective compound, which are obtained by repeating the injection of a fixed volume of the standard solution. This method is available only in the case that the calibration curve is a straight line, and passes through the origin when the absolute calibration curve method is employed. In this method, all procedures must be carried out under a strictly constant condition.

Method for peak measuring

Generally, the following methods are used.

(1) Peak height measuring method

(i) Peak height method: Measure the distance between the maximum of the peak and the intersecting point of a perpendicular line from the maximum of the peak to the horizontal axis of recording paper with a tangent linking the baselines on either side of the peak.

(ii) Automatic peak height method: Measure the signals from the detector as the peak height using a data processing

system.

(2) Peak area measuring method

(i) Width at half-height method: Multiply the peak width at the half-height by the peak height.

(ii) Automatic integration method: Measure the signals from the detector as the peak area using a data processing system.

Terminology

The definition of terms described under Liquid Chromatography <2.01> shall apply in Gas Chromatography <2.02>.

Note: Avoid the use of authentic specimens, internal standards, reagents or solvents containing substances that may interfere with the determination.

Among the operating conditions specified in the individual monograph, inside diameter and length of the column, particle size of the column packing material, concentration of the stationary phase, column temperature, and flow rate of carrier gas may be modified within limits which allow the required elution order, resolution, symmetry factor and relative standard deviation to be obtained. The sample injection port and the operating conditions for headspace may be also modified within limits which allow the accuracy and precision more than those of a prescribed method to be obtained.

2.03 Thin-layer Chromatography

Thin-layer Chromatography is a method to separate each ingredient by developing a mixture in a mobile phase, using a thin-layer made of a suitable stationary phase, and is applied for identification, purity test, etc. of substances.

Preparation of thin-layer plate

Generally, proceed by the following method.

A smooth and uniformly thick glass plate having a size of 50 mm × 200 mm or 200 mm × 200 mm is used for preparing a thin-layer plate. Using a suitable apparatus, apply a water suspension of powdered solid substance for the stationary phase, directed in the monograph, on one side of the glass plate to make a uniform layer of 0.2 to 0.3 mm in thickness. After air-drying, dry further by heating at a fixed temperature between 105°C and 120°C for 30 to 60 minutes. A suitable plastic plate may be used instead of the glass plate. Preserve the dried plate with protection from moisture.

Procedure

Unless otherwise specified, proceed by the following method.

Designate a line about 20 mm distant from the bottom of the thin-layer plate as the starting line, spot 2 to 6 mm in diameter the directed volumes of the sample solution or the standard solution in the monograph using micropipets at points on this line, separated by more than 10 mm, and air-dry. Unless otherwise specified, attach the filter paper along with the inside wall of the container, and wet the filter paper with the developing solvent. In the container, the developing solvent is placed up to about 10 mm in height from the bottom beforehand, seal the container closely, and allow it to stand for 1 hour at ordinary temperature. Place the plate in the container, avoiding contact with the inside wall, and seal the container. Develop it at ordinary temperature.

When the solvent front has ascended from the starting line

to the distance directed in the monograph, remove the plate from the container. Immediately put a mark at the solvent front. After air-drying, observe the location, color, etc., of each spot by the method specified in the monograph. Calculate the *Rf* value by using the following equation:

$$Rf = \frac{\text{distance from the starting line to the center of the spot}}{\text{distance from the starting line to the solvent front}}$$

Spectroscopic Methods

2.21 Nuclear Magnetic Resonance Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is based on the phenomenon that specific radio frequency radiation is absorbed by magnetic nuclei in a sample placed in a magnetic field; target nuclei are ^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P , etc. These nuclei have intrinsic spin angular momentum, of which the magnitude is given by $I(I+1)/h/2\pi$, where I is the spin quantum number and is integral or half-integral ($I=1/2$ for ^1H and ^{13}C). When the magnetic nuclei are placed in a magnetic field, they are oriented in $2I+1$ possible orientations corresponding to $2I+1$ equally spaced energy levels (two energy levels for ^1H and ^{13}C). The transition between two successive quantized energy levels corresponding to adjacent orientations can be induced by electromagnetic radiation with a suitable frequency. The precise relation between the field strength and the resonant frequency ν is given by

$$\nu = \gamma \cdot \frac{H_0}{2\pi}$$

where H_0 is the strength of the applied external magnetic field and γ is the gyromagnetic ratio, a constant characterizing a particular isotope. The absorption of radiation (NMR signal) can occur only when the irradiating radio frequency satisfies the resonance condition. Since the absorption coefficient (the transition probability) does not depend on the environment in which the nuclei are located, the intensity is basically proportional to the number of nuclei. The excess spins shifted to the higher energy levels by the transition process return to the thermal equilibrium state at various rates determined by a characteristic time constant (known as the relaxation time).

A nucleus is shielded from the applied magnetic field by the electrons belonging to its own atom and to the molecule. Therefore nuclei in different environments are shielded to different extents and resonate at different frequencies. The difference in resonance frequencies is defined as chemical shift (δ), which is independent of the strength of the magnetic field, and is given by

$$\delta = \frac{\nu_S - \nu_R}{\nu_R} + \delta_R$$

where,

- ν_S : The resonance frequency of the observed signal,
- ν_R : The resonance frequency of the reference signal,
- δ_R : The chemical shift of the reference signal (in the case of the value not being 0).

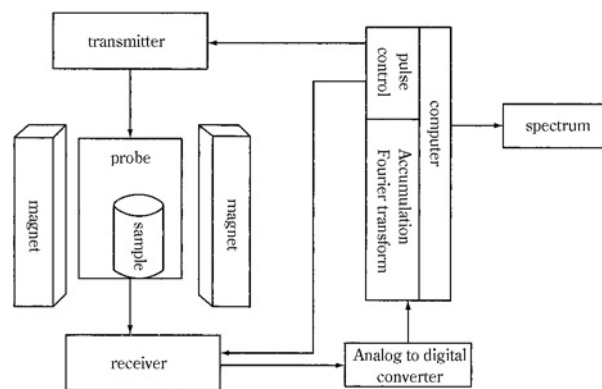


Fig. 2.21-1 FT-NMR spectrometer

The chemical shifts are normally expressed in ppm, a dimensionless unit, by assuming the chemical shift of the reference compound as 0 ppm. When the chemical shift of the reference compound is not assumed to be 0 ppm, chemical shifts of samples are corrected accordingly.

In addition to the shielding due to electrons, the nucleus is subjected to effects due to the spin orientations of other magnetic nuclei through chemical bonds, resulting in an additional splitting of the signal. The spacing between two adjacent components of the signal is known as the spin-spin coupling constant (J). Coupling constants are measured in hertz and are independent of the strength of the external magnetic field. The increased number of interacting nuclei will make the multiplet pattern more complex.

From the NMR spectrum the following four parameters can be obtained: chemical shift, spin-spin coupling constant, resonance intensity (intensities of ^1H are proportional to the number of nuclei and those of ^{13}C and others are susceptible to the nuclear Overhauser effect (NOE) and relaxation) and relaxation time. These parameters are useful for structural determination, identification and quantitative analysis of molecules. Spin decoupling, NOE, and two-dimensional NMR techniques are also available for structural analysis.

Spectrometer

There are two types of spectrometers.

(1) Fourier transform NMR (FT-NMR) spectrometers (Fig. 2.21-1)

Target nuclei are simultaneously excited in all frequency range of the nuclei by means of an intense radio frequency pulse. The FID (free induction decay) after the pulse is detected, which is a time domain signal, is converted to a frequency domain spectrum by Fourier transformation. Number of data points suitable for the spectral range, flip angle, acquisition time, delay time and number of scans should be set appropriately.

Recently FT-NMR is commonly used because of its high sensitivity and various advanced applications.

(2) Continuous wave NMR (CW-NMR) spectrometers (Fig. 2.21-2)

In the case of the CW method, a spectrum is obtained by sweeping the radio frequency or magnetic field continuously over the frequency range of the nuclei being observed.

Measurement

Prior to measurements, the sensitivity and resolution of the

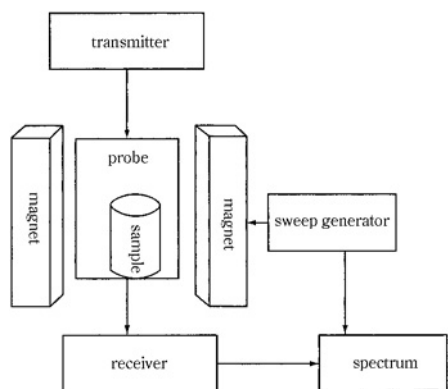


Fig. 2.21-2 CW-NMR spectrometer

instrument must be adjusted to the optimum levels using a standard sample (ethylbenzene, 1,2-dichlorobenzene or acetaldehyde) dissolved in an appropriate NMR solvent.

(1) The sample dissolved in a suitable solvent is transferred into an NMR tube. The reference compound can be added directly to the sample solution (internal reference), or a sealed capillary tube containing the reference compound can be inserted into the NMR tube (external reference). The sample solutions should be completely homogeneous. In particular, solid contaminants should be removed in order to obtain good spectra. Various deuterated NMR solvents are commonly used for NMR measurement and the following points should be considered in selecting an appropriate solvent: (i) The solvent signals do not overlap with the sample signals. (ii) The sample must be soluble in the solvent selected. (iii) The solvent does not react with the sample. Furthermore, it should be noted that chemical shifts can depend upon the solvent employed, sample concentration and deuterium ion concentration, and that viscous solutions usually give rather broad, poorly resolved spectra.

(2) For the reference standards use the reagents for nuclear magnetic resonance spectroscopy. For ^1H and ^{13}C spectra, tetramethylsilane (TMS) is usually used as the reference compound for samples dissolved in organic solvents. For samples dissolved in deuterium oxide, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or sodium 3-(trimethylsilyl)propionate (TSP) is used. For other nuclei, nitromethane, trichlorofluoromethane and phosphoric acid are used as reference compounds for ^{15}N , ^{19}F and ^{31}P , respectively. Furthermore, chemical shifts of residual protons in deuterated solvents and ^{13}C in the solvent instead of a reference compound can be used for ^1H and ^{13}C NMR.

Record of apparatus and measurement conditions

Type of instrument, frequency, solvent, temperature, sample concentration, reference compound, experimental technique, etc. should be recorded to allow appropriate comparison of spectra, because NMR spectra depend on the measurement conditions.

Identification

The sample solution is prepared and tested by the method directed in each monograph. Usually in the case of ^1H NMR, the sample is identified by the following method.

(1) Identification by the use of chemical shift, multiplicity and relative intensity

When chemical shifts, multiplicities and relative intensities

of signals are defined, the sample can be identified as being the same substance when all chemical shifts, multiplicities and relative intensities are the same as those prescribed.

(2) Identification by the use of a Reference Standard

Measurement conditions should be the same as those used in the case of the Reference Standard. When the spectra of a sample and the Reference Standard exhibit the same multiplicities and relative intensities of signal at the same chemical shifts, the sample can be identified as being the same substance as the Reference Standard.

Experimental techniques of ^1H and ^{13}C NMR spectroscopy

NMR spectroscopy includes one-, two- and multi-dimensional techniques, which are used for various purposes.

Spin decoupling, and NOE are available in one-dimensional ^1H spectroscopy. Spin decoupling can assign coupling correlations. As NOE can observe correlations among spatially proximate protons, the configuration and the conformation can be analyzed.

Broadband decoupling, INEPT and DEPT are usually applied in one-dimensional ^{13}C spectroscopy. The broadband decoupling technique simplifies a spectrum and achieves enhancement of sensitivity. INEPT (insensitive nuclei enhanced by polarization transfer) and DEPT (distortionless enhancement of polarization transfer) enhance the sensitivity of ^{13}C by means of polarization transfer from directly bonded ^1H with a large magnetic moment. They can be applied to identify primary, secondary, tertiary or quaternary carbon.

Two-dimensional spectroscopy can observe all correlation peaks between nuclei through spin-spin coupling or NOE in a single experiment, and there are many techniques for homonuclear and heteronuclear measurements. Representative techniques are described below.

COSY (2D correlation spectroscopy), HOHAHA (homonuclear Hartmann-Hahn spectroscopy) or TOCSY (total correlation spectroscopy): Correlation between protons through scalar spin-spin coupling is obtained and intramolecular connectivities of hydrogen atoms are revealed.

NOESY (2D nuclear Overhauser enhancement and exchange spectroscopy): NOE is measured by a two-dimensional technique. Approximate distances between spatially proximate hydrogen atoms are obtained to analyze the three-dimensional structure.

INADEQUATE (incredible natural abundance double quantum transfer experiment): Although this technique is insensitive because it involves double quantum transfer by ^{13}C - ^{13}C scalar coupling in a sample with natural isotopic abundance, the connectivities of all neighboring ^{13}C nuclei can be obtained to analyze the carbon skeleton.

HMQC (heteronuclear multiple quantum coherence): This technique observes correlations between ^1H and ^{13}C with direct spin-spin coupling using ^1H detection and reveals intramolecular chemical bonds between hydrogen and carbon atoms.

HMBC (heteronuclear multiple bond connectivity): This technique observes correlations between ^1H and ^{13}C with long range spin-spin coupling using ^1H detection and reveals intramolecular connectivities of hydrogen and carbon atoms.

There are many other techniques such as DQF-COSY (double quantum filtered COSY) and HSQC (heteronuclear single quantum coherence). Furthermore, multidimensional NMR techniques are used to analyze macromolecules.

2.22 Fluorometry

Fluorometry is a method to measure the intensity of fluorescence emitted from a solution of fluorescent substance irradiated with an exciting light in a certain wavelength range. This method is also applied to the phosphorescent substances.

Fluorescence intensity F in a dilute solution is proportional to the concentration c in mol per liter of the solution and the pathlength l of light through the solution in centimeter.

$$F = kI_0\phi\epsilon cl$$

k : Constant

I_0 : Intensity of exciting light

ϕ : Quantum yield of fluorescence or phosphorescence

$$\phi = \frac{\text{number of quanta emitted as fluorescence or phosphorescence}}{\text{number of quanta absorbed}}$$

ϵ : Molar extinction coefficient of the substance at the excitation wavelength

Apparatus

Spectrofluorometer is usually used. Generally, a xenon lamp, laser, an alkaline halide lamp, etc. which provide stable exciting light are used as light source. Usually, a non-fluorescent quartz cell (1 cm \times 1 cm) with four transparent sides is used as the container for sample solution.

Procedure

Excitation spectrum is obtained by measuring fluorescence intensities of sample solution with varying excitation wavelengths at a fixed emission wavelength (in the vicinity of the fluorescence maximum) and drawing a curve showing the relationship between the excitation wavelength and the fluorescence intensity. Fluorescence spectrum is obtained by measuring fluorescence intensities of sample solution with varying emission wavelengths at a fixed excitation wavelength (in the vicinity of the excitation maximum) and drawing the same curve as described for the excitation spectrum. If necessary, the spectra are corrected with regard to the optical characteristics of the apparatus.

The fluorescence intensity is usually measured at the excitation and the emission wavelengths in the vicinity of excitation and emission maxima of the fluorescent substance. The fluorescence intensity is expressed as a value relative to that of a standard solution, because it is readily affected even by a slight change in the condition for the measurement.

Unless otherwise specified, the instrument is operated as follows with standard, sample, and reference solutions prepared as directed in the monograph: Fix the excitation and fluorescence wavelength scales at the designated positions, adjust the dark current to zero, put the quartz cell containing the standard solution in the light path, and adjust the instrument so that the standard solution shows the fluorescence intensity of 60% to 80% of full scale. Then perform the measurements with the cells containing the sample solution and the control solution, and read the fluorescence intensity as % under the same condition. Set the width of the wavelength properly unless otherwise specified.

Note: The fluorescence intensity is readily affected by the concentration, temperature and pH of the solution, and na-

ture and purity of solvents or reagents used.

2.23 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry is a method to determine the amount or the concentration of an element in a sample specimen being examined, by utilizing the phenomenon that atoms being in the ground state absorb the light of specific wavelength, characteristic of the respective atom, when the light passes through an atomic vapor layer of the element to be determined.

Apparatus

Usually, the apparatus consists of a light source, a sample atomizer, a spectroscope, a photometer and a recording system. Some are equipped with a background compensation system. As a light source, usually a hollow cathode lamp specified for each element is used and sometimes a discharge lamp is also used. There are three types of sample atomizer: the flame type, the electrothermal type, and the cold-vapor type. The first one is composed of a burner and a gas-flow regulator, the second one is composed of an electric furnace and a power source, and the third one is composed of a mercury generator and an absorption cell. The third one is further classified into two subtypes, which differ in the atomizing method for mercury containing-compounds: one utilizes chemical reduction-vaporization and the other utilizes a thermal reduction-vaporization method.

For the selection of an appropriate analytical wavelength in a spectroscope, a grating for light diffraction or an interference filter can be used. A recording system is composed of a display and a recording device. A background compensation system is employed for the correction of atmospheric effects on the measuring system. Several principles can be utilized for background compensation, using continuous spectrum sources, the Zeeman splitted spectrum, the non-resonance spectrum, or self-inversion phenomena.

Another special options such as a hydride generator and a heating cell, can also be used for analyzing such as selenium. As a hydride generator, a batch method and/or a continuous flow method can be applied. While as a heating cell, there are two kinds of cell: one for heating by flame and the other for heating by electric furnace.

Procedure

Unless otherwise specified, proceed by any of the following methods.

(1) **Flame type**—Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Next, a mixture of a combustible gas and a supporting gas is ignited and the gas flow rate and/or pressure should be adjusted to optimum conditions. The zero adjustment of the detecting system must be done through nebulizing the blank solvent into the flame. After setting up the measuring system, the sample solution prepared by the specified procedure is introduced into the flame and the light absorption at the characteristic wavelength of the element to be determined is measured.

(2) **Electrothermal type**—Fit the specific light source to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and slit-width. Further, set an electric furnace to the appropriate temperature, electric current, and heating program, as directed separately in the monograph. When a suitable amount of sample is injected into the heated furnace with an appropriate stream of inert gas, the sample is dried and ashed, simultaneously with atomization of the metallic compound included in the specimen. The atomic absorption specified is observed and the intensity of absorption is measured. Details of the sample preparation method are provided separately in the monograph.

(3) **Cold vapor type**—Fit the mercury lamp to the lamp housing and switch on the instrument. After lighting the lamp and selecting the analytical wavelength specified in the monograph, set an appropriate electric current and a slit-width. In the chemical atomization-vaporization method, a mercury containing compound in the sample solution, prepared by the specified procedure, is chemically reduced to metallic mercury by adding a proper reducing reagent to the closed vessel and the generated mercury is vaporized and introduced into the absorption cell with a flow of inert gas. In the thermal atomization-vaporization method, the sample specimen on a quartz dish is heated electrically and the generated atomic mercury is vaporized and introduced into the absorption cell with a flow of inert gas. Thus, in both methods, the generated atomic mercury is carried into the absorption cell as cold vapor and the intensity of the characteristic atomic absorption of mercury is measured.

Determination

Usually, proceed by any of the following methods. In the determination, the possibility of interference for various reasons and the background effect must be considered and avoided if possible.

(1) **Calibration curve method**—Prepare standard solutions at more than 3 concentration levels, measure the specific absorption due to these standard solutions, and prepare the calibration curve of the atomic absorption against the concentration. Then measure the atomic absorption due to the sample specimen, in which the concentration of the element to be determined should be adjusted to be within the concentration range of the standard solutions, and determine the amount or the concentration of the element to be examined using the calibration curve.

(2) **Standard addition method**—To equal volumes of more than 3 sample solutions, prepared as directed in the monograph, add a measured quantity of the standard solutions to produce a series of solutions containing increasing amounts of the element to be examined, and further add a solvent to make up a constant volume. Measure the atomic absorption for the respective solutions, and plot the obtained values on a graph with the added amount or the concentration on the abscissa and the absorbance on the ordinate. Extrapolate the linear plot obtained by linking the data points, and determine the amount or the concentration of the element to be examined from the distance between the origin and the point where the plot intersects with the abscissa. This method is available only when the calibration curve obtained by Method (1) is confirmed to be linear and to pass through the origin.

(3) **Internal standard method**—Prepare a series of standard solutions of the element to be determined, each containing a definite amount of the internal standard element directed in the monograph. For these standard solutions, measure the atomic absorption due to the standard element and the internal standard element separately at the respective wavelengths under the same operating conditions, and obtain the ratio of absorbance by the standard element to that by the internal standard element. Prepare a calibration curve for the element to be determined, with the amount or the concentration of the standard element on the abscissa and the above-mentioned ratio of the absorbance on the ordinate. Then prepare sample solutions, adding the same amount of the internal standard element as contained in the standard solutions. Measure the ratio of the absorbance due to the element to be determined to that due to the internal standard element under the same conditions as employed for preparing the calibration curve, and determine the amount or the concentration of the element being examined by using the calibration curve.

Note: Reagents, test solutions, and gases used in this test should not interfere in any process of the measurement.

2.24 Ultraviolet-visible Spectrophotometry

Ultraviolet-visible Spectrophotometry is a method to measure the degree of absorption of light between the wavelengths of 200 nm and 800 nm by substances for the tests of their identity and purity, and for assay. When an atomic absorption spectrophotometer is used for these purposes, proceed as directed under Atomic Absorption Spectrophotometry <2.23 >. When monochromatic light passes through a substance in the solution, the ratio of transmitted light intensity I to incident light intensity I_0 is called transmittance t ; transmittance expressed in the percentage is called percent transmittance T , and common logarithm of the reciprocal of transmittance is called absorbance A .

$$t = \frac{I}{I_0} \quad T = \frac{I}{I_0} \times 100 = 100t \quad A = \log \frac{I_0}{I}$$

The absorbance A is proportional to the concentration c of a substance in the solution and the length l of the layer of the solution through which light passes.

$$A = kcl \quad (k: \text{constant})$$

The absorbance, calculated on the basis that l is 1 cm and c is 1 mol/L, is called molar absorption coefficient ϵ . The molar absorption coefficient at the wavelength of maximum absorption is expressed as ϵ_{\max} .

When a light beam passes through a substance in the solution, the absorbance by the sample differs depending on the wavelength of the light. So, an absorption spectrum is obtained by determining the absorbances of a light beam at various wavelengths and by graphically plotting the relation between absorbance and wavelength. From the absorption spectrum, it is possible to determine the wavelength of maximum absorption λ_{\max} and that of minimum absorption λ_{\min} .

The absorption spectrum of a substance in the solution is characteristic, depending on its chemical structure. Therefore, it is possible to identify a substance by comparing the

spectrum of a sample within the specified wavelength range with the Reference Spectrum or the spectrum of Reference Standard, by determining the wavelengths of maximum absorption, or by measuring the ratio of absorbances at two specified wavelengths. For the purpose of assay, the absorbance by a sample solution with a certain concentration is measured at the wavelength of the maximum absorption λ_{\max} and compared it with the absorbance of a standard solution with a certain concentration.

Apparatus and adjustment

A spectrophotometer or a photoelectric photometer is used for the measurement of absorbance.

After adjusting the spectrophotometer or photoelectric photometer based on the operation manual of the apparatus, it should be confirmed that the wavelength and the transmission rate meet the specifications of the tests described below.

The calibration of wavelength should be carried out as follows. Using an optical filter for wavelength calibration, measure the transmission rate in the vicinity of the standard wavelength value shown in the test results form, under the test conditions given in the test results form attached to each of the filters. When performing a test to determine the wavelength which shows minimal transmission rate, the difference between the measured wavelength and the standard wavelength value should be within ± 0.5 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm. It is also possible to carry out the test using a low-pressure mercury lamp at bright line wavelengths of 253.65 nm, 365.02 nm, 435.84 nm and 546.07 nm, or a deuterium discharge lamp at bright line wavelengths of 486.00 nm and 656.10 nm. In the case of these tests, the difference between the measured wavelength and the wavelength of the bright line should be within ± 0.3 nm. When the measurement is repeated three times, each value obtained should be within the mean ± 0.2 nm.

The calibration of transmission rate or absorbance should be carried out as follows. Using an optical filter for transmission rate calibration, determine the transmission rate at the standard wavelength value under the test conditions given in the test results form attached to each of the filters. The difference between the measured transmission rate and the standard transmission rate value should be within the range of from 1% larger of the upper limit to 1% smaller of the lower limit for the relative accuracy shown in the test results form. When the measurement is repeated three times, each absorbance obtained (or calculated from the transmission rate) should be within the mean ± 0.002 when the absorbance is not more than 0.500, and within the mean ± 0.004 when the absorbance is more than 0.500. In addition, it will be desirable to confirm the linearity of transmission rate at the same wavelength using several optical filters for calibration of transmission rate with different transmission rates.

Procedure

After adjusting the apparatus as directed in the Apparatus and adjustment, select and set the light source, detector, mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed.

Subsequently, allow the apparatus to stand for a certain time to confirm its stability. Then, usually adjust the apparatus so that the transmittance is 0% at measuring wavelength or over measuring wavelength range after shutting the sample side of light path. Then open the shutter and adjust the trans-

mittance to 100% (the absorbance is zero). Adjusting the transmittance to 100% is usually done by putting cells containing the control solution in both light paths. For the control solution, unless otherwise specified, blank solvent is used.

Then perform the measurement with the cell containing the sample solution, and read the absorbance at measuring wavelength, or measure the spectrum over measuring wavelength range. Unless otherwise specified, a cell with a path length of 1 cm, made of quartz for ultraviolet range and of quartz or glass for visible range, is used. Special consideration is needed with the absorption of solvents in the ultraviolet range; use a solvent which does not disturb accurate measurement.

Specific absorbance

In the Japanese Pharmacopoeia, the absorbance, calculated on the basis that l is 1 cm and c (concentration of a medicament) is 1 w/v%, is called specific absorbance, and is expressed as $E_{1\text{cm}}^{1\%}$.

$$E_{1\text{cm}}^{1\%} = \frac{A}{c \times l}$$

l : Length of the layer of the solution (cm)

A : Absorbance value

c : Concentration of the sample in the solution (w/v%)

The description of, for example, “ $E_{1\text{cm}}^{1\%}$ (241 nm): 500–530 (after drying, 2 mg, methanol, 200 mL)” in the monograph, indicates that observed $E_{1\text{cm}}^{1\%}$ value is between 500 and 530, when the test is performed in the following manner: The sample is dried under the conditions specified in the Test for Loss on Drying, and about 2 mg of the sample is weighed accurately with a microbalance, and dissolved in methanol to make exactly 200 mL, then the absorbance of the solution is measured as directed in the Procedure at a wavelength of 241 nm using a cell with a path length of 1 cm.

Identification

Prepare the sample solution as directed in the monograph, and test as directed in the Procedure. Usually, the test is performed by a single method or in a combination of a few methods in the following methods using the absorbance or absorption spectrum obtained from the sample solution. Subtle differences in the absorption spectrum arising from differences in the apparatus used may be neglected.

(1) Identification using Reference Spectrum

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the Reference Spectrum, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

Reference spectrum: Reference spectra are specified under the Ultraviolet-visual Reference Spectra, which are used as the reference for the test of identification specified in the monograph.

(2) Identification using Reference Standard

When the absorption spectrum obtained from the sample solution exhibits similar intensities of absorption at the same wavelengths as those of the spectrum obtained from the Reference Standard, the identity of the sample and the reference may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Refer-

ence Spectrum. When the relevant Reference Spectrum is not available, the range is that specified in the monograph.

(3) Identification using absorption wavelength

When maximum absorption wavelengths of the spectrum obtained from the sample solution match the wavelengths specified in the monograph, the identity of the substance may be confirmed. In this case, the range of the wavelength to be compared is the range shown on the Reference Spectrum.

(4) Identification using the ratio of the absorbances obtained at two or more wavelengths

When the ratios of absorbances at the specified wavelengths in the spectrum obtained from the sample solution meet the specifications in the monograph, the identity of the substance may be confirmed.

Assay

Prepare the control solution, the sample solution and the standard solution as directed in the monograph, measure the absorbances of the sample solution and the standard solution according to the method described in the Procedure, and determine the amount of the substance to be assayed in the sample by comparing the absorbances.

2.25 Infrared Spectrophotometry

Infrared Spectrophotometry is a method of measurement of the extent, at various wave numbers, of absorption of infrared radiation when it passes through a layer of a substance. In the graphic representation of infrared spectra, the plot usually shows units of wave numbers as the abscissa and units of transmittance or absorbance as the ordinate. Wave number and transmittance or absorbance at each absorption maximum may be read graphically on an absorption spectrum and/or obtained by a data-processor. Since the wave number and the respective intensity of an absorption maximum depend on the chemical structure of a substance, this measurement can be used to identify or determine a substance.

Instrument and adjustment

Several models of dispersive infrared spectrophotometers or Fourier-transform infrared spectrophotometers are available.

The instruments, adjusted according to the instruction manual of each individual instrument, should comply with the following test for resolving power, transmittance reproducibility and wave number reproducibility. When the spectrum of a polystyrene film about 0.04 mm thick is recorded, the depth of the trough from the maximum absorption at about 2850 cm^{-1} to the minimum at about 2870 cm^{-1} should be not less than 18% transmittance and that from the maximum at about 1583 cm^{-1} to the minimum at about 1589 cm^{-1} should be not less than 12% transmittance.

The wave number (cm^{-1}) scale is usually calibrated by the use of several characteristic absorption wave numbers (cm^{-1}) of a polystyrene film shown below. The number in parentheses indicates the permissible range.

3060.0 (± 1.5)	2849.5 (± 1.5)	1942.9 (± 1.5)
1601.2 (± 1.0)	1583.0 (± 1.0)	1154.5 (± 1.0)
1028.3 (± 1.0)		

When the dispersive infrared spectrophotometer is used,

the permissible range of the absorption wave numbers at 1601.2 cm^{-1} and at 1028.3 cm^{-1} should be both within $\pm 2.0 \text{ cm}^{-1}$.

As the repeatability of transmittance and wave number, the difference of transmittance should be within 0.5% when the spectrum of a polystyrene film is measured twice at several wave numbers from 3000 to 1000 cm^{-1} , and the difference of wave number should be within 5 cm^{-1} at about 3000 cm^{-1} and within 1 cm^{-1} at about 1000 cm^{-1} .

Preparation of samples and measurement

Unless otherwise specified, when it is directed to perform the test "after drying the sample", use a sample dried under the conditions specified in the monograph. Prepare the specimen for the measurement according to one of the following procedures so that the transmittance of most of the absorption bands is in the range of 5% to 80%. Single crystals of sodium chloride, potassium bromide, etc. are available for the optical plate. Generally, the reference cell or material is placed in the reference beam for double-beam instruments, while for single-beam instruments, it is placed in the same optical path in place of the specimen and measured separately under the same operating conditions. The composition and preparation of the reference depend on the sample preparation methods, and sometimes the background absorption of the atmosphere can be utilized.

Unless otherwise specified in the monograph, the spectrum is usually recorded between 4000 cm^{-1} and 400 cm^{-1} . The spectrum should be scanned using the same instrumental conditions as were used to ensure compliance with the requirements for the resolving power and for the precision of wave number scale and of wave numbers.

(1) *Potassium bromide disk or potassium chloride disk method*—Powder 1 to 2 mg of a solid sample in an agate mortar, triturate rapidly with 0.10 to 0.20 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption, and compress the mixture with a press in a suitable die (disk-forming container) to make the sample disk. If necessary to obtain a transparent disk, press the mixture under vacuum in a die with pressure applied to the die of 50 to 100 kN per cm^2 for 5 to 8 minutes. Prepare a potassium bromide reference disk or a potassium chloride reference disk in the same manner as the sample disk.

(2) *Solution method*—Place the sample solution prepared by the method directed in each monograph in a fixed cell for liquid, and usually measure the spectrum against the reference solvent used for preparing the sample solution. The solvent used in this method should not show any interaction or chemical reaction with the specimen to be examined and should not damage the optical plate. The thickness of the fixed cell is usually 0.1 mm or 0.5 mm.

(3) *Paste method*—Powder 5 to 10 mg of a solid specimen in an agate mortar, and, unless otherwise specified, triturate the specimen with 1 to 2 drops of liquid paraffin to give a homogeneous paste. After spreading the paste to make a thin film in the center of an optical plate, place the plate upon another optical plate with precautions against intrusion of air, bubbles in the film, and examine its absorption spectrum.

(4) *Liquid film method*—Examine 1 to 2 drops of a liquid specimen as a thin film held between two optical plates. When the absorption intensity is not sufficient, place spacers of

aluminum foil, etc., between the two optical plates to make a thicker liquid film.

(5) *Film method*—Examine a thin film just as it is or a prepared thin film as directed in each monograph.

(6) *Gas sampling method*—Put a sample gas in a gas cell previously evacuated under the pressure directed in the monograph, and examine its absorption spectrum. The path length of the gas cell is usually 5 cm or 10 cm, but, if necessary, may exceed 1 m.

(7) *ATR method*—Place a specimen in close contact with an attenuated total reflectance (ATR) prism, and examine its reflectance spectrum.

(8) *Diffuse reflectance method*—Powder 1 to 3 mg of a solid specimen into a fine powder of not more than about 50 μm particle size in an agate mortar, and triturate rapidly with 0.05 to 0.10 g of potassium bromide or potassium chloride for infrared spectrophotometry with precautions against moisture absorption. Place the mixture in a sample cup, and examine its reflectance spectrum.

Identification

When the spectrum of a specimen and the Reference Spectrum of the substance expected to be found or the spectrum of the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the substance expected to be found. Furthermore, when several specific absorption wave numbers are specified in the monograph, the identification of a specimen with the substance expected to be found can be confirmed by the appearance of absorption bands at the specified wave numbers.

(1) Identification by the use of a Reference Standard

When the spectra of a specimen and the Reference Standard exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance as the Reference Standard. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with that of the Reference Standard, treat the specimen being examined and the Reference Standard in the same manner as directed in the monograph, then repeat the measurement.

(2) Identification by the use of a Reference Spectrum

When the spectra of a specimen and the Reference Spectrum exhibit similar intensities of absorption at the same wave numbers, the specimen can be identified as being the same substance associated with the Reference Spectrum. When a sample treatment method for a solid specimen is indicated in the monograph in the case of nonconformity of the spectrum with the Reference Spectrum, treat the specimen being examined as directed in the monograph, then repeat the measurement.

(3) Identification by the use of absorption wave number

When several specific absorption wave numbers of the substance being examined are specified in the monograph, a specimen can be identified as being the same substance as the expected substance by confirmation of clear appearance of the absorption bands at all the specified wave numbers.

Reference spectra

Infrared Reference Spectra, in the range between 4000 cm^{-1} and 400 cm^{-1} , are shown at the end of this book for the monographs requiring the identification test by *Infrared Spectrophotometry* <2.25>, except for monographs in which "Identification by absorption wave number" is specified.

Other Physical Methods

2.41 Loss on Drying Test

Loss on Drying Test is a method to measure the loss in mass of the sample, when dried under the conditions specified in each monograph. This method is applied to determine the amount of water, all or a part of water of crystallization, or volatile matter in the sample, which is removed during the drying.

The description, for example, "not more than 1.0% (1 g, 105°C, 4 hours)" in a monograph, indicates that the loss in mass is not more than 10 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed and dried at 105°C for 4 hours, and "not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours)," indicates that the loss in mass is not more than 5 mg per 1 g of the substance in the test in which about 1 g of the substance is accurately weighed, transferred into a desiccator (phosphorus (V) oxide), and dried in vacuum for 4 hours.

Procedure

Weigh accurately a weighing bottle that has been dried for 30 minutes according to the method specified in the monograph. Take the sample within the range of $\pm 10\%$ of the amount directed in the monograph, transfer into the weighing bottle, and, unless otherwise specified, spread the sample so that the layer is not thicker than 5 mm, then weigh it accurately. Place the loaded bottle in a drying chamber, and dry under the conditions specified in the monograph. When the size of the sample is large, convert it to small particles having a size not larger than 2 mm in diameter by quick crushing, and use the crushed sample for the test. After drying, remove from the drying chamber, and reweigh accurately. When the sample is dried by heating, the temperature is within the range of $\pm 2^\circ\text{C}$ of that directed in the monograph, and, after drying the bottle, the sample is allowed to cool in a desiccator (silica gel) before weighing.

If the sample melts at a temperature lower than that specified in the monograph, expose the sample for 1 to 2 hours to a temperature between 5°C and 10°C below the melting temperature, dry under the conditions specified in the monograph. Use a desiccant specified in the monograph, and renew frequently.

2.42 Congealing Point Determination

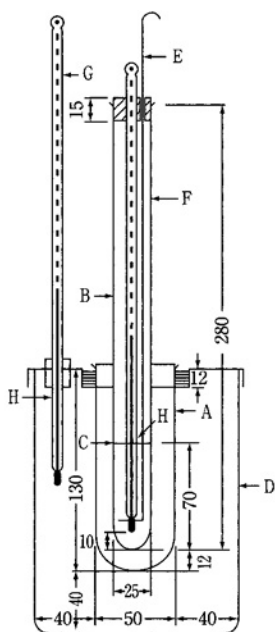
The congealing point is the temperature measured by the following method.

Apparatus

Use the apparatus illustrated in Fig. 2.42-1.

Procedure

Transfer the sample into sample container B up to the marked line C. When the sample is solid, melt the sample by heating to a temperature not higher than 20°C above the expected congealing point, and transfer to B. Fill the glass or



The figures are in mm.

- A: Cylinder made of glass (the tube is painted with silicone oil on both sides of the wall to prevent clouding).
 B: Sample container (a hard glass test tube, which is painted with silicone oil to prevent clouding, except at the region of the wall in contact with the sample; insert it into cylinder A, and fix with cork stopper).
 C: A marked line.
 D: Bath made of glass or plastics.
 E: Stirring rod made of glass or stainless steel (3 mm in diameter, the lower end part of it is bent to make a loop, about 18 mm in diameter).
 F: Thermometer with an immersion line.
 G: Thermometer with an immersion line or a total immersion thermometer.
 H: Immersion line

Fig. 2.42-1

plastic bath D with water at a temperature about 5°C below the expected congealing point. When the sample is liquid at room temperature, fill bath D with water at a temperature between 10°C and 15°C lower than the expected congealing point.

Insert the sample container B containing the sample into cylinder A. Adjust the immersion line H of thermometer F to the same level of the meniscus of the sample. After cooling the sample to about 5°C above the expected congealing point, move vertically the stirrer E at the rate of about 60 to 80 strokes per minute, and observe the thermometer readings at 30-second intervals. The temperature falls gradually. Discontinue stirring, when an appreciable amount of crystals has formed and the temperature is constant or has begun to rise. Usually, read the maximum temperature (reading of F), that is constant for a while after a rise of temperature. If no rise of temperature occurs, read the temperature that is constant for a while. The average of not less than four consecutive readings that lie within a range of 0.2°C constitutes the congealing point.

Note: If a state of super cooling is anticipated, rub the inner wall of bath B or put a small fragment of the solid sample into bath B for promoting the congealment, when the temperature approaches near the expected congealing point.

2.43 Loss on Ignition Test

Loss on Ignition Test is a method to measure the loss in mass when the sample is ignited under the conditions specified in each monograph. This method is usually applied to inorganic drugs which lose a part of the components or impurities during ignition.

The description, for example, “40.0 – 52.0% (1 g, $450 - 550^{\circ}\text{C}$, 3 hours)” in a monograph, indicates that the loss in mass is 400 to 520 mg per g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited between 450°C and 550°C for 3 hours.

Procedure

Previously ignite a crucible or a dish of platinum, quartz or porcelain to constant mass, at the temperature directed in the monograph, and weigh accurately after cooling.

Take the sample within the range of $\pm 10\%$ of the amount directed in the monograph, transfer into the above ignited container, and weigh it accurately. Ignite under the conditions directed in the monograph, and, after cooling, reweigh accurately. Use a desiccator (silica gel) for the cooling.

2.44 Residue on Ignition Test

This test is harmonized with the Sulphated Ash Test of the European Pharmacopoeia and the Residue on Ignition Test of the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\blacklozenge \blacklozenge).

\blacklozenge The Residue on Ignition Test is a method to measure the amount of residual substance not volatilized from a sample when the sample is ignited in the presence of sulfuric acid according to the procedure described below. This test is usually used for determining the content of inorganic impurities in an organic substance.

The description, for example, “not more than 0.1% (1 g)”, in a monograph, indicates that the mass of the residue is not more than 1 mg per 1 g of the substance in the test in which about 1 g of the substance is weighed accurately and ignited by the procedure described below, and “after drying” indicates that the sample is tested after being dried under the conditions specified in the test for Loss on drying. \blacklozenge

Procedure

Ignite a suitable crucible (for example, silica, platinum, quartz or porcelain) at $600 \pm 50^{\circ}\text{C}$ for 30 minutes, cool the crucible in a desiccator (silica gel or other suitable desiccant) and weigh it accurately.

Take the amount of test sample specified in the individual monograph in the crucible and weigh the crucible accurately.

Moisten the sample with a small amount (usually 1 mL) of sulfuric acid, then heat gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moisten the residue with a small amount (usually 1 mL) of sulfuric acid, heat gently until white fumes are no lon-

ger evolved, and ignite at $600 \pm 50^\circ\text{C}$ until the residue is completely incinerated. Ensure that flames are not produced at any time during the procedure. Cool the crucible in a desiccator (silica gel or other suitable desiccant), weigh accurately and calculate the percentage of residue.

Unless otherwise specified, if the amount of the residue so obtained exceeds the limit specified in the individual monograph, repeat the moistening with sulfuric acid, heating and ignition as before, using a 30-minute ignition period, until two consecutive weighings of the residue do not differ by more than 0.5 mg or until the percentage of residue complies with the limit in the individual monograph.

2.45 Refractive Index Determination

Refractive Index Determination is a method to measure the ratio of the velocity of light in air to that in the sample. Generally, when light proceeds from one medium into another, the direction is changed at the boundary surface. This phenomenon is called refraction. When light passes from the first isotropic medium into the second, the ratio of the sine of the angle of incidence, i , to that of the angle of refraction, r , is constant with regard to these two media and has no relation to the angle of incidence. This ratio is called the refractive index of the second medium with respect to the first, or the relative refractive index, n .

$$n = \frac{\sin i}{\sin r}$$

The refractive index obtained when the first medium is a vacuum is called the absolute refractive index, N , of the second medium.

In isotropic substances, the refractive index is a characteristic constant at a definite wavelength, temperature, and pressure. Therefore, this measurement is applied to purity test of substances, or to determination of the composition of homogeneous mixtures of two substances.

The measurement is usually carried out at 20°C , and the D line of the sodium spectrum is used for irradiation. This value is expressed as n_D^{20} .

Procedure

For the measurement of refractive index, usually the Abbé refractometer is used at a temperature in the range of $\pm 0.2^\circ\text{C}$ of that directed in the monograph. Use of the Abbé refractometer permits direct reading of n_D under incandescent light, with a measurable range from 1.3 to 1.7, and an attainable precision of 0.0002.

2.46 Residual Solvents Test

Residual Solvents Test is a test to determine the amounts of residual organic solvents in pharmaceuticals by using the gas chromatography to monitor adherence to the limits which are advised for the safety of patients by "Guideline for Residual Solvents: ICH Harmonized Tripartite Guideline".

Unless otherwise specified, the limit of the residual solvents is described in ppm in the individual monograph, and unless

otherwise specified, the limit should be not more than the limit advised in the Guideline.

Apparatus, Procedure, and Test Method

Prepare the sample solution and the standard solution as directed in the relevant monograph, and perform the test as directed under Gas Chromatography <2.02>.

In monographs, the quantity for the test of sample and reference standard (reference substances), the method for preparation of the sample and standard solutions, the injection amount of the sample and standard solutions for the gas chromatography, the operating conditions for the head-space apparatus and the gas chromatography, the system suitability, the calculation formula, and other items concerning the test are specified.

2.47 Osmolarity Determination

Osmolarity Determination is a method for measuring the osmotic concentration of the sample solution from the extent of the freezing-point depression.

When a solution and a pure solvent are separated by a semipermeable membrane, through which the solvent can pass freely, but the solute cannot, a part of the solvent passes into the solution compartment through the membrane. The pressure difference produced between the two compartments concomitantly with the solvent migration through the membrane, is defined as the osmotic pressure Π (Pa). The osmotic pressure is a physical quantity depending on the total of the molecular species present, including neutral molecules and ions, and does not depend on the kind of solute. A solution property, such as osmotic pressure, freezing-point depression, boiling-point elevation etc., which depends not on the kind of solute, but on the total number of all molecular species, is called a colligative property of a solution.

The osmotic pressure of a polymer solution can be measured directly as the hydrostatic pressure difference between two compartments separated by a semipermeable membrane, such as a cellulose membrane. However, this is not applicable to a solution containing low molecular species, which can pass through a semipermeable membrane. Though the osmotic pressure of such a solution cannot be measured directly, the direction and extent of solvent migration through biological membranes can be predicted from the total number of all molecular species present when the solution is placed under physiological conditions. Other colligative properties of a solution such as freezing-point depression, boiling-point elevation, vapor-pressure depression, etc. can be directly obtained by observing changes of temperature and/or pressure, etc. These solution properties depend on the total number of ionic and neutral species in the solution in the same way as the osmotic pressure, and the molecular particle concentration is defined as the osmotic concentration. The osmotic concentration can be defined in two ways, one being mass-based concentration (osmolality, mol/kg) and the other, volume-based concentration (osmolarity, mol/L). In practice, the latter is more convenient.

Unless otherwise specified, the freezing-point depression method is used for measuring the osmotic concentration. The method is based on the linear dependency of the freezing-point depression ΔT ($^\circ\text{C}$) upon the osmolality m (mol/kg), as expressed in the following equation,

$$\Delta T = K \cdot m$$

In this equation, K is the molal freezing-point depression constant, and it is known to be $1.86^\circ\text{C kg/mol}$ for water. Since the constant K is defined on the basis of molarity, the molar osmotic concentration can be obtained from the above equation. In the dilute osmotic concentration range, osmolality m (mol/kg) can be assumed to be numerically equal to osmolarity c (mol/L). Thus, the conventional osmolarity (mol/L) and the unit of osmole (Osm) are adopted in this test method. One Osm means that the Avogadro number ($6.022 \times 10^{23}/\text{mol}$) of species is contained in 1 L of solution. Usually the osmotic concentration is expressed as the submultiple milliosmole (mOsm, mosmol/L) in the Pharmacopoeia.

Apparatus

Usually, the osmotic concentration of a solution can be obtained by measuring the extent of the freezing-point depression. The apparatus (osmometer) is composed of a sample cell for a fixed volume of sample solution and a cell holder, a cooling unit and bath with a temperature regulator, and a thermistor for detecting temperature.

Procedure

A fixed volume of the test solution is introduced into the sample cell, as indicated for the individual apparatus.

The apparatus must first be calibrated by the two-point calibration method by using osmolal standard solutions. For the calibration, select two different standard solutions just covering the expected osmolar concentration of a sample solution. Other than the indicated osmolal standard solutions in the *Table* below, water can also be used as a standard solution (0 mOsm) for measuring low osmolar sample solutions (0 – 100 mOsm). Next, after washing the sample cell and the thermistor as indicated for the individual apparatus, measure the degree of the freezing-point depression caused by a sample solution. Using the above-mentioned relation of osmolar concentration m and ΔT , the osmolarity of a sample solution can be obtained, and it is assumed to be numerically equal to the osmolarity.

In the case of higher osmolar solutions over 1000 mOsm, dilute the sample by adding distilled water and prepare n times diluted sample solution (1 in n). Measure the osmolarity of the diluted solution, as described above. In this case, it is necessary to state that the calculated osmolarity for the sample (see below) is an apparent osmolarity obtained by the dilution method. When the dilution method is applied, the dilution number should be selected so that the expected osmolarity is nearly equal to that of physiological saline solution.

In the case of solid samples, such as freeze-dried medicines, prepare a sample solution by dissolving the solid using the indicated solution for dissolution.

Suitability of the apparatus

After the calibration of the apparatus, a suitability test must be done by repeating the measurement of osmolarity for one of the standard solutions not less than 6 times. In performing the test, it is advisable that the osmolarity of a sample solution and the selected standard solution are similar to each other. In this test, the repeatability of measured values and the deviation of the average from the indicated value should be less than 2.0% and 3.0%, respectively. When the requirement is not met, calibrate the apparatus again by the

two-point calibration method, and repeat the test.

Preparation of the osmolar standard solutions

Weigh exactly an amount indicated in Table 2.47-1 of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve the weighed sodium chloride in exactly 100 g of water to make the corresponding osmolar standard solution.

Table 2.47-1

Standard solution for osmometer calibration (milliosmoles)	Amount of sodium chloride (g)
100	0.309
200	0.626
300	0.946
400	1.270
500	1.593
700	2.238
1000	3.223

Osmolar ratio

In this test method the osmolar ratio is defined as the ratio of osmolarity of a sample solution to that of the isotonic sodium chloride solution. The ratio can be used as a measure of isotonicity of sample solution. Since the osmolarity of the isotonic sodium chloride solution ($\text{NaCl } 0.900 \text{ g}/100 \text{ mL}$) c_S (mOsm) is assumed to be constant (286 mOsm), the osmolar ratio of a sample solution, of which the osmolarity is c_T (mOsm), can be calculated by means of the following equation,

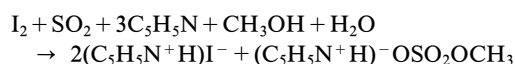
$$\text{Osmolar ratio} = \frac{c_T}{c_S}$$

c_S : 286 mOsm

When the measurement is done by the dilution method, because the sample has an osmolarity over 1000 mOsm, the apparent osmolarity of the sample solution c_T can be calculated as $n \cdot c'_T = c_T$, in which n is the dilution number and c'_T is the measured osmolarity for the diluted solution. In this calculation, a linear relation between osmolarity and solute concentration is assumed. Thus when the dilution measurement is performed, the dilution number must be stated as (1 in n).

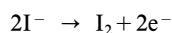
2.48 Water Determination (Karl Fischer Method)

Water Determination is a method to determine water content in sample materials, utilizing the fact that water reacts with iodine and sulfur dioxide quantitatively in the presence of a lower alcohol such as methanol, and an organic base such as pyridine. The reaction proceeds in the manner shown in the following equation:



In this measurement there are two methods different in iodine-providing principle: one is the volumetric titration method and the other, the coulometric titration method. In the former, iodine is previously dissolved in a reagent for water determination, and water content is determined by

measuring the amount of iodine consumed as a result of reaction with water. In the latter, iodine is produced by electrolysis of Karl Fischer reagent containing iodide ion. Based on the quantitative reaction of the generated iodine with water, the water content in a sample specimen can be determined by measuring the quantity of electricity which is required for the production of iodine during the titration.



1. Volumetric titration

Apparatus

Generally, the apparatus consists of automatic burettes, a titration flask, a stirrer, and equipment for amperometric titration at constant voltage or potentiometric titration at constant current.

The Karl Fischer TS is extremely hygroscopic, so the apparatus should be designed to be protected from atmospheric moisture. Desiccants such as silica gel or calcium chloride for water determination are used for moisture protection.

Reagents

(1) Chloroform for water determination—To 1000 mL of chloroform add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of chloroform. Preserve the chloroform, protecting it from moisture. The water content of this chloroform should not be more than 0.1 mg per mL.

(2) Methanol for water determination—To 1000 mL of methanol add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of methanol. Preserve the methanol, protecting it from moisture. The water content of this methanol should not be more than 0.1 mg per mL.

(3) Propylene carbonate for water determination—To 1000 mL of propylene carbonate add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear propylene carbonate layer. Preserve this protecting from moisture. The water content should not be more than 0.3 mg per mL.

(4) Diethylene glycol monoethyl ether for water determination—To 1000 mL of diethylene glycol monoethyl ether add 30 g of synthetic zeolite for drying, stopper tightly, allow to stand for about 8 hours with occasional gentle shaking, then allow to stand for about 16 hours, and collect the clear layer of diethylene glycol monoethyl ether. Preserve the diethylene glycol monoethyl ether, protecting it from moisture. The water content of this diethylene glycol monoethyl ether should not be more than 0.3 mg per mL.

(5) Pyridine for water determination—Add potassium hydroxide or barium oxide to pyridine, stopper tightly, and allow to stand for several days. Distill and preserve the purified and dried pyridine, protecting it from moisture. The water content of this pyridine should not be more than 1 mg per mL.

(6) Imidazole for water determination—Use imidazole for thin-layer chromatography, of which the water content should not be more than 1 mg per g.

(7) 2-Methylaminopyridine for water determination—Distill and preserve 2-methylaminopyridine, protecting it from moisture. The water content of this 2-methylaminopyri-

dine should not be more than 1 mg per mL.

Preparation of test solutions and standard solutions

(1) Karl Fischer TS for water determination

Prepare according to the following method (i), (ii) or (iii).

(i) Preparation 1

Dissolve 63 g of iodine in 100 mL of pyridine for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 32 g. Then make up to 500 mL by adding chloroform for water determination or methanol for water determination, and allow to stand for more than 24 hours before use.

(ii) Preparation 2

Dissolve 102 g of imidazole for water determination in 350 mL of diethylene glycol monoethyl ether for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through this solution until the mass increase of the solution reaches 64 g, keeping the temperature between 25°C and 30°C. Then dissolve 50 g of iodine in this solution, and allow to stand for more than 24 hours before use.

(iii) Preparation 3

Pass dried sulfur dioxide gas through 220 mL of propylene carbonate until the mass increase of the solvent reaches 32 g. To this solution, cooled in ice bath, add 180 mL of propylene carbonate or diethylene glycol monoethyl ether for water determination, in which 81 g of 2-methylaminopyridine for water determination is dissolved. Then dissolve 36 g of iodine in this solution, and allow to stand for more than 24 hours before use.

The Karl Fischer TS, prepared by any one of the methods described above, must be standardized before every use, because its activity for water determination changes with the lapse of time. Further preserve the TS in a cold place, protecting it from light and moisture.

Standardization—According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the solvent with a Karl Fischer TS to make the content of the flask anhydrous. Then, add quickly about 30 mg of water weighed accurately to the solution in the flask, and titrate the water dissolved in the solvent with a Karl Fischer TS to the end point, under vigorous stirring. Calculate the water equivalence factor, f (mg/mL), corresponding to the amount of water (H_2O) in mg per 1 mL of the test solution by using the following equation:

$$f(\text{mg/mL}) = \frac{\text{Amount of water (H}_2\text{O) (mg)}}{\text{Volume of Karl Fischer TS consumed for titration of water (H}_2\text{O) (mL)}}$$

(2) Standard water-methanol solution

Preparation—Take 500 mL of methanol for water determination in a dried 1000-mL volumetric flask, add 2.0 mL of water, and adjust with the methanol to make 1000 mL.

Perform the standardization of this solution, followed by the procedure for Karl Fischer TS. Preserve it in a cool place, protecting it from light and moisture.

Standardization—According to the procedure described below, take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Then, add exactly 10 mL of Karl Fischer TS to this solution in the flask, and titrate it with the prepared

standard water-methanol solution to the end point. Calculate the water concentration in the standard water-methanol solution, f' (mg/mL), by using the following equation:

$$f'(\text{mg/mL}) = \frac{f(\text{mg/mL}) \times 10 (\text{mL})}{\text{Volume of the standard water-methanol solution consumed for titration (mL)}}$$

Procedure

As a rule, the titration of water with a Karl Fischer TS should be performed at the same temperature as that at which the standardization was done, with protection from moisture. The apparatus is equipped with a variable resistor in the circuit, and this resistor is manipulated so as to maintain a constant voltage (mV) between two platinum electrodes immersed in the solution to be titrated. The variable current (μA) can be measured (Amperometric titration at constant voltage). During titration with Karl Fischer TS, the current in the circuit varies noticeably, but returns to the original value within several seconds. At the end of a titration, the current stops changing and persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

Otherwise, the manipulation of the resistor serves to pass a definite current between two platinum electrodes. The variable potential (mV) can be measured (Potentiometric titration at constant current). With the progress of titration of water with a Karl Fischer TS, the value indicated by the potentiometer in the circuit decreases suddenly from a polarization state of several hundreds (mV) to the non-polarization state, but it returns to the original value within several seconds. At the end of titration, the non-polarization state persists for a certain time (usually, longer than 30 seconds). When this electric state has been attained, it is designated as the end point of titration.

In the case of back titration, when the amperometric titration method is used at constant voltage, the needle of microammeter is out of scale during excessive presence of Karl Fischer TS, and it returns rapidly to the original position when the titration system has reached the end point. In the case of the potentiometric titration method at constant current in the back titration mode, the needle of the millivoltmeter is at the original position during excessive presence of Karl Fischer TS. Finally a definite voltage is indicated when the titration system has reached the end point.

Unless otherwise specified, the titration of water with Karl Fischer TS can be performed by either one of the following methods. Usually, the end point of the titration can be observed more clearly in the back titration method, compared with the direct titration method.

(1) Direct titration

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in a dried titration flask, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a quantity of sample specimen containing 5 to 30 mg of water, transfer it quickly into the titration flask, dissolve by stirring, and titrate the solution to be examined with Karl Fischer TS to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount of the sample containing 5 to 30 mg of water, and transfer it quickly into the titration vessel, stir the mixture for 5 – 30 minutes, protecting

it from moisture, and perform a titration under vigorous stirring. Alternatively, in the case of a sample specimen which is insoluble in the solvent for water determination or which interfere with the Karl Fischer reaction, water in the sample can be removed by heating under a stream of nitrogen gas, and introduced into the titration vessel by using a water evaporation technique.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water (H}_2\text{O) \%} = \frac{\text{Volume of Karl Fischer TS consumed for titration (mL)} \times f(\text{mg/mL})}{\text{Amount of sample (mg)}} \times 100$$

(2) Back titration

Unless otherwise specified, proceed by the following method. Take a suitable quantity of methanol for water determination in the dried titration vessel, and titrate the water contaminated with Karl Fischer TS to make the content of the flask anhydrous. Take a suitable quantity of sample specimen having 5 – 30 mg of water, transfer the sample quickly into the titration vessel, dissolve it in the solution by stirring, add an excessive and definite volume of Karl Fischer TS, and then titrate the solution with the standard water-methanol solution to the end point under vigorous stirring.

In the case of an insoluble sample specimen, powder the sample quickly, weigh a suitable amount accurately, transfer it quickly into the titration vessel, and add an excessive and definite volume of Karl Fischer TS. After stirring for 5 – 30 minutes, with protection from moisture, perform the titration under vigorous stirring.

Water (H₂O) % =

$$\frac{\left\{ \left[\begin{array}{l} \text{Volume of Karl} \\ \text{Fischer TS} \\ \text{added (mL)} \end{array} \right] \times f(\text{mg/mL}) \right.}{\left. - \left[\begin{array}{l} \text{Volume of the standard water-} \\ \text{methanol solution consumed} \\ \text{for titration (mL)} \end{array} \right] \times f'(\text{mg/mL}) \right\}}{\text{Amount of sample (mg)}} \times 100$$

2. Coulometric titration

Apparatus

Usually, the apparatus is comprised of a titration flask equipped with an electrolytic cell for iodine production, a stirrer, and a potentiometric titration system at constant current. The iodine production system is composed of an anode and a cathode, separated by a diaphragm. The anode is immersed in the anolyte solution for water determination and the cathode is immersed in the catholyte solution for water determination. Both electrodes are usually made of platinum-mesh.

Because both the anolyte and the catholyte solutions for water determination are strongly hygroscopic, the titration system should be protected from atmospheric moisture. For this purpose, silica gel or calcium chloride for water determination can be used.

Preparation of anolyte and catholyte solutions for water determination

Electrolytic solutions shall consist of an anolyte solution and a catholyte solution, the preparations of which are described below.

Preparation—Any of methods (1), (2), and (3) can be used for the preparation of the electrolytes for coulometric titration.

(1) Preparation 1

Anolyte for water determination—Dissolve 102 g of imidazole for water determination in 900 mL of methanol for water determination, cool the solution in ice bath, and pass dried sulfur dioxide gas through the solution, which is kept below 30°C. When the mass increase of the solution has reached 64 g, the gas flow is stopped and 12 g of iodine is dissolved by stirring. Then drop a suitable amount of water into the solution until the color of liquid is changed from brown to yellow, and add methanol for water determination to make up 1000 mL.

Catholyte for water determination—Dissolve 24 g of diethanolamine hydrochloride in 100 mL of methanol for water determination.

(2) Preparation 2

Anolyte for water determination—Dissolve 40 g of 1,3-di(4-pyridyl)propane and 30 g of diethanolamine in about 200 mL of methanol for water determination, and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 25 g, the gas flow is stopped. Add 50 mL of propylene carbonate, and dissolve 6 g of iodine in the solution. Then make up the solution to 500 mL by addition of methanol for water determination and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination—Dissolve 30 g of choline hydrochloride into methanol for water determination and adjust the volume to 100 mL by adding the methanol.

(3) Preparation 3

Anolyte for water determination—Dissolve 100 g of diethanolamine in 900 mL of methanol for water determination or a mixture of methanol and chloroform for water determination (3 : 1), and pass dried sulfur dioxide gas through the solution. When the mass increase of the solution has reached 64 g, the gas flow is stopped. Dissolve 20 g of iodine in the solution, and drop in a suitable amount of water until the color of liquid is changed from brown to yellow.

Catholyte for water determination—Dissolve 25 g of lithium chloride in 1000 mL of a mixture of methanol for water determination and nitromethane (4 : 1).

Procedure

Take a suitable volume of an anolyte for water determination in the titration vessel, immerse in this solution a pair of platinum electrodes for potentiometric titration at constant current. Then immerse the iodine production system filled with a catholyte for water determination in the anolyte solution. Switch on the electrolytic system and make the content of the titration vessel anhydrous. Next take an accurately weighed amount of a sample specimen containing 0.2 – 5 mg of water, add it quickly to the vessel, dissolve by stirring, and perform the titration to the end point under vigorous stirring.

When a sample specimen cannot be dissolved in the anolyte, powder it quickly, and add an accurately weighed amount of the sample estimated to contain 0.2 – 5 mg of

water to the vessel. After stirring the mixture for 5 – 30 minutes, with protection from atmospheric moisture, perform the titration under vigorous stirring. Alternatively, in the case of an insoluble solid or a sample containing a component which interferes with the Karl Fisher reaction, water in the sample can be removed by heating, and carried by a nitrogen gas flow into the titration vessel, by using a water evaporation technique.

Determine the quantity of electricity (C) [= electric current (A) × time (s)] required for the production of iodine during the titration, and calculate the water content (%) in the sample specimen by use of the following equation.

Though the titration procedure should be performed under atmospheric conditions at low humidity, if the effect of atmospheric moisture cannot be avoided, for instance, if a long time is required for extraction and titration of water, a blank test must be done under the same conditions as used for the sample test, and the data must be corrected, accordingly.

$$\text{Water (H}_2\text{O) \%} = \frac{\text{Quantity of electricity required for iodine production (C)}}{10.72 \text{ (C/mg)} \times \text{Amount of sample (mg)}} \times 100$$

10.72: quantity of electricity corresponding to 1 mg of water (C/mg)

2.49 Optical Rotation Determination

Optical Rotation Determination is a method for the measurement of the angular rotation of the sample using a polarimeter.

Generally, the vibrations of light take place on planes perpendicular to the direction of the beam. In the case of ordinary light, the directions of the planes are unrestricted. In the case of plane polarized light, commonly designated as polarized light, however, the vibrations take place on only one plane that includes the direction of the beam (plane of polarization). Some drugs in the solid state or in solution have the property of rotating the plane of the polarized light either to the right or to the left. This property is referred to as optical activity or optical rotation, and is inherently related to the chemical constitution of the substance.

The extent of the rotation, expressed in degrees of rotation of the angle of the plane of polarized light caused by the optically active substance or its solution, is measured with a polarimeter. This value is proportional to the length of the polarimeter tube, and is related to the concentration of the solution, the temperature and the wavelength. The character of the rotation is indicated by placing a plus sign (+) for that which rotates the plane of the polarized light to the right, when facing the direction of the beam, referred to as dextrorotatory, or a minus sign (–) for that which rotates the plane to the left, referred to as levorotatory, before the number indicating the degrees of rotation, as like as +20°, meaning 20° to the right, or –20°, meaning 20° to the left.

The angular rotation α_x^t is that which is measured with specific monochromatic light of x (described in terms of the wavelength or the name) at a temperature of $t^\circ\text{C}$. Usually the measurement is performed at 20°C, with a polarimeter tube

of 100 mm in length, and with the D line of sodium as the light source.

The specific rotation is represented by the following equation:

$$[\alpha]_x^t = \frac{100 \alpha}{lc}$$

t: The temperature of measurement.

x: The wavelength or the name of the specific monochromatic light of the spectrum used (in the case of the D line, described as D).

α : The angle, in degrees, of rotation of the plane of the polarized light.

l: The thickness of the layer of sample solution, i.e., the length of the polarimeter tube (mm).

c: For the purpose of the Japanese Pharmacopoeia, the number of grams of a drug present in 1 mL of the solution. When an intact liquid drug is used for determination, not in solution, *c* represents the density. However, unless otherwise specified, the specific gravity is used instead of the density.

The description, for example, “[α]_D²⁰: -33.0 - -36.0° (after drying, 1 g, water, 20 mL, 100 mm),” in a monograph, indicates that the [α]_D²⁰ is between -33.0° and -36.0° in the determination in which the substance is dried under the conditions described in the test for Loss on Drying, and about 1 g of the substance is accurately weighed, and dissolved by adding water to make exactly 20 mL, then the solution is measured with a polarimeter tube 100 mm in length.

2.50 Endpoint Detection Methods in Titrimetry

Titrimetry is a method or a procedure for volumetric analysis, which is usually classified into acid-base titration (neutralization titration or pH titration), precipitation titration, complexation titration, oxidation-reduction titration, etc., according to the kind of reaction or the nature of the phenomenon occurring between the titrate and the titrant (standard solution for volumetric analysis). Furthermore, titration performed in a nonaqueous solvent is generally called nonaqueous titration, which is frequently used for volumetric analysis of weak acids, weak bases, and their salts. The endpoint in titrimetry can be detected by color changes of indicators and/or by changes of electrical signals such as electrical potential or electrical current.

The indicator method is one of the endpoint detection methods in titrimetry. In this method the color of an indicator dye, dissolved in the titrate, changes dramatically in the vicinity of the equivalence point due to its physico-chemical character, and this property is used for visual endpoint detection. Selection of an indicator and specification of the color change induced in the respective titration system, should be described in the individual monograph. An appropriate indicator should change color clearly, in response to a slight change in physico-chemical properties of the titrate, such as pH, etc., in the vicinity of the equivalence point.

Regarding the electrical endpoint detection methods, there are an electrical potential method and an electrical current method, which are called potentiometric and amperometric

titration methods, respectively. They are generically named electrometric titration. In the potentiometric titration method, the endpoint of a titration is usually determined to be the point at which the differential potential change becomes maximum or minimum as a function of the quantity of titrant added. In the amperometric titration method, unless otherwise specified, a bi-amperometric titration method is used, and the endpoint is determined by following the change of microcurrent during the course of a titration. Furthermore, the quantity of electricity (electrical current \times time) is often used as another electrochemical signal to follow a chemical reaction, as described in Coulometric Titration under Water Determination <2.48>.

The composition of a titration system, such as amount of specimen, solvent, standard solution for volumetric analysis, endpoint detection method, equivalent amount of substance to be examined (mg)/standard solution (mL), should be specified in the individual monograph. Standardization of the standard solution and titration of a specimen are recommended to be done at the same temperature. When there is a marked difference in the temperatures at which the former and the latter are performed, it is necessary to make an appropriate correction for the volume change of the standard solution due to the temperature difference.

Indicator Method

Weigh an amount of a specimen in a flask or a suitable vessel as directed in the monograph or in “*Standard Solutions for Volumetric Analysis*”, and add a specified quantity of solvent to dissolve the specimen. After adding a defined indicator to the solution to prepare the titrate, titrate by adding a standard solution for volumetric analysis by using a buret. In the vicinity of the endpoint, observe the color change induced by the cautious addition of 0.1 mL or less of the titrant. Calculate the quantity of titrant added from the readings on the scale of the buret used for the titration at the starting point and at the endpoint at which the specified color change appears, as directed in the individual monograph or in the “*Standard Solutions for Volumetric Analysis*”. Although addition of the volumetric standard solution by buret is usually done manually, an automatic buret can also be used.

Unless otherwise specified, perform a blank determination according to the following method, and make any necessary correction.

Measure a specified quantity of solvent, as directed in the monograph or in the “*Standard Solutions for Volumetric Analysis*”, and titrate as directed. The required quantity of the standard solution added to reach a specified color change, is assumed to be the blank quantity for the titration system. However, when the blank quantity is too small to evaluate accurately, the quantity can be assumed to be zero.

Electrical Endpoint Detection Methods

1. Potentiometric titration

(1) Apparatus

The apparatus consists of a beaker to contain the specimen, a buret for adding a standard solution, an indicator electrode and a reference electrode, a potentiometer for measuring potential difference between the electrodes or an adequate pH meter, a recorder, and a stirrer for gentle stirring of the solution to be examined. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

In this titration method, unless otherwise specified, indica-

tor electrodes designated in Table 2.50-1 are used according to the kind of titration. As a reference electrode, usually a silver-silver chloride electrode is used. Besides the single indicator electrodes as seen in Table 2.50-1, a combined reference electrode and indicator electrode can also be used.

Table 2.50-1 Kind of Titration and Indicator Electrode

Kind of titration	Indicator electrode
Acid-base titration (Neutralization titration, pH titration)	Glass electrode
Precipitation titration (Titration of halogen ion by silver nitrate)	Silver electrode. A silver-silver chloride electrode is used as a reference electrode, which is connected with the titrate by a salt bridge of saturated potassium nitrate solution.
Oxidation-reduction titration (Diazo titration, etc.)	Platinum electrode
Complexation titration (Chelometric titration)	Mercury-mercury chloride (II) electrode
Nonaqueous titration (Perchloric acid titration, Tetramethylammonium hydroxide titration)	Glass electrode

When the potentiometric titration is carried out by the pH measurement method, the pH meter should be adjusted according to the pH Determination <2.54>.

(2) Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the monograph. After the potential difference E (mV) or the pH value of the solvent to be used for titration has reached a stable value, immerse both reference and indicator electrodes, which have previously been washed with the solvent being used, in the solution to be examined, and titrate with a standard solution for volumetric analysis with gentle stirring of the solution. During the titration, the tip of the buret should be dipped into the solution, to be examined. The endpoint of titration is determined by following the variation of the potential difference between two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of a titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained potential values along the ordinate and the quantity of a titrant added V (mL) along the abscissa to draw a titration curve, and obtain the endpoint from the maximum or the minimum value of $\Delta E/\Delta V$ or from the value of electromotive force or pH corresponding to the equivalence point.

Unless otherwise specified, the decision of the endpoint in this method is usually made by either of the following methods.

(i) Drawing method

Usually, draw two parallel tangent lines with a slope of about 45° to the obtained titration curve. Next, draw a 3rd parallel line at the same distance from the previously drawn two parallel lines, and decide the intersection point of this line with the titration curve. Further, from the intersection point, draw a vertical line to the abscissa, and read the quan-

tity of titrant added as the endpoint of the titration.

Separately, the endpoint of the titration can also be obtained from the maximum or the minimum of the differential titration curve ($\Delta E/\Delta V$ vs. V).

(ii) Automatic detection method

In the case of potentiometric titration using an automatic titration system, the endpoint can be determined by following the respective instrumental indications. The endpoint is decided either by following the variation of the differential potential change or the absolute potential difference as a function of the quantity of titrant added: in the former case the quantity given by the maximum or the minimum of the differential values, and in the latter the quantity given by the indicator reaching the endpoint potential previously set for the individual titration system, are assumed to be the endpoint volumes, respectively.

2. Amperometric titration

(1) Apparatus

The apparatus consists of a beaker for holding a specimen, a buret for adding a standard solution for volumetric analysis, two small platinum plates or wires of the same shape as the indicator electrode, a device to load direct current microvoltage between two electrodes, a microammeter to measure the indicator current between the two electrodes, a recorder, and a stirrer which can gently stir the solution in a beaker. Separately, an automatic titration apparatus assembled from suitable units and/or parts, including a data processing system, can also be used.

(2) Procedure

Weigh a defined amount of a specimen in a beaker, and add an indicated quantity of solvent to dissolve the specimen, as directed in the individual monograph. Next, after washing the two indicator electrodes with water, immerse both electrodes in the solution to be examined, apply a constant voltage suitable for measurement across two electrodes by using an appropriate device, and titrate the solution with a standard solution for volumetric analysis. During the titration, the tip of the buret should be dipped into the solution to be examined. The endpoint of titration is determined by following the changes of microcurrent between the two electrodes as a function of the quantity of titrant added. In the vicinity of the endpoint, the amounts of the titrant added should be 0.1 mL or less for adequate titrimetry. Plot the obtained current values along the ordinate and the quantity of the titrant added V (mL) along the abscissa to draw a titration curve, and usually take the inflection point of the titration curve (the point of intersection given by the extrapolation of two straight lines before and after the inflection) as the endpoint in amperometric titration.

The blank test in this titration is usually performed as follows: Take a volume of the solvent specified in the individual monograph or in the "Standard Solution for Volumetric Analysis", and use this as the sample solution. Determine the amount of the volumetric standard solution needed for giving the endpoint, and use this volume as the blank. If this volume is too small to determine accurately, the blank may be considered as 0 (mL).

Unless otherwise specified, the endpoint in this titration is decided by either of the following methods.

(i) Drawing method

Usually, extrapolate the two straight lines before and after the inflection, and obtain the inflection point of the titration

curve. Next, read the quantity of titrant added at the inflection point, and assume this point to be the endpoint.

(ii) Automatic detection method

In the case of amperometric titration using an automatic titration system, the endpoint can be determined by following the instrumental indications. The endpoint is decided by following the variation of the indicator current during the course of a titration, and the quantity of titrant added is assumed to be that at which the current has reached the endpoint current set previously for the individual titration system.

When atmospheric carbon dioxide or oxygen is expected to influence the titration, a beaker with a lid should be used, and the procedure should be carried out in a stream of an inert gas, such as nitrogen gas. Further, when a specimen is expected to be influenced by light, use a light-resistant container to avoid exposure of the specimen to direct sunlight.

2.51 Conductivity Measurement

Conductivity Measurement is a method for the measuring the flowability of electric current in an aqueous solution. The measurement is made with a conductivity meter or a resistivity meter, and is used, for example, in the purity tests in monographs. The method is applied to evaluate the test item "Conductivity (Electrical Conductivity)" specified in the monographs. Further it is also used for monitoring the quality of water in the preparation of highly purified water. However, when applying this method for monitoring the quality of water, the details of measurement should be specified by the user, based on the principles described here.

Conductivity of a solution $\kappa(\text{S}\cdot\text{m}^{-1})$ is defined as the reciprocal of resistivity $\rho(\Omega\cdot\text{m})$, which is an indicator of the strength of ionic conductivity for a fluid conductor. Resistivity is defined as the product of electrical resistance per unit length and cross-sectional area of a conductor. When resistivity is ρ , cross-section area $A(\text{m}^2)$, and length $l(\text{m})$, resistance $R(\Omega)$ can be expressed by the following equation.

$$R = \rho(l/A)$$

Thus, conductivity κ is expressed as follows,

$$\kappa = 1/\rho = (1/R)(l/A)$$

If l/A is known, the conductivity κ can be obtained by measuring resistance R or conductance $G (= R^{-1})$.

In the International System (SI), the unit of conductivity is the Siemens per meter ($\text{S}\cdot\text{m}^{-1}$). In practice, conductivity of a solution is generally expressed by $\mu\text{S}\cdot\text{cm}^{-1}$, and resistivity by $\Omega\cdot\text{cm}$.

Unless otherwise specified, the reference temperature for the expression of conductivity or resistivity is 20°C.

Apparatus

A conductivity meter or a resistivity meter is composed of an indicator part (operating panel, display, recording unit) and a detector part, the latter of which includes a conductivity cell. In the conductivity cell a pair of platinum electrodes is embedded. The cell is immersed in a solution, and the resistance or the resistivity of the liquid column between the electrodes is measured. Alternating current is supplied to this

apparatus to avoid the effects of electrode polarization. Further, a temperature compensation system is generally contained in the apparatus.

Conductivity measurement is generally performed by using an immersion-type cell. A pair of platinum electrodes, the surfaces of which are coated with platinum black, is fixed in parallel. Both electrodes are generally protected by a glass tube to prevent physical shocks.

When the surface area of the electrode is $A(\text{cm}^2)$, and the separation distance of the two electrodes is $l(\text{cm})$, the cell constant $C(\text{cm}^{-1})$ is given by the following equation.

$$C = \alpha \cdot (l/A)$$

α is a dimensionless numerical coefficient, and it is characteristic of the cell design.

In addition to the immersion-type cell, there are flow-through-type and insert-in-pipe-type cells. These cells are set or inserted in an appropriate position in the flow system for monitoring the quality of water continuously or intermittently, during the preparation of highly purified water.

Standard Solution of Potassium Chloride

After pulverizing an appropriate amount of potassium chloride for conductivity measurement, dry it at 500 – 600°C for 4 hours. Take an indicated amount of the dried potassium chloride, as shown in Table 2.51-1, dissolve it in distilled or purified water (conductivity less than $2\mu\text{S}\cdot\text{cm}^{-1}$), previously boiled and cooled, and adjust to make 1000.0 g, for preparation of the standard solutions. The conductivity and the resistivity of the respective standard solutions at 20°C are indicated in Table 2.51-1. These standard solutions should be kept in tightly closed polyethylene or hard glass bottles.

Table 2.51-1. Conductivity and Resistivity of the Standard Solutions of Potassium Chloride at 20°C

Concentration (g/1000.0 g)	Conductivity κ ($\mu\text{S}\cdot\text{cm}^{-1}$)	Resistivity ρ ($\Omega\cdot\text{cm}$)
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

When measurement at 20°C can not be done, the indicated value of conductivity for the respective standard solution (Table 2.51-1), can be corrected by using the equation below. However, the equation is valid only within the range of 20 ± 5°C.

$$\kappa_T = \kappa_{20}[1 + 0.021(T - 20)]$$

T : Measuring temperature specified in the monograph

κ_T : Calculated conductivity of the KCl standard solution at $T^\circ\text{C}$

κ_{20} : Conductivity of the KCl standard solution at 20°C

Operating Procedure

(1) Cell Constant

An appropriate conductivity cell should be chosen according to the expected conductivity of the sample solution. The higher the expected conductivity, the larger the cell constant required for the conductivity cell, so that the electrical resistance is within the measuring range of the apparatus being used. Conductivity cells with a cell constant of the order of 0.1cm^{-1} , 1cm^{-1} , or 10cm^{-1} , are generally used.

For determination or confirmation of the cell constant, an

appropriate KCl standard solution should be chosen and prepared, taking account of the expected conductivity of the sample solution to be measured. Rinse the cell several times with distilled water. Next, after rinsing the cell 2–3 times with the standard solution used for the cell constant determination, immerse the cell in the standard solution contained in a measuring vessel. After confirming that the temperature of the standard solution is maintained at $20 \pm 0.1^\circ\text{C}$ or at the temperature specified in the monograph, measure the resistance R_{KCl} or the conductance G_{KCl} of the standard solution, and calculate the cell constant C (cm^{-1}) by use of the following equation.

$$C = R_{\text{KCl}} \cdot \kappa_{\text{KCl}} \quad \text{or} \quad C = \kappa_{\text{KCl}} / G_{\text{KCl}}$$

R_{KCl} : Measured resistance ($\text{M}\Omega$)

G_{KCl} : Measured conductance (μS)

κ_{KCl} : Conductivity of the standard solution being used ($\mu\text{S} \cdot \text{cm}^{-1}$)

The measured cell constant should be consistent with the given value within 5%. If it is not consistent, coat the electrodes with platinum black again, or replace the cell with a new one.

(2) Suitability Test for the Apparatus

Using an appropriate KCl standard solution according to the expected conductivity of the sample solution, perform the suitability test for the apparatus. Rinse the conductivity cell several times with distilled water, and rinse again 2–3 times with the selected standard solution. Fill the standard solution in the measuring vessel. After confirming that the temperature of the measuring system is maintained at $20 \pm 0.1^\circ\text{C}$, measure the conductivity of the standard solution. When this measuring procedure is repeated several times, the average conductivity should be consistent with an indicated value in Table 1 within 5%. Further, the relative standard deviation should be less than 2%.

(3) Measurement

After confirmation of the suitability of the apparatus, perform the conductivity measurement for the sample solution. Unless otherwise specified, the preparation method for sample solution should be as specified in the respective monograph. Rinse the conductivity cell several times with distilled water, and rinse again 2–3 times with sample solution. Immerse the cell in the sample solution placed in a measuring vessel. If necessary, agitate gently the sample solution. After confirming that the temperature of the sample solution is maintained at $20 \pm 0.1^\circ\text{C}$ or at the temperature specified in the monograph, measure the resistance R_{T} ($\text{M}\Omega$) or conductance G_{T} (μS) of the sample solution, and calculate the conductivity κ_{T} by using the following equation.

$$\kappa_{\text{T}} = CG_{\text{T}} \quad \text{or} \quad \kappa_{\text{T}} = C/R_{\text{T}}$$

Items such as the sample preparation method, the necessity of blank correction, the calculation method, the specification value, and the measuring temperature should be described in the monograph, if necessary.

2.52 Thermal Analysis

“Thermal Analysis” is a generic term for a variety of techniques to measure the physical properties of a substance as a function of temperature and/or time.

Among the physical properties, phase transitions such as solid/liquid phase transition (melting, freezing) and crystal polymorphism or thermal behavior such as heat evolution or absorption accompanying thermal degradation or chemical reaction can be detected by the techniques of differential thermal analysis (DTA) or differential scanning calorimetry (DSC). DTA is a method for detecting the thermal behavior of a specimen in terms of the temperature change, while DSC employs the heat quantity (enthalpy) change. There is also a method, thermogravimetry (TG), in which the mass change of a specimen caused by dehydration, adsorption, elimination or oxidation etc., is detected as a function of temperature and/or time.

Among the above three different methods, TG can be used as an alternative method for “Loss on Drying <2.41>” or “Water Determination <2.48>”. However, it must be confirmed beforehand that no volatile component except for water is included in the test specimen when TG is used as an alternative method for “Water Determination”.

Method 1 Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC)

Apparatus Apparatus for DTA or DSC is usually composed of a heating furnace, a temperature-controller, a detector, a device for controlling the atmosphere, and an indicator/recorder.

Differential Thermal Analysis (DTA) In a DTA apparatus, a sample specimen and an inert reference material placed in the heating furnace are heated or cooled at a constant rate, and the temperature difference evolved between the sample and reference material is detected continuously by a device such as a thermocouple and recorded as a function of time and/or temperature. As an inert reference material, α -Alumina for thermal analysis is usually adopted.

Differential Scanning Calorimetry (DSC) Two kinds of DSC apparatus, based upon different principles are available as shown below.

1. Input compensation-type differential scanning calorimetry (Input compensation DSC)

A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference, which is detected by a device such as a platinum resistance thermometer, is kept at null by controlling the heating unit with a compensation feed-back circuit. The instrument is designed to measure and record continuously the balance of thermal energy applied to each furnace as a function of temperature and/or time.

2. Heat flux-type differential scanning calorimetry (Heat flux DSC)

A sample specimen and the reference material in twin furnaces are programmed to be heated or cooled at a constant rate, and the temperature difference between the sample and the reference is detected as a difference of heat flux and recorded as a function of temperature and/or time. In heat flux DSC, thermal conductors are adopted so that the heat flux between the sample and the heat reservoir is proportional to the temperature difference between them.

In usual DSC analysis, α -Alumina is used as a reference material, both in Input compensation DSC and in Heat flux DSC. But in some cases, an empty sample container can also be used without any reference material.

Procedure

A sample specimen and the reference material are put in sample pans, and the furnace is heated or cooled under a controlled temperature program. As the temperature changes, the temperature difference (DTA) or heat quantity change (DSC) that develops between the specimen and the reference is detected and recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

A preliminary experiment is needed to determine the appropriate temperature range of measurement, within which a predicted physical change such as melting or polymorphic phase transition will occur, and to confirm that unpredicted thermal changes are not induced in a specimen in that temperature range. In this preliminary test, a wide temperature range (room temperature-the temperature at which degradation begins) can be scanned at a rapid heating rate (10 – 20°C/min). Thereafter, tests by DSC or DTA should be performed at a low heating rate, usually 2°C/min, in the chosen temperature range. However, when a clear heat change cannot be observed, such as in a case of glass-transition, the heating rate may be changed to a higher or a lower rate, as appropriate for the kind of physical change being observed. By analyzing the measured DTA-curve or DSC-curve, a quantity of heat change and/or a specific temperature (ignition, peak or end temperature) that accompanies a physical change, such as melting or polymorphic phase transition, can be obtained.

Calibration of the apparatus**1. Temperature calibration for DTA and DSC**

Temperature calibration for DTA and/or DSC apparatus can be performed by using reference substances having an intrinsic thermal property, such as melting point of pure metals or organic substances, or phase transition point of crystalline inorganic salts or oxides. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

2. Heat-quantity calibration for DSC

For accurate estimation of a quantity of heat change (enthalpic change) of a sample specimen, caused by a certain physical change accompanying a temperature change, it is necessary to calibrate the apparatus by using appropriate reference substances. As indicated in the section of Temperature calibration, heat-quantity calibration for DSC apparatus can be performed by using appropriate reference substances having a known definite enthalpic change caused by such physical changes as melting of pure metals and/or organic substances, or phase transition of crystalline inorganic salts. Melting points of Indium for thermal analysis and/or Tin for thermal analysis are usually employed for calibration.

Notes on operating conditions

When DTA or DSC measurements are made, the following items must be recorded: sample size, discrimination of open or closed-type sample container, heating or cooling rate, measuring temperature range, and kind and flow rate of atmospheric gas.

Method 2 Thermogravimetry (TG)

Apparatus The construction of a TG apparatus is fundamentally similar to that of DTA or DSC apparatus. However, the detector for TG is a balance, called a thermobalance, which can be classified to hanging-, Roberval's-,

and horizontal-type balances. The TG apparatus is designed to detect small mass changes of a specimen, placed at a fixed position on a thermobalance, caused by temperature change of the furnace under a controlled temperature program. Mass change with time and/or temperature is recorded continuously.

Procedure

A specimen is put in a sample container, which is placed at a fixed position of the thermobalance, then the heating furnace is run under a controlled temperature program. During this temperature change of the furnace, the mass change of a specimen with time and/or temperature is recorded continuously. Apparatus equipped with a data-processor is operated according to the instruction manual provided with the instrument.

When TG is used as an alternative method for "Loss on Drying" or "Water Determination", the measurement starts at room temperature and ends at a temperature above which no further mass change due to drying and/or vaporization of water can be observed. The standard heating rate is usually 5°C/min, and a linear heating program is recommended. However heating conditions (rate and time span) can be changed as necessary, depending on the kind of specimen and the extent of the measuring temperature range. Further, in TG measurement, dry air or dry nitrogen is usually passed through the heating furnace to ensure rapid elimination of evolved water or other volatile components and to avoid the occurrence of any chemical reaction, such as oxidation. By analyzing the TG curve plotted against time and/or temperature, absolute mass change and/or relative mass change with respect to the initial quantity(%) is obtained.

When the mass change caused by oxidation or degradation of a specimen is measured, a specific temperature range has to be determined beforehand so that stable baselines can be obtained before and after a targeted chemical reaction. Subsequent operating procedures are the same as described above.

Calibration of the apparatus**1. Temperature calibration**

The Curie temperature of a ferromagnetic substance such as pure Nickel can be used for temperature calibration for TG, based on the occurrence of an apparent mass change at the Curie point. In the case of a TG apparatus capable of simultaneously conducting DSC and DTA, the same reference substances as those for DTA and DSC can be adopted.

2. Scale calibration and confirmation

The scale calibration for TG must be done by using reference masses for chemical balances and/or semimicrobalances in the appropriate range. This is called a primary scale calibration, and is performed under ordinary temperature and pressure when the apparatus is set up initially and periodically, thereafter.

In usual measurement by TG, scale calibration or confirmation is done by using Calcium Oxalate Monohydrate Reference Standard to take account of such effects as buoyancy and convection due to atmospheric gas flow in the real measurement state. This is called secondary scale calibration, and is performed under the standard operation conditions stated below by using the above-mentioned Reference Standard, with a certified water content. When the difference of water content between the measured value and the certified one for the Reference Standard is less than 0.3%, normal

operation of the apparatus is confirmed. However, when the difference is more than 0.3%, scale calibration for TG must be done, based on the certified water content of the Reference Standard.

The standard operation conditions are as follows,

Amount of Calcium Oxalate Monohydrate Reference Standard: 0.01 g

Heating rate: 5°C/min

Temperature range: from room temperature to 250°C

Atmospheric gas: dried Nitrogen or dried Air

Flow rate of atmospheric gas,

hanging- or Roberval's-type balance: 40 mL/min

horizontal-type balance: 100 mL/min

Notes on operating conditions

In TG measurement, the following operation conditions must be recorded: sample size, heating rate, temperature range, kind and flow rate of atmospheric gas, etc.

2.53 Viscosity Determination

Viscosity Determination is a method to determine the viscosity of liquid samples using a viscometer.

When a liquid moves in a definite direction, and the liquid velocity has a gradient with respect to the direction rectangular to that of flow, a force of internal friction is generated along both sides of a hypothetical plane parallel to the movement. This flow property of a liquid is expressed in terms of viscosity. The internal friction per unit area on the parallel plane is called slip stress or shear stress, and the velocity gradient with respect to the direction rectangular to that of flow is called slip velocity or shear velocity. A liquid of which the slip velocity is proportional to its slip stress is called a Newtonian liquid. The proportionality constant, η , is a characteristic of a liquid at a certain temperature and is called viscosity. The viscosity is expressed in the unit of Pascal second (Pa·s), and usually milli-Pascal second (mPa·s).

A liquid whose slip velocity is not proportional to its slip stress is called a non-Newtonian liquid. Since the viscosity for a sample of a non-Newtonian liquid changes with its slip velocity, the viscosity measured at a certain slip velocity is called an apparent viscosity. In that case, the value of slip stress divided by the corresponding slip velocity is called an apparent viscosity. Thus, the relationship between apparent viscosity and slip velocity will permit characterization of the flow properties of a given non-Newtonian liquid.

The value of the viscosity, η , divided by the density, ρ , at the same temperature is defined as a kinematic viscosity, ν , which is expressed in the unit of meters squared per second (m²/s), and usually millimeters squared per second (mm²/s).

The viscosity of a liquid is determined either by the following *Method I* or *Method II*.

Method I Viscosity measurement by capillary tube viscometer

For measuring the viscosity of a Newtonian liquid, a capillary tube viscometer is usually used, in which the downflowing time of a liquid, t (s), required for a definite volume of the liquid to flow through a capillary tube is measured and the kinematic viscosity, ν , is calculated according to the following equation.

$$\nu = Kt$$

Further, the viscosity, η , is calculated from the next equation:

$$\eta = \nu\rho = Kt\rho$$

where ρ (g/mL) is the density of the liquid measured at the same temperature, t (°C).

The parameter K (mm²/s²) represents the viscometer constant and is previously determined by using the *Standard Liquids for Calibrating Viscometers* with known kinematic viscosity. In the case of a liquid having a similar viscosity to water, water itself can be used as a reference standard liquid for the calibration. The kinematic viscosity of water is 1.0038 mm²/s at 20°C. In the cases of liquids having a slightly higher viscosity than water, the *Standard Liquids for Calibrating Viscometers* should be used for the calibration.

The intrinsic viscosity, $[\eta]$ (dL/g), of a polymer solution is obtained by plotting the relation of viscosity versus concentration and extrapolating the obtained straight line to zero concentration. Intrinsic viscosity shows the degree of molecular expansion of a polymer substance in a given solvent (sample solution) and is also a measure of the average molecular mass of the polymer substance.

The downflowing time t (s) for a polymer solution, whose concentration is c (g/dL), and t_0 (s) for the solvent used for dissolving the polymer, are measured by using the same viscometer, and then the intrinsic viscosity of a given polymer substance, $[\eta]$, is calculated according to the following equation:

$$[\eta] = \lim_{c \rightarrow 0} \frac{\left(\frac{t}{t_0}\right) - 1}{c} \quad \text{or} \quad [\eta] = \lim_{c \rightarrow 0} \frac{\ln \frac{t}{t_0}}{c}$$

When the concentration dependency of $\{(t/t_0) - 1\}/c$ is not large, the value of $\{(t/t_0) - 1\}/c$ at a concentration directed in the respective monograph can be assumed to be the intrinsic viscosity for a given substance.

Unless otherwise specified, the viscosity of a sample solution is measured with the following apparatus and procedure.

Apparatus

For measurement of the kinematic viscosity in the range of 1 to 100,000 mm²/s, the Ubbelohde-type viscometer illustrated in Fig. 2.53-1 can be used. The approximate relations between kinematic viscosity range and inside diameter of the capillary tube suitable for the measurement of various liquids with different viscosity, are given in Table 2.53-1. Although a capillary tube viscometer other than the Ubbelohde-type one specified in Table 2.53-1 can also be used, a viscometer should be selected in which the downflowing time, t (s), of a sample solution to be determined would be between 200 s and 1000 s.

Procedure

Place a sample solution in a viscometer from the upper end of *tube 1*, so that the meniscus of the solution is at a level between the two marked lines of *bulb A*. Place the viscometer vertically in a thermostatted bath maintained at a specified temperature within 0.1°C, until *bulb C* is fully immersed, and let it stand for about 20 minutes to attain the specified temperature. Close *tube 3* with a finger and pull the sample solution up to the middle part of *bulb C* by gentle suction from the top of *tube 2*, taking care not to introduce any bubbles into *tube 2*, and stop the suction. Open the end of *tube 3*,

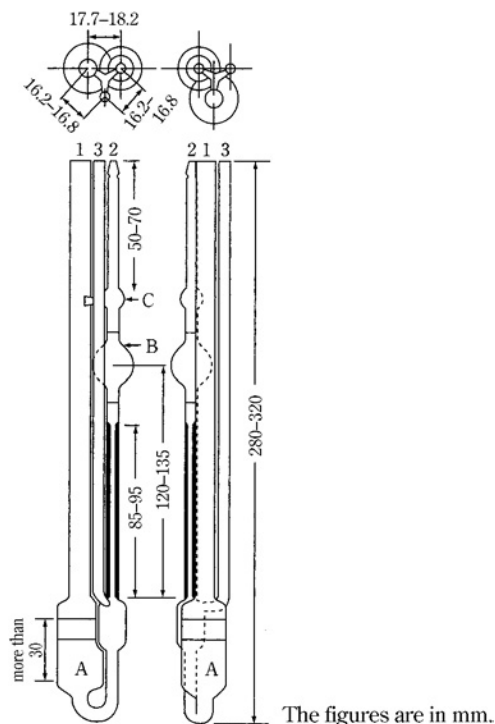


Fig. 2.53-1 Capillary tube viscometer

and immediately close the end of tube 2. After confirming

Table 2.53-1 Specifications of the Ubbelohde-type viscometer

Viscometer constant K (mm^2/s^2)	Inner diameter of capillary tube (mm) Permissible tolerance $\pm 10\%$	Volume of bulb B (mL) Permissible tolerance $\pm 10\%$	Measuring range of kinematic viscosity (mm^2/s)
0.005	0.46	3.0	1 - 5
0.01	0.58	4.0	2 - 10
0.03	0.73	4.0	6 - 30
0.05	0.88	4.0	10 - 50
0.1	1.03	4.0	20 - 100
0.3	1.36	4.0	60 - 300
0.5	1.55	4.0	100 - 500
1.0	1.83	4.0	200 - 1000
3.0	2.43	4.0	600 - 3000
5.0	2.75	4.0	1000 - 5000
10.0	3.27	4.0	2000 - 10,000
30.0	4.32	4.0	6000 - 30,000
50.0	5.20	5.0	10,000 - 50,000
100	6.25	5.0	20,000 - 100,000

that the liquid column is cut off at the lowest end of the capillary tube, open the end of tube 2 to make the sample solution flow down through the capillary tube. Record the time, t (s), required for the meniscus of the sample solution to fall from the upper to the lower marked line of bulb B.

Determine the viscometer constant K previously, using the *Standard Liquids for Calibrating Viscometers* under the same conditions. The temperature at which the calibration is conducted must be identical with that specified in the monograph.

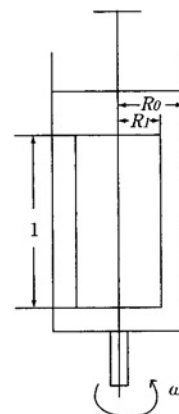


Fig. 2.53-2a Coaxial double cylinder-type rotational viscometer

Method II Viscosity measurement by rotational viscometer

A rotational viscometer is usually used for measuring the viscosity of Newtonian or non-Newtonian liquids. The measuring principle of a rotational viscometer generally consists in the detection and determination of the force acting on a rotor (torque), when it rotates at a constant angular velocity in a liquid. The extent of torque generated by the rotation can be detected in terms of the torsion of a spring and the liquid viscosity is calculated from the scale-indicated value corresponding to the degree of torsion.

The viscosity of a sample solution is measured with the following apparatus and procedure.

Apparatus

Viscosity measurement is performed by using any one of the following three types of rotational viscometers.

(1) Coaxial double cylinder-type rotational viscometer (Couette type viscometer)

In the coaxial double cylinder-type rotational viscometer, viscosity is determined by placing a liquid in the gap between the inner and the outer cylinders, which share the same central axis and rotate separately, and the generated torque acting on one cylinder surface when the other cylinder is rotated, and the corresponding angular velocity, are measured.

As shown in Fig. 2.53-2a, the inner cylinder is hung by a wire whose twist constant is designated as k . In Fig. 2.53-2a, half the outer diameter of the inner cylinder and inner diameter of the outer cylinder are designated as R_i and R_o , respectively, and the length of the inner cylinder immersed in a liquid is designated as l . When a liquid is introduced into the gap between the two cylinders, and the outer cylinder is made to rotate at a constant angular velocity, ω , the inner cylinder is also forced to rotate due to the viscosity of the liquid. Consequently, torque, T , is generated by the forced rotation in a viscous liquid, and in the steady state the torque is balanced by the torsion of the wire, as indicated by the degree of rotation θ . Then, the relationship can be expressed by $T = k\theta$ and the viscosity of a liquid, η , is determined from the following equation by measuring the relationship between ω and θ . Conversely, viscosity measurement can also be performed by rotating the inner cylinder, and the same relationship holds.

$$\eta = \frac{100T}{4\pi l\omega} \left[\frac{1}{R_i^2} - \frac{1}{R_o^2} \right]$$

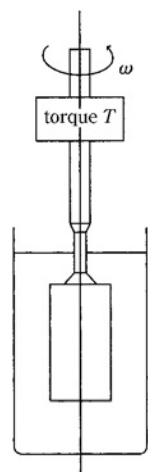


Fig. 2.53-2b Single cylinder-type rotational viscometer

- η : viscosity of a liquid (mPa·s)
 π : circumference/diameter ratio
 l : length of the inner cylinder (cm)
 ω : angular velocity (rad/s)
 T : torque acting on cylinder surface (10^{-7} N·m)
 R_i : 1/2 of outer diameter of the inner cylinder (cm)
 R_o : 1/2 of inner diameter of the outer cylinder (cm)

(2) Single cylinder-type rotational viscometer (Brookfield type viscometer)

In the single cylinder-type rotational viscometer, viscosity is determined by measuring the torque acting on the cylinder surface when the cylinder immersed in a liquid is rotated at a given angular velocity. Use an apparatus of the type illustrated in Fig. 2.53-2b. If the apparatus constant K_B is previously determined experimentally by using the *Standard Liquids for Calibrating Viscometers*, the viscosity of a liquid, η , can be obtained from the following equation.

$$\eta = K_B \frac{T}{\omega}$$

- where, η : viscosity of a liquid (mPa·s)
 K_B : apparatus constant of viscometer (rad/cm³)
 ω : angular velocity (rad/s)
 T : torque acting on cylinder surface (10^{-7} N·m)

(3) Cone-flat plate-type rotational viscometer (Cone-plate type viscometer)

In the cone-flat plate-type rotational viscometer, viscosity is determined by placing a liquid in the gap between a flat disc and a cone with a large vertical angle sharing the same rotational axis, and the torque and the corresponding angular velocity are measured, when either the disc or the cone is rotated in a viscous liquid.

As shown in Fig. 2.53-2c, a liquid is introduced to fill the gap between a flat disc and a cone forming an angle α (rad). When either the flat disc or the cone is rotated at a constant angular velocity or a constant torque, the torque acting on the disc or cone surface rotated by the viscous flow and the corresponding angular velocity in the steady state, are measured. The viscosity of the liquid, η , can be calculated from the following equation.

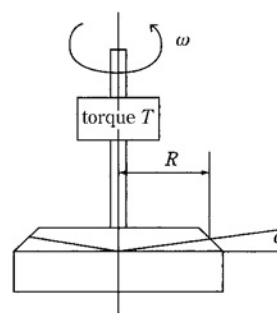


Fig. 2.53-2c Cone-flat plate-type rotational viscometer

$$\eta = \frac{3\alpha}{2\pi R^3} \cdot \frac{100T}{\omega}$$

- η : viscosity of a liquid (mPa·s)
 π : circumference/diameter ratio
 R : radius of cone (cm)
 α : angle between flat disc and cone (rad)
 ω : angular velocity (rad/s)
 T : torque acting on flat disc or cone surface (10^{-7} N·m)

Procedure

Set up the viscometer so that its rotational axis is perpendicular to the horizontal plane. Place a sufficient quantity of a sample solution in the viscometer, and allow the measuring system to stand until a specified temperature is attained, as directed in the monograph. Where it is desired to measure the viscosity within a precision of 1%, measuring temperature should be controlled within 0.1°C. Next, after confirming that the sample solution is at the designated temperature, start operating the rotational viscometer. After the forced rotation induced by the viscous flow has reached a steady state and the indicated value on the scale, which corresponds to the rotational frequency or the torque, has become constant, read the value on the scale. Then, calculate the viscosity η by using the respective equation appropriate to the type of viscometer being used. Determination or confirmation of the apparatus constant should be conducted beforehand by using the *Standard Liquids for Calibrating Viscometers*, and the validation of the apparatus and operating procedure should also be performed by using those standard liquids.

In the case of a non-Newtonian liquid, repeat the procedure for measuring the viscosity of the liquid with variation of the rotation velocity or torque from one measurement to another. From a series of such viscosity measurements, the relationship between the slip velocity and the slip stress of a non-Newtonian liquid, *i.e.*, the flow characteristics of a non-Newtonian liquid, can be obtained.

Calibration of a rotational viscometer is conducted by using water and the *Standard Liquids for Calibrating Viscometers*. These standard liquids are used for the determination or confirmation of the apparatus constant of the rotational viscometer. They are also used for periodic recalibration of the viscometer to confirm maintenance of a specified precision.

2.54 pH Determination

pH is defined as the reciprocal of the common logarithm of hydrogen ion activity, which is the product of hydrogen ion concentration and the activity coefficient. Conventionally it is used as a scale of hydrogen ion concentration of a sample solution.

pH of a sample solution is expressed by the following equation in relation to the pH of a standard solution (pH_s), and can be measured by a pH meter using a glass electrode.

$$\text{pH} = \text{pH}_s + \frac{E - E_s}{2.3026 RT/F}$$

pH_s: pH value of a pH standard solution.

E: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a sample solution:

Glass electrode | sample solution | reference electrode

*E*_s: Electromotive force (volt) induced on the following galvanic cell composed of a glass electrode and suitable reference electrode in a pH standard solution:

Glass electrode | standard pH solution | reference electrode

R: Gas constant

T: Absolute temperature

F: Faraday's constant

The value of $2.3026 RT/F$ (V) in the above equation means the degree of electromotive force (V) per one pH unit and it is dependent on the temperature as shown in Table 2.54-1:

Table 2.54-1 Temperature dependency of the electromotive force (V)

Temperature of solution (°C)	2.3026 <i>RT/F</i> (V)	Temperature of solution (°C)	2.3026 <i>RT/F</i> (V)
5	0.05519	35	0.06114
10	0.05618	40	0.06213
15	0.05717	45	0.06313
20	0.05817	50	0.06412
25	0.05916	55	0.06511
30	0.06015	60	0.06610

pH Standard solution

The pH standard solutions are used as a standard of pH, for standardization of a pH meter. To prepare water for preparation of the pH standard solutions, distill purified water, boil the distillate for more than 15 minutes, and cool in a container fitted with a carbon dioxide-absorbing tube (soda lime). Next, prepare individually 6 kinds of pH standard solutions shown in Table 2.54-2.

Store the pH standard solutions in hard glass or polyethylene bottles. For storage of alkaline pH standard solutions, it is preferable to use a bottle fitted with a carbon dioxide-absorbing tube. Since the pH may change gradually during storage over a long period, it is necessary to ascertain whether the expected pH value is held or not by comparison with newly prepared standard, when the solution is used after long storage.

(1) Oxalate pH standard solution—Pulverize potassium trihydrogen dioxalate dihydrate for pH determination, and

dry in a desiccator (silica gel). Weigh 12.71 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(2) Phthalate pH standard solution—Pulverize potassium hydrogen phthalate for pH determination, and dry at 110 °C to constant mass. Weigh 10.21 g (0.05 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(3) Phosphate pH standard solution—Pulverize potassium dihydrogen phosphate for pH determination and disodium hydrogen phosphate for pH determination, and dry at 110 °C to constant mass. Weigh 3.40 g (0.025 mole) of potassium dihydrogen phosphate and 3.55 g (0.025 mole) of disodium hydrogen phosphate dodecahydrate accurately, and dissolve in water to make exactly 1000 mL.

(4) Borate pH standard solution—Allow sodium tetraborate for pH determination to stand in a desiccator (saturated sodium bromide aqueous solution) until it reaches constant mass. Weigh 3.81 g (0.01 mole) of it accurately, and dissolve in water to make exactly 1000 mL.

(5) Carbonate pH standard solution—Dry sodium hydrogen carbonate for pH determination in a desiccator (silica gel) to constant mass, and weigh 2.10 g (0.025 mole) of it accurately. Dry sodium carbonate for pH determination between 300 °C and 500 °C to constant mass, and weigh 2.65 g (0.025 mole) of it accurately. Dissolve both reagents in water to make exactly 1000 mL.

(6) Calcium hydroxide pH standard solution—Reduce calcium hydroxide for pH determination to a fine powder, transfer 5 g to a flask, add 1000 mL of water, shake well, and allow the solution to become saturated at a temperature between 23 °C and 27 °C. Then filter the supernatant at the same temperature and use the clear filtrate (about 0.02 mol/L).

The pH values of these pH standard solutions at various temperatures are shown in the *Table* below. pH values at an arbitrary temperature not indicated in Table 2.54-2 can be calculated by the interpolation method.

Apparatus

A pH meter generally consists of an electrode system of a glass electrode and a reference electrode, an amplifier and an indicating unit for controlling the apparatus and for displaying the measured value of electromotive force. The indicating unit is usually fitted with dials for zero and span (sensitivity) adjustment. Sometimes a temperature compensation dial is included.

The reproducibility of a pH meter should be within 0.05 pH unit, when measurements for an arbitrary pH standard solution are repeated five times, following the procedure described below. After each measurement it is necessary to wash the detecting unit well with water.

Procedure

Immerse the glass electrode previously in water for more than several hours. Start the measurement after confirming stable running of the apparatus. Rinse well the detecting unit with water, and remove the remaining water gently with a piece of filter paper.

To standardize the pH meter, two pH standard solutions are usually used as follows. Immerse the detection unit in the phosphate pH standard solution and adjust the indicated pH to the pH value shown in the *Table*. Next, immerse the detection system in the second pH standard solution, which should be selected so that the expected pH of the sample solution to be determined is between the pH values of the two pH standard solutions, and measure the pH under the same condi-

Table 2.54-2 pH values of six pH standard solutions

Temperature (°C)	Oxalate pH standard solution	Phthalate pH standard solution	Phosphate pH standard solution	Borate pH standard solution	Carbonate pH standard solution	Calcium hydroxide pH standard solution
0	1.67	4.01	6.98	9.46	10.32	13.43
5	1.67	4.01	6.95	9.39	10.25	13.21
10	1.67	4.00	6.92	9.33	10.18	13.00
15	1.67	4.00	6.90	9.27	10.12	12.81
20	1.68	4.00	6.88	9.22	10.07	12.63
25	1.68	4.01	6.86	9.18	10.02	12.45
30	1.69	4.01	6.85	9.14	9.97	12.30
35	1.69	4.02	6.84	9.10	9.93	12.14
40	1.70	4.03	6.84	9.07		11.99
50	1.71	4.06	6.83	9.01		11.70
60	1.73	4.10	6.84	8.96		11.45

tions as used for the first pH standard solution. Adjust the indicated pH to the defined pH value using the span adjustment dial, when the observed pH is not identical with that tabulated. Repeat the above standardization procedure until both pH standard solutions give observed pH values within 0.02 pH unit of the tabulated value without further adjustments. When a pH meter is fitted with a temperature compensation dial, the standardization procedure is done after the setting of the temperature to that of the pH standard solution to be measured.

In the case of using an apparatus having an auto-calibration function, it is necessary to confirm periodically that the pH values of two pH standard solutions are identical with the tabulated values within 0.05 pH unit.

After finishing the standardization procedure described above, rinse well the electrodes with water, remove the attached water using a filter paper, immerse the electrode system in the sample solution, and read the indicated pH value after confirming the value is stable. If necessary, a sample solution can be agitated gently.

In the pH determination, the temperature of a sample solution must be controlled to be the same as that of the pH standard solutions with which the pH meter was standardized (within 2°C). When a sample solution is alkaline, the measurement should be done in a vessel with a cover and if necessary, in a stream of inert gas such as nitrogen. Furthermore for a strongly alkaline solution above pH 11 containing alkali metal ions, an alkali error may be induced in the pH measurement. Thus, in such a case, an electrode with less alkali error should be used and an appropriate correction should be applied to the measured value.

Note: Construction and treatment in detail are different for different pH meters.

2.55 Vitamin A Assay

Vitamin A Assay is a method to determine vitamin A in Retinol Acetate, Retinol Palmitate, Vitamin A Oil, Cod Liver Oil and other preparations. Method 1 is for the assay of synthetic vitamin A esters, using the ultraviolet-visible spectrophotometry (Method 1-1) or the liquid chromatography (Method 1-2). Method 2 is for the assay of vitamin A of natural origin, containing many geometrical isomers, using

the ultraviolet-visible spectrophotometry to determine vitamin A as vitamin A alcohol obtained by saponification in an alkaline solution and extraction.

One Vitamin A Unit (equal to 1 vitamin A I.U.) is equivalent to 0.300 µg of vitamin A (all-*trans* vitamin A alcohol).

Procedure

All procedures should be carried out quickly and care should be taken as far as possible to avoid exposure to light, air, oxidants, oxidizing catalysts (e.g. copper, iron), acids and heat. If necessary, light-resistant vessels may be used.

Generally, for synthetic vitamin A esters apply Method 1-1 or Method 1-2, but if the assay conditions required for Method 1-1 are not suitable, apply Method 2.

Method 1-1

Weigh accurately about 0.1 g of the sample, and dissolve in 2-propanol for vitamin A assay to make exactly 50 mL. Dilute this solution with 2-propanol for vitamin A assay to make a solution so that each mL contains 10 to 15 vitamin A Units, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution between 220 nm and 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> to obtain the wavelength of the maximum absorption and the absorbances at 300 nm, 310 nm, 320 nm, 326 nm, 330 nm, 340 nm and 350 nm. When the maximum absorption lies between 325 nm and 328 nm, and the ratios, A_{λ_i}/A_{326} , of each absorbance, A_{λ_i} , at 300 nm, 310 nm, 320 nm, 330 nm, 340 nm and 350 nm to the absorbance, A_{326} , at 326 nm are within the range of ± 0.030 of the values in the Table, the potency of vitamin A in Units per g of the sample is calculated from the following equation.

$$\text{Units of vitamin A in 1 g} = \frac{A_{326}}{W} \times \frac{V}{100} \times 1900$$

A_{326} : Absorbance at 326 nm

V : Total volume (mL) of the sample solution

W : Amount (g) of sample in V mL of the sample solution
1900: Conversion factor from specific absorbance of retinol ester to IU (Unit/g)

This method is applied to drugs or preparations containing vitamin A esters (retinol acetate or retinol palmitate) as the main component. However, when the wavelength of maximum absorption does not lie between 325 nm and 328 nm, or when the absorbance ratio A_{λ_i}/A_{326} is not within the range of ± 0.030 of the values in Table 2.55-1, apply Method 2.

Table 2.55-1 Absorbance Ratio, A_{λ_i}/A_{326} , of retinol acetate and retinol palmitate

λ_i (nm)	A_{λ_i}/A_{326}	
	Retinol acetate	Retinol palmitate
300	0.578	0.590
310	0.815	0.825
320	0.948	0.950
330	0.972	0.981
340	0.786	0.795
350	0.523	0.527

Method 1-2

Proceed with an appropriate amount of sample as directed under Liquid Chromatography <2.01>.

For the assay of retinol acetate and retinol palmitate use Retinol Acetate Reference Standard and Retinol Palmitate

Reference Standard, respectively, and fix appropriately the operating procedure, the operating conditions and the system suitability based on the characteristics of the substance to be tested and the species and amount of coexisting substances.

Method 2

Unless otherwise specified, weigh accurately a sample containing not less than 500 Units of vitamin A, and not more than 1 g of fat, transfer to a flask, and add 30 mL of aldehyde-free ethanol and 1 mL of a solution of pyrogallol in ethanol (95) (1 in 10). Then add 3 mL of a solution of potassium hydroxide (9 in 10), attach a reflux condenser, and heat on a water bath for 30 minutes to saponify. Cool quickly to ordinary temperature, add 30 mL of water, transfer to a separator A, wash the flask with 10 mL of water and then 40 mL of diethyl ether, transfer the washings to the separator A, shake well, and allow to stand. Transfer the water layer so obtained to a separator B, wash the flask with 30 mL of diethyl ether, add the washing to the separator B, and extract by shaking. Transfer the water layer to a flask, add the diethyl ether layer to the separator A, transfer the water layer in the flask to the separator B, add 30 mL of diethyl ether, and extract by shaking. Transfer the diethyl ether layer so obtained to the separator A, add 10 mL of water, allow the separator A to stand after gentle turning upside-down 2 or 3 times, and remove the water layer. Wash the content of the separator A with three 50-mL portions of water with increasingly vigorous shaking as the washing proceeds. Further wash with 50-mL portions of water until the washing no longer shows a pink color with phenolphthalein TS, and allow to stand for 10 minutes. Remove remaining water as far as possible, transfer the diethyl ether to an Erlenmeyer flask, wash the separator with two 10-mL portions of diethyl ether, add the washings to the flask, add 5 g of anhydrous sodium sulfate to the flask, mix by shaking, and transfer the diethyl ether to a round-bottomed flask by decantation. Wash the remaining sodium sulfate in the flask with two or more 10-mL portions of diethyl ether, and transfer the washings to the flask. Evaporate the diethyl ether in a water bath at 45°C while swirling the flask, using an aspirator, to about 1 mL, immediately add an exactly measured amount of 2-propanol for vitamin A assay to make a solution containing 6 to 10 vitamin A Units per mL, and designate the solution as the sample solution. Determine the absorbances, A_{310} at 310 nm, A_{325} at 325 nm, and A_{334} at 334 nm, of the sample solution as directed under Ultraviolet-visible Spectrophotometry.

Units of vitamin A in 1 g of the sample

$$= \frac{A_{325}}{W} \times \frac{V}{100} \times f \times 1830$$

$$f = 6.815 - 2.555 \times \frac{A_{310}}{A_{325}} - 4.260 \times \frac{A_{334}}{A_{325}}$$

A_{325} : Absorbance at 325 nm

V : Total volume (mL) of the sample solution

W : Amount (g) of sample in V mL of the sample solution

f : Correction factor

1830: Conversion factor from specific absorbance of retinol alcohol to IU (Unit/g)

2.56 Determination of Specific Gravity and Density

The density ρ (g/mL or g/cm³) means the mass per unit volume, and the relative density means the ratio of the mass of a sample specimen to that of an equal volume of a standard substance. The relative density is also called the specific gravity.

The specific gravity, d'_i , means the ratio of the mass of the sample specimen at t' °C to that of an equal volume of water (H₂O) at t °C. Unless otherwise specified, the measurement is to be performed by Method 1, Method 2 or Method 4. When the specified value is accompanied with the term "about" in the monograph, Method 3 is also available.

Method 1. Measurement using a pycnometer

A pycnometer is a glass vessel with a capacity of usually 10 mL to 100 mL, having a ground-glass stopper fitted with a thermometer, and a side inlet-tube with a marked line and a ground-glass cap.

Weigh a pycnometer, previously cleaned and dried, to determine its mass W . Remove the stopper and the cap. Fill the pycnometer with the sample solution, keeping them at a slightly lower temperature by 1°C to 3°C than the specified temperature t' °C, and stopper them, taking care not to leave bubbles. Raise the temperature gradually, and when the thermometer shows the specified temperature, remove the portion of the sample solution above the marked line through the side tube, cap the side tube, and wipe the outside surface thoroughly. Measure the mass W_1 of the pycnometer filled with the sample solution. Perform the same procedure, using the same pycnometer containing water, and note the mass W_2 at the specified temperature t °C. The specific gravity d'_i can be calculated by use of the following equation.

$$d'_i = \frac{W_1 - W}{W_2 - W}$$

Further, when measurements for a sample solution and water are performed at the same temperature (t °C = t' °C), the density of the sample solution at the temperature t' °C (ρ'_T) can be calculated from the measured specific gravity d'_i and the density of water at the temperature t' °C (ρ'_{S1}) indicated in Table 2.56-1 by using the following equation.

$$\rho'_T = \rho'_{S1} d'_i$$

Method 2. Measurement using a Sprengel-Ostwald pycnometer

A Sprengel-Ostwald pycnometer is a glass vessel with a capacity of usually 1 mL to 10 mL. As shown in Fig. 2.56-1, both ends are thick-walled fine tubes (inside diameter: 1 - 1.5 mm, outside diameter: 3 - 4 mm), one of which, tube A, has a line C marked on it. Determine the mass of a pycnometer, W , previously cleaned and dried, by hanging it on the arm of a chemical balance with a platinum or aluminum wire D. Immerse the fine tube B in the sample solution, which is at a lower temperature by 3°C to 5°C than the specified temperature t' °C. Attach rubber tubing or a ground-glass tube to the end of A, and suck up the sample solution until the meniscus is above the marked line C, taking care to prevent bubble formation. Immerse the pycnometer in a water bath kept at the specified temperature t' °C for about 15 minutes, and then,

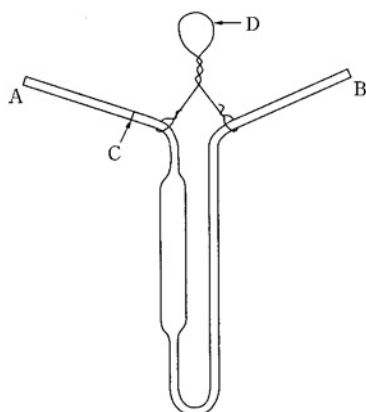


Fig. 2.56-1 Sprengel-Ostwald pycnometer

by attaching a piece of filter paper to the end of B, adjust the level of the sample solution to the marked line C. Take the pycnometer out of the water bath, wipe thoroughly the outside surface and determine the mass W_1 . By use of the same pycnometer, perform the same procedure for the standard solution of water. Weigh the pycnometer containing water at the specified temperature $t^\circ\text{C}$, and note the mass W_2 . Calculate the specific gravity d'_s , according to the equation described in Method 1.

Further, when measurements of specific gravity for a sample solution and water are performed at the same temperature ($t^\circ\text{C} = t^\circ\text{C}$), the density of sample solution at temperature $t^\circ\text{C}$ can be calculated by using the equation described in Method 1.

Method 3. Measurement using a hydrometer

Clean a hydrometer with ethanol (95) or diethyl ether. Stir the sample well with a glass rod, and float the hydrometer in the well. When the temperature is adjusted to the specified temperature $t^\circ\text{C}$ and the hydrometer comes to a standstill, read the specific gravity d'_s or the density ρ'_T at the upper brim of the meniscus. Here the temperature $t^\circ\text{C}$ indicates the temperature at which the hydrometer is calibrated. If specific instructions for reading the meniscus are supplied with the hydrometer, the reading must be in accordance with the instructions.

Further, when measurement of the specific gravity for a sample solution is performed at the same temperature ($t^\circ\text{C} = t^\circ\text{C}$), at which the hydrometer is calibrated, the density of a sample solution at $t^\circ\text{C}$, ρ'_T , can be calculated by using the specific gravity d'_s and the equation shown in Method 1.

Method 4. Measurement using an oscillator-type density meter

Density measurement with an oscillator-type density meter is a method for obtaining the density of liquid or gas by measuring the intrinsic vibration period T (s) of a glass tube cell filled with sample specimen. When a glass tube containing a sample is vibrated, it undergoes a vibration with an intrinsic vibration period T in proportion to the mass of the sample specimen. If the volume of the vibrating part of the sample cell is fixed, the relation of intrinsic oscillation period and density of the sample specimen shall be linear.

Before measuring a sample density, the respective intrinsic oscillation periods T_{S1} and T_{S2} for two reference substances

(density: ρ_{S1} , ρ_{S2}) must be measured at a specified temperature $t^\circ\text{C}$, and the cell constant K_r ($\text{g}\cdot\text{cm}^{-3} \text{ s}^{-2}$) must be determined by using the following equation.

$$K_r = \frac{\rho'_{S1} - \rho'_{S2}}{T_{S1}^2 - T_{S2}^2}$$

Usually, water and dried air are chosen as reference substances. Here the density of water at $t^\circ\text{C}$, ρ'_{S1} , is taken from the attached Table, and that of dried air ρ'_{S2} is calculated by using the following equation, where the pressure of dried air is at p kPa.

$$\rho'_{S2} = 0.0012932 \times \{273.15/(273.15 + t')\} \times (p/101.325)$$

Next, introduce a sample specimen into a sample cell having a cell constant K_r , the intrinsic vibration period, T_T , for the sample under the same operation conditions as employed for the reference substances. The density of a sample specimen at $t^\circ\text{C}$, ρ'_T , is calculated by use of the following equation, by introducing the intrinsic oscillation period T_{S1} and the density of water at a specified temperature $t^\circ\text{C}$, ρ'_{S1} , into the equation.

$$\rho'_T = \rho'_{S1} + K_r (T_T^2 - T_{S1}^2)$$

Further, the specific gravity of a sample specimen d'_s against water at a temperature $t^\circ\text{C}$ can be obtained by using the equation below, by introducing the density of water at a temperature $t^\circ\text{C}$, ρ'_{S1} , indicated in Table 2.56-1.

$$d'_s = \frac{\rho'_T}{\rho'_{S1}}$$

Apparatus

An oscillator-type density meter is usually composed of a glass tube cell of about 1 mL capacity, the curved end of which is fixed to the vibration plate, an oscillator which applies an initial vibration to the cell, a detector for measuring the intrinsic vibration period, and a temperature controlling system.

A schematic illustration of the apparatus is depicted in Fig. 2.56-2.

Procedure

A sample cell, water, and a sample specimen are previously adjusted to a specified temperature $t^\circ\text{C}$. Wash the sample cell with water or an appropriate solvent, and dry it thoroughly with a flow of dried air. Stop the flow of dried air, confirm that the temperature is at the specified value, and then measure the intrinsic oscillation period T_{S2} given by the dried air. Separately, the atmospheric pressure p (kPa) must be measured at the time and place of the examination. Next, introduce water into the sample cell and measure the intrinsic oscillation period T_{S1} given by water. Using these values of the intrinsic oscillation period and the atmospheric pressure, the sample cell constant K_r can be determined by use of the above-mentioned equation.

Next, introduce a sample specimen into the glass cell, confirm the specified temperature, and measure the intrinsic oscillation period T_T given by the sample specimen. Using the intrinsic oscillation periods for water and the sample specimen, the density of water ρ'_{S1} , and the cell constant K_r , the density of the sample specimen ρ'_T can be obtained by use of the above equation. If necessary, the specific gravity of the sample specimen d'_s against water at a temperature $t^\circ\text{C}$, can be calculated by using the density of water ρ'_{S1} shown in

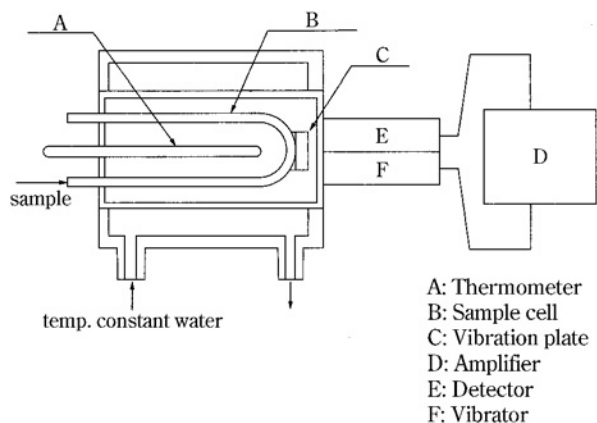
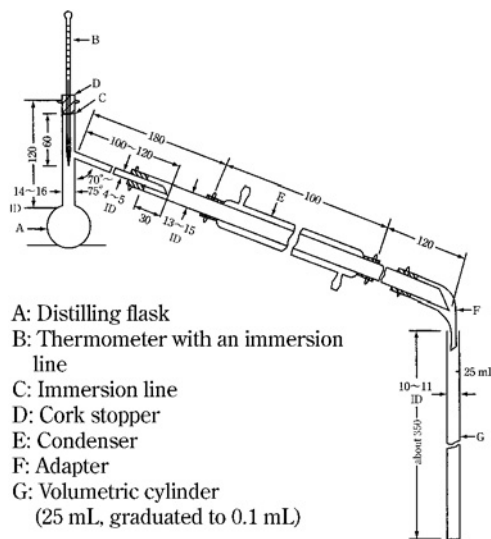


Fig. 2.56-2 Oscillator-type density meter



The figures are in mm.

Fig. 2.57-1

Table 2.56-1.

In this measurement, avoid the occurrence of bubble formation in the sample cell, when a sample specimen or water is introduced into the cell.

Table 2.56-1 Density of water

Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL	Temp. °C	Density g/mL
0	0.999 84	10	0.999 70	20	0.998 20	30	0.995 65
1	0.999 90	11	0.999 61	21	0.997 99	31	0.995 34
2	0.999 94	12	0.999 50	22	0.997 77	32	0.995 03
3	0.999 96	13	0.999 38	23	0.997 54	33	0.994 70
4	0.999 97	14	0.999 24	24	0.997 30	34	0.994 37
5	0.999 96	15	0.999 10	25	0.997 04	35	0.994 03
6	0.999 94	16	0.998 94	26	0.996 78	36	0.993 68
7	0.999 90	17	0.998 77	27	0.996 51	37	0.993 33
8	0.999 85	18	0.998 60	28	0.996 23	38	0.992 97
9	0.999 78	19	0.998 41	29	0.995 94	39	0.992 59
10	0.999 70	20	0.998 20	30	0.995 65	40	0.992 22

* In this Table, although the unit of density is represented by g/mL in order to harmonize with the unit expression in the text, it should be expressed in g/cm³ seriously.

2.57 Boiling Point and Distilling Range Test

The boiling point and distilling range are determined by Method 1 or Method 2 as described herein, unless otherwise specified. Boiling point is the temperature shown between when the first 5 drops of distillate leave the tip of the condenser and when the last liquid evaporates from the bottom of the flask. Distilling range test is done to determine the volume of the distillate which has been collected in the range of temperature directed in the monograph.

Method 1 This method is applied to a sample for which the permissible range of boiling temperature is smaller than 5°C.

(1) Apparatus

Use the apparatus illustrated in Fig. 2.57-1.

(2) Procedure

Measure 25 mL of the sample, whose temperature is previously noted, using a volumetric cylinder G graduated in 0.1

mL, and transfer it to a distilling flask A of 50- to 60-mL capacity. Use this cylinder as the receiver for the distillate without rinsing out any of the adhering liquid. Put boiling chips into the distilling flask A, insert a thermometer B with an immersion line so that its immersion line C is on a level with the lower end of cork stopper D and the upper end of its mercury bulb is located in the center of the delivery tube, and connect condenser E with the distilling flask A and adapter F with the condenser E. Insert the open end of F into the mouth of cylinder G (receiver) so that air can pass through slightly. Use a hood with a height sufficient to shield A, and heat A with a suitable heat source. When direct flame is applied as the heat source, put A on a hole of a fire-resistant, heat-insulating board [a board consisting of a fire-resistant, heat-insulating material, 150 mm square and about 6 mm thick (or a wire gauge of 150 mm square bonded to fire-resistant, heat-insulation materials in about 6 mm thickness), having in its center a round hole 30 mm in diameter].

Unless otherwise specified, distil the liquid sample by the application of heat, at a rate of 4 to 5 mL per minute of distillate in the case of liquids whose boiling temperature to be determined is lower than 200°C and at a rate of 3 to 4 mL per minute in the case of liquids whose boiling temperature is 200°C or over, and read the boiling point. For the distilling range test, bring the temperature of distillate to the temperature at which the volume was originally measured, and measure the volume of distillate.

Liquids that begin to distil below 80°C are cooled to between 10°C and 15°C before measuring the volume, and the receiving cylinder is kept immersed in ice up to a point 25 mm from the top during the distillation.

Correct the observed temperature for any variation in the barometric pressure from the normal (101.3 kPa), by allowing 0.1 degree for each 0.36 kPa of variation, adding if the pressure is lower, or subtracting if higher than 101.3 kPa.

Method 2 This method is applied to the sample for which the permissible range of boiling temperature is 5°C or more.

(1) Apparatus

The same apparatus as described in Method 1 is used.

However, use a 200-mL distilling flask A with a neck 18 to 24 mm in inside diameter having a delivery tube 5 to 6 mm in inside diameter. The fire-resistant, heat-insulating board used for direct flame heating should have in its center a round hole 50 mm in diameter.

(2) Procedure

Measure 100 mL of the sample, whose temperature is previously noted, using a volumetric cylinder graduated in 1 mL, and carry out the distillation in the same manner as in Method 1.

2.58 X-Ray Powder Diffraction Method

X-Ray Powder Diffraction Method is a method for measuring characteristic X-ray diffraction angles and intensities from randomly oriented powder crystallites irradiated by a monochromated X-ray beam. In this diffraction process, electrons in the component atoms of crystallites are forced to vibrate by the incident X-ray beam, and make sharp coherent scatters of the X-ray. The diffraction pattern from a crystalline specimen is specific for the crystal form of the specimen. Therefore the X-ray powder diffraction method can be used for qualitative and quantitative evaluation of crystal forms, formation of polymorphism, and crystalline solvates. The random arrangement of molecules in non-crystalline substances makes them poor interference scatters of X-rays, resulting in broad, diffuse maxima in the diffraction patterns. Thus, for non-crystalline substances and/or materials with poor crystallinity, the utility of this method is limited. However, the X-ray diffraction method can be used for estimating the crystallinity of materials like plastics.

In crystalline materials, the molecular and atomic species are ordered in a three-dimensional array, called a lattice. The smallest lattice is designated the unit cell in crystallography. A plane in the crystal can be defined by three arbitrary lattice points and there is a family of planes separated by a certain interplanar spacing from each other. Thus a family of planes is called a crystalline plane and is usually indexed by using Miller indices (hkl). These indices are the reciprocals, reduced to smallest integers, of the intercepts that a plane makes along the three axes composing the unit cell. Interplanar spacing for a set of parallel planes (hkl) is denoted by d_{hkl} .

The occurrence of X-ray diffraction and the direction of the diffracted beam by a crystallographic plane obey the Bragg's law. According to this law, X-ray diffraction occurs only when the scattered beams in a specific direction travel distances that differ by integral multiples (n), the order of the reflection, of the incident X-ray wavelength (λ). This condition is described by the equation,

$$2 d_{hkl} \sin \theta = n\lambda$$

in which d_{hkl} denotes the interplanar spacing and θ is the angle of diffraction. The diffracted angle 2θ due to a plane (hkl) is specified by the intrinsic interplanar spacing, defined by the lattice constants, and the wavelength of the incident X-ray beam.

The intensity of a coherent X-ray diffraction is dependent upon structure factors, temperature factor, degree of crystal-

linity, volume and density of the powdered specimen, absorption characteristics, intensity and wavelength of the incident X-ray beam, polarization factor, multiplicity, Lorentz factor, etc. Among these factors, the polarization factor is dependent upon the monochromatizing method of the incident X-ray beam, the Lorentz factor upon geometrical factors of the apparatus, multiplicity factor upon the crystalline systems, absorption factor upon the component atoms of the sample, temperature factor and crystallinity upon experimental temperature and physical properties of the specimen, and structural factor upon the position of each atom in the unit cell and atomic species.

Apparatus

Usually an X-ray diffractometer equipped with a radiation detector is used for X-ray powder diffraction measurement. The diffractometer is composed of an X-ray source, goniometer, radiation detector, and the combined controller and data processor.

X-Ray radiation sources are usually vacuum tubes, in which heated electrons are emitted from a cathode, and impinge violently against an anode under a high electric field. Since the wavelength of the generated X-rays depend upon the type of metal utilized as the anode, an appropriate anode has to be selected for the specimen to be analyzed. In general, since the generated X-rays have a wide spectrum of wavelength, an appropriate filter element or crystal monochromator must be chosen so that a monochromated beam is practically used for the diffraction analysis.

The goniometer is an angle scanning apparatus for adjusting two angles, that between the X-ray beam and the specimen surface, and that between the specimen surface and the detector. It is usually scanned with the above two angles being held equal, which is called the symmetrical reflection technique. The goniometer is equipped with a filter or a monochromator for selecting a specific X-ray beam. Further equipment may be included for heating or cooling sample specimens.

A counting apparatus is composed of a detector and a computing apparatus, of which the former converts the intensity of scattered X-rays to electrical signals, while the latter converts the obtained signals to diffraction intensity values. A proportional counter or a scintillation counter is usually used as the detector.

A combination of controller and data processor is used for controlling the goniometer angle, for recording diffraction intensity, and for data processing.

Operation Procedure

The following variables have to be selected and/or determined before performing a diffraction measurement for a given specimen: anode type, electric current and voltage for the X-ray vacuum tube, scanning speed and range of the goniometer, time constant and so on. A copper anode is most commonly employed for organic substances and polymers.

A powdered specimen is usually packed and prepared in a specimen holder made of aluminum or glass. As a rule, the orientation of sample crystallites have to be randomized before packaging. The specimen may be ground in an agate mortar to a fine powder in order to randomize the orientation of crystallites. However, this grinding method is sometimes inappropriate due to the physical characteristics of a specimen or the measurement object.

In setting up the specimen and apparatus, coplanarity of

the specimen surface with the specimen holder surface and the setting of the specimen holder at the position of symmetric reflection geometry have to be assured. Further it should be noted that the grinding procedure may affect the crystallinity of the specimen and the packaging pressure on the specimen holder may induce orientation of the crystallites.

Identification and/or Judgement

Identification of the specimen with the standard material can be accomplished by comparing the X-ray powder diffraction patterns with each other. Judgement of polymorphism and crystalline solvates can be done by comparison of the diffraction pattern obtained for the specimen with that of the reference material or the same material measured previously.

Comparison of two X-ray diffraction patterns should be based on the intensity ratio of diffracted peaks, and the interplanar spacings d . The intensity ratio is defined by the ratio of the peak intensity at a particular diffraction angle to the intensity of the standard peak, for which the strongest maximum on the diffraction pattern is usually selected. However, the diffraction angle 2θ can be used as a basis for the identification, where the same wavelength of the radiation beam is utilized for the diffraction measurement of the sample and reference material. The scanning angle range for diffraction measurement is usually between 5° and 40° for ordinary organic substances, unless otherwise specified in Monographs. Based on the obtained X-ray diffraction patterns, the identification of a specimen with a standard material can be confirmed, if the diffraction pattern for the specimen gives diffraction peaks of the same intensity at the same diffraction angle 2θ , as those of the standard. If two powder crystallites ascribed to the same substance have the same crystal form, the X-ray diffraction angles should agree within $\pm 0.2^\circ$.

Assay

A quantitative analysis by X-ray powder diffraction does not give a sufficiently precise result. Thus, the quantitative application of this method is limited to a few analytical problems: numerical estimation of degree of polymorphism, solvation number for crystalline solvates, and degree of crystallinity.

For a quantitative analysis of polymorphism and/or solvate, an appropriate diffraction peak has to be selected. Usually, the calibration curve method can be applied to the quantitative estimation by the X-ray analysis. Before measurement of the diffraction intensity for a sample specimen at a selected diffraction peak, a calibration curve must be prepared under the same conditions, using a series of standard samples containing known amounts of the objective substance.

Alternatively the internal standard method can also be effective in place of the above standard method. A known amount of the internal standard is usually added to weighed amounts of a sample to be analyzed. Diffraction intensity ratios of the specimen to the internal standard are measured. Separately, a calibration curve for the intensity ratio against the mixing ratio of the reference material to the internal standard are prepared under the same conditions. By using the calibration curve, a quantitative analysis is possible in X-ray powder diffraction measurement. If more than two diffraction peaks ascribed to different lattice planes (hkl) are used, the influence of orientation of crystallites can be detected. The internal standard should have approximately the same density as the specimen and similar absorption characteristics

with regard to the X-ray beam. Further the diffraction peak given by the standard should not overlap with that of the specimen to be analyzed.

Caution: Handle the apparatus with great care since X-ray may affect the human health.

2.59 Test for Total Organic Carbon

Test for Total Organic Carbon is a method for measuring the amount of organic carbon, which forms organic compounds, in water. Normally, organic carbon can be oxidized to carbon dioxide by a dry decomposition method, where organic compounds are oxidized by combustion, or by a wet decomposition method, where organic compounds are oxidized by applying ultraviolet rays or by adding oxidizing agent. The amount of carbon dioxide generated in the decomposition process is measured using an appropriate method such as infrared gas analysis, electric conductivity measurement, or resistivity measurement. The amount of organic carbon in water can be calculated from the amount of carbon dioxide measured in one of the above methods.

There are two types of carbon in water: organic carbon and inorganic carbon. For measuring the amount of organic carbon, two approaches can be taken. One method is to measure the amount of total carbon in water, then to subtract the amount of inorganic carbon from that of total carbon. The other method is to remove inorganic carbon from the test water, then to measure the amount of remaining organic carbon.

Instrument

The instrument consists of a sample injection port, a decomposition device, a carbon dioxide separation block, a detector, and a data processor or a recorder. The instrument should be capable of measuring the amount of organic carbon down to 0.050 mg/L.

The sample injection port is designed to be able to accept a specific amount of sample injected by a microsyringe or other appropriate sampling devices. The decomposition device for the dry decomposition method consists of a combustion tube and an electric furnace to heat the sample. Both devices are adjusted to operate at specified temperatures. The decomposition device for the wet decomposition method consists of an oxidizing reaction box, an ultraviolet ray lamp, a decomposition aid injector, and a heater. The decomposition device for either method should be capable of generating not less than 0.450 mg/L of organic carbon when using a solution of sodium dodecylbenzenesulfonate (theoretical value of total organic carbon in this solution is 0.806 mg/L) as the sample. The carbon dioxide separation block removes water from carbon dioxide formed in the decomposition process or separates carbon dioxide from the decomposed gas. An infrared gas analyzer, electric conductivity meter or specific resistance meter is used as the detector which converts the concentration of carbon dioxide into electric signal. The data processor calculates the concentration of the total organic carbon in the sample based on the electric signal converted by the detector. The recorder records the electric signal intensity converted by the detector.

Reagents and standard solutions

Water used for measuring organic carbon (water for measurement): This water is used for preparing standard solutions or decomposition aid or for rinsing the instrument. The amount of organic carbon in this water, when collected into a sample container, should be not more than 0.250 mg/L.

Standard potassium hydrogen phthalate solution: The concentration of this standard solution is determined as specified for the instrument. Dry potassium hydrogen phthalate (standard reagent) at 105°C for 4 hours, and allow it to cool in a desiccator (silica gel). Weigh accurately a prescribed amount of dried potassium hydrogen phthalate, and dissolve it in the water for measurement to prepare the standard solution.

Standard solution for measuring inorganic carbon: The concentration of this standard solution is determined as specified for the instrument. Dry sodium hydrogen carbonate in a desiccator (sulfuric acid) for not less than 18 hours. Dry sodium carbonate decahydrate separately between 500°C and 600°C for 30 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately prescribed amounts of these compounds so that the ratio of their carbon content is 1:1, and dissolve them in the water for measurement to prepare the standard solution.

Decomposition aid: Dissolve a prescribed amount of potassium peroxodisulfate or other substances that can be used for the same purpose, in the water for measurement up to the concentration as specified for the instrument.

Gas for removing inorganic carbon or carrier gas: Nitrogen, oxygen, or other gases that can be used for the same purpose.

Acid for removing inorganic carbon: Dilute hydrochloric acid, phosphoric acid or other acids that can be used for the same purpose, with the water for measurement down to the concentration as specified for the instrument.

Apparatus

Sample container and reagent container: Use a container made of the material which does not release organic carbon from its surface, such as hard glass. Soak the container before use in a mixture of diluted hydrogen peroxide solution (1 in 3) and dilute nitric acid (1:1), and wash well with the water for measurement.

Microsyringe: Wash a microsyringe with a mixture of a solution of sodium hydroxide (1 in 20) and ethanol (99.5) (1:1), or diluted hydrochloric acid (1 in 4), and rinse well with the water for measurement.

Procedure

Employ an analytical method suitable for the instrument used. Calibrate the instruments using the standard potassium hydrogen phthalate solution with the test procedure specified for the instrument.

It is recommended that this instrument be incorporated into the manufacturing line of the water to be tested.

Otherwise, this test should be performed in a clean circumstance where the use of organic solvents or other substances that may affect the result of this test is prohibited, using a large sample container to collect a large volume of the water to be tested. The measurement should be done immediately after the sample collection.

(1) Measurement of organic carbon by subtracting inorganic carbon from total carbon

According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measur-

ing the expected amount of total carbon into the instrument from sample injection port, and decompose organic and inorganic carbon in the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of total carbon in the sample using a data processor or a recorder. Change the setting of the instrument for measuring inorganic carbon exclusively, and measure the amount of inorganic carbon in the same manner as total carbon. The amount of organic carbon can be obtained by subtracting the amount of inorganic carbon from that of total carbon.

(2) Measurement of organic carbon after removing inorganic carbon

Remove inorganic carbon by adding the acid for removing inorganic carbon to the sample, followed by bubbling the gas for removing inorganic carbon (e.g. nitrogen) into the sample. According to the test procedure specified for the instrument used, inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, and decompose the sample. Detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon in the sample using a data processor or a recorder.

For the instrument where the removal of inorganic carbon is performed in the instrument, first inject a suitable volume of the sample for measuring the expected amount of organic carbon into the instrument from sample injection port, according to the test procedure specified for the instrument used. Then, remove inorganic carbon by adding the acid for removing inorganic carbon to the sample in the decomposition device, followed by bubbling the gas for removing inorganic carbon into the sample. Decompose organic carbon, detect the generated carbon dioxide with the detector, and calculate the amount of organic carbon using a data processor or a recorder.

2.60 Melting Point Determination

The melting point is defined to be the temperature at which a crystalline substance melts during heating, when the solid phase and the liquid phase are in an equilibrium. However, in this Pharmacopoeia it is conventionally defined to be the temperature at which the remaining solid sample melts completely when it is subjected to continuous heating and the change of the sample state that accompanies heating is accurately observed. Since a pure substance has an intrinsic melting point, it is used for the identification and/or confirmation of a substance and also as an indicator of the purity of a substance.

The melting point is determined by either of the following methods: Method 1 is applied to those substances of which the purity is comparably high and which can be pulverized, Method 2 to those substances which are insoluble in water and can not be readily pulverized, and Method 3 to petrolatums.

Unless otherwise specified, measurement is performed by Method 1.

Method 1

This method is applied to those substances of which the purity is comparably high and which can be pulverized.

Apparatus

Use the apparatus illustrated in the Fig. 2.60-1.

Alternatively, apparatus in which some of the procedures, such as stirring, heating, and cooling are automated, can be used.

Bath fluid: Usually use clear silicone oil having a viscosity of 50 to 100 mm²/s at an ordinary temperature.

Thermometer with an immersion line: There are six types of thermometers, Type 1—Type 6, which are specified by an appropriate measuring temperature range. For melting points lower than 50°C, use a thermometer Type 1; for 40°C to 100°C, Type 2; for 90°C to 150°C, Type 3; for 140°C to 200°C, Type 4; for 190°C to 250°C, Type 5; for 240°C to 320°C, Type 6.

Capillary tube: Use a hard glass capillary tube 120 mm long, 0.8 to 1.2 mm in inner diameter and 0.2 to 0.3 mm thick, with one end closed.

Procedure

Pulverize the sample to a fine powder, and, unless otherwise specified, dry in a desiccator (silica gel) for 24 hours. When it is specified to do the test after drying, dry the sample under the conditions specified in the monograph before measurement. Place the sample in a dried capillary tube H, and pack it tightly so as to form a layer about 2.5–3.5 mm high by dropping the capillary repeatedly, with the closed end of H down, through a glass tube, about 70 cm long, held vertically on a glass or porous plate.

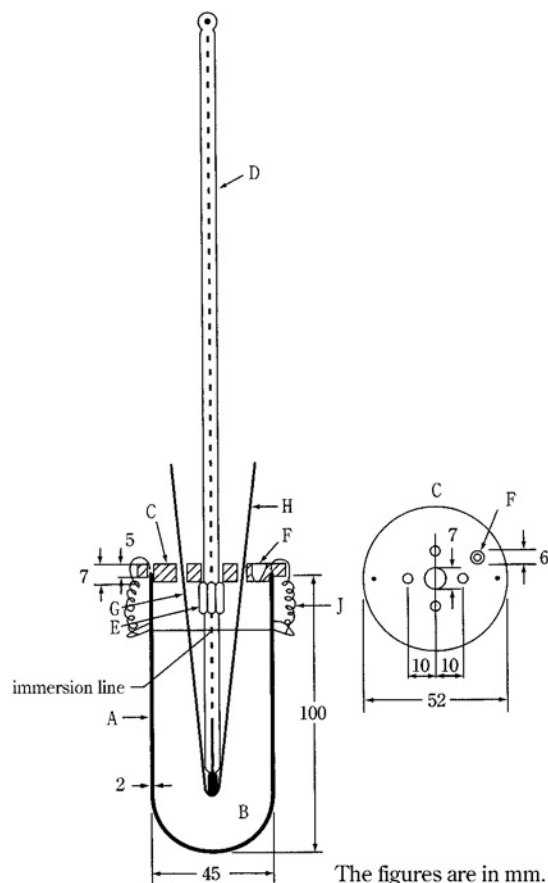
Heat the bath fluid B until the temperature rises to about 10°C below the expected melting point, place the thermometer D in the bath with the immersion line at the same level as the meniscus of the bath fluid, and insert capillary tube H into a coil spring G so that the packed sample is placed in a position corresponding to the center of the mercury bulb of the thermometer D. Continue heating to raise the temperature at a rate of approximately 3°C per minute until the temperature rises to 5°C below the expected melting point, then carefully regulate the rate of temperature increase to 1°C per minute.

Read the thermometer indication of the instantaneous temperature at which the sample liquefies completely and no solid is detectable in the capillary, and designate the indicated temperature as the melting point of the sample specimen.

System suitability test—Confirmation of the system suitability of the apparatus should be done periodically by using the Melting Point Standards. The Reference Standard is prepared for the suitability test of the apparatus when it is used with Type 2—Type 5 thermometers, and consists of 6 highly purified substances: acetanilide, acetophenetidine, caffeine, sulfanilamide, sulfapyridine, and vanillin. The label shows the certified melting points of the respective substances (the end point of the melting change), MP_f.

After selecting one of the thermometers and the appropriate Melting Point Standard based upon the expected melting point of a sample specimen, perform a melting point measurement of the selected Reference Standard, according to the above procedure. When the value of the obtained melting point of the Reference Standard is within MP_f ± 0.5°C in the case of vanillin and acetanilide, within MP_f ± 0.8°C in the case of acetophenetidine and sulfanilamide, and within MP_f ± 1.0°C in the case of sulfapyridine and caffeine, the apparatus is assumed to be suitable.

The above-mentioned measurement is repeated 3 times and the average is determined to be the melting point of the Reference Standard tested. When the above suitability test



- The figures are in mm.
- A: Heating vessel of hard glass
 - B: Bath fluid
 - C: Teflon stopper
 - D: Thermometer with an immersion line
 - E: Thermometer-fastening spring
 - F: Vent for adjustment of the bath fluid volume
 - G: Coil spring
 - H: Capillary tube
 - J: Spring for fastening Teflon stopper

Fig. 2.60-1 Melting point determination apparatus

criteria are not met in a certain melting point measurement system of an apparatus and a Reference Standard, do the test again, after checking the packing of the sample specimen into the capillary tube, the locations and positioning of the thermometer and the capillary tube, the heating and stirring of the bath fluid, and the control of the temperature increasing rate. When a melting point measurement system does not meet the suitability test criteria again after checking these measuring conditions, the thermometer with an immersion line should be calibrated again or replaced with a new one.

Method 2

This method is applied to substances such as fats, fatty acids, paraffins or waxes.

Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line. Furthermore, the capillary tube should be

the same as specified in Method 1, except that both ends of the tube are open.

Procedure

Carefully melt the sample at as low a temperature as possible, and, taking care to prevent bubbles, introduce it into a capillary tube to a height of about 10 mm. Allow the capillary containing the sample to stand for 24 hours at below 10°C, or for at least 1 hour in contact with ice, holding the capillary so that the sample can not flow out. Then attach the capillary to the thermometer by means of a rubber band so that the absorbed sample is located at a position corresponding to the center of the mercury bulb. Adjust the capillary tube in a water-containing beaker to such a position that the lower edge of the sample is located 30 mm below the water surface. Heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of temperature increase to 1°C per minute. The temperature at which the sample begins floating in the capillary is taken as the melting point of the sample specimen.

Method 3

This method is applied to petrolatums.

Apparatus

Instead of the apparatus specified in Method 1, use a water-containing beaker as a bath fluid and a heating vessel. In this measurement, total immersion mercury-filled thermometers can also be used in place of the thermometer with an immersion line.

Procedure

Melt the sample slowly by heating, with thorough stirring, until the temperature reaches 90 – 92°C. Discontinue the heating, and allow the sample to cool to 8 – 10°C above the expected melting point. Chill the bulb of the thermometer to 5°C, wipe and dry, and, while still cold, stick half of the thermometer bulb into the melted sample. Withdraw it immediately, hold vertically, cool until the attached sample becomes turbid, then dip the sample-bearing bulb for 5 minutes in water having a temperature below 16°C. Next, fix the thermometer securely in a test tube by means of a cork stopper so that the lower end is located 15 mm above the bottom. Suspend the test tube in a water-containing beaker held at a temperature about 16°C, and raise the temperature of the water bath to 30°C at a rate of 2°C per minute, then continue heating carefully at a rate of 1°C per minute until it reaches the melting point. Read the thermometer indication of the instantaneous temperature at which the first drop of the sample leaves the thermometer. If the variations between three repeated determinations are not more than 1°C, take the average of the three as the melting point. If any variation is greater than 1°C, make two additional measurements, and take the average of the five as the melting point.

3. Powder Property Determinations

3.01 Determination of Bulk and Tapped Densities

Determination of Bulk and Tapped Densities is a method

to determine the bulk densities of powdered drugs under loose and tapped packing conditions respectively. Loose packing is defined as the state obtained by pouring a powder sample into a vessel without any consolidation, and tapped packing is defined as the state obtained when the vessel containing the powder sample is to be repeatedly dropped a specified distance at a constant drop rate until the apparent volume of sample in the vessel becomes almost constant. The bulk density is expressed in mass per unit apparent volume of powder (g/mL). Because the bulk density is one of the measures of packing properties, compressibility and flow properties, and is dependent on the “history” of the powder, it is essential to report the bulk density to specify how the determination was made.

Bulk density

The bulk density is an apparent density obtained by pouring a powder sample into a vessel without any consolidation. The determination of bulk density is achieved by measuring the apparent volume of a powder sample having a known mass in a graduated cylinder (Method 1) or by measuring the mass of powder in a vessel having a known volume (Method 2).

Method 1 (Constant mass method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000- μ m (No.16) sieve to break up agglomerates that may have formed during storage. Weigh accurately about 30 g of test sample, and pour it into a dry 100-mL graduated glass cylinder (readable to 1 mL). Carefully level the powder without consolidation, if necessary, and read the unsettled apparent volume, V_0 , to the nearest graduated unit. Calculate the bulk density ρ_B by the formula:

$$\rho_B = M/V_0$$

ρ_B : Bulk density by constant mass method (g/mL)

M : Mass of powder sample (g)

V_0 : Apparent volume of powder sample (mL)

Record the average of 3 determinations using 3 different powder samples. If a 30-g sample is too large to determine, adjust the mass of sample so as to provide an apparent volume of 60 – 100 mL.

Method 2 (Constant volume method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000- μ m (No.16) sieve to break up agglomerates that may have formed during storage. Allow an excess of sample powder to pour into the measuring vessel having the volume of V and mass of M_0 . Carefully scrape excess powder from the top of the vessel using the edge of a slide glass or other tool by smoothly moving across it. Remove any material from the sides of the vessel, and determine the total mass M_t . Calculate the bulk density ρ_B by the formula:

$$\rho_B = (M_t - M_0)/V$$

ρ_B : Bulk density by constant volume method (g/mL)

M_t : Total mass of powder and measuring vessel (g)

M_0 : Mass of measuring vessel (g)

V : Volume of measuring vessel (mL)

Record the average of 3 determinations using 3 different powder samples.

Tapped density

Tapped density is an apparent density obtained by mechanically tapping a measuring vessel containing a powder sample. The determination of tapped density is achieved by measuring the apparent volume of a powder sample having a known mass in a vessel after tapping (Method 1) or by measuring the mass of powder in a vessel having a known volume after tapping (Method 2).

Method 1 (Constant mass method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000- μm (No.16) or a 710- μm (No.22) sieve to break up agglomerates that may have formed during storage. Weigh accurately about 100 g of test sample, and pour it into a 250-mL graduated glass cylinder (readable to 2 mL) without consolidation. If it is not possible to use 100 g, proceed according to the same procedure as that described above by using a 100-mL graduated glass cylinder (readable to 1 mL). It is essential to select appropriate masses of the cylinder support, holder and cylinder so as to ensure the dynamic stability of the apparatus during tapping. After attaching the glass cylinder containing the powder sample to the tapping apparatus, carry out tapping under the measuring conditions (tapping rate and drop height) specified for each apparatus.

Unless otherwise specified, repeat increments of 50 taps or 1 minute until the difference between succeeding measurements is less than 2%, and determine the final apparent volume, V_f . Calculate the tapped density ρ_T by the formula:

$$\rho_T = M / V_f$$

ρ_T : Tapped density by constant mass method (g/mL)

M : Mass of powder sample (g)

V_f : Final apparent volume of sample after tapping (mL)

Record the average of 3 determinations using 3 different powder samples.

Method 2 (Constant volume method)

Unless otherwise specified, pass a quantity of sample sufficient to complete the test through a 1000- μm (No.16) sieve to break up agglomerates that may have formed during storage. Attach a supplementary cylinder to the stainless steel measuring vessel having a known mass of M_0 and a volume of V (Fig. 3.01-1), and then pour an excess of the sample into the vessel. After setting up the vessel in an adequate tapping apparatus with a fixed drop height, carry out tapping at the rate and cumulative tap number specified for each apparatus. Then remove the supplementary cylinder from the vessel and carefully scrape excess powder from the top of the vessel by smoothly moving across it the edge of a slide glass or other tool. Remove any material from the sides of the vessel, and determine the total mass M_t . Calculate the tapped density ρ_T by the formula:

$$\rho_T = (M_t - M_0) / V$$

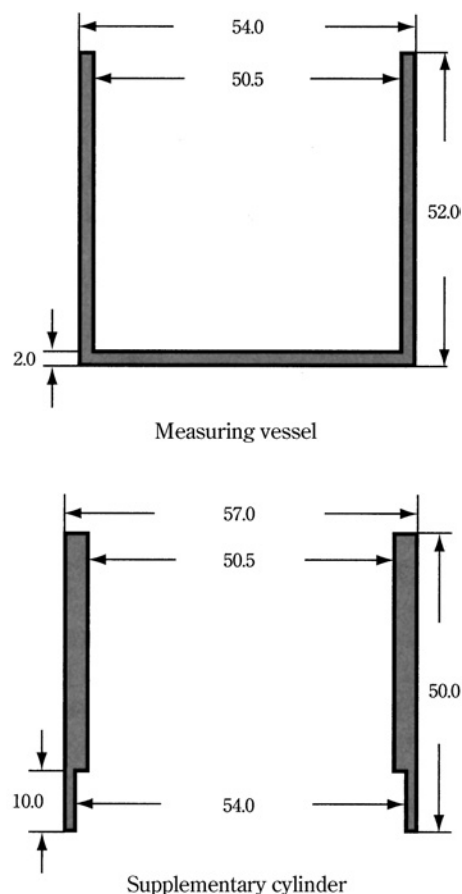
ρ_T : Tapped density by constant volume method (g/mL)

M_t : Total mass of powder and measuring vessel (g)

M_0 : Mass of measuring vessel (g)

V : Volume of measuring vessel (mL)

Record the average of 3 determinations and the relative standard deviation using 3 different powder samples. If the relative standard deviation is not less than 2%, repeat the test



The figures are in mm used for a 100-mL measuring vessel and supplementary cylinder for example.

Fig. 3.01-1 Measuring vessel (upper) and supplementary cylinder (down)

with further tapping.

Balances: Use balances readable to the nearest 0.1 g.

3.02 Specific Surface Area by Gas Adsorption

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\blacklozenge \blacklozenge).

\blacklozenge The specific surface area determination method is a method to determine specific surface area (the total surface area of powder per unit mass) of a pharmaceutical powder sample by using gas adsorption method. \blacklozenge The specific surface area of a powder is determined by physical adsorption of a gas on the surface of the solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface. Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbate gas molecules and the adsorbent surface of the test powder. The determination is usually carried out at the temperature of liquid nitrogen. The amount of gas adsorbed can be measured by a volumetric or continuous flow procedure.

MULTI-POINT MEASUREMENT

When the gas is physically adsorbed by the powder sample, the following relationship (Brunauer, Emmett and Teller (BET) adsorption isotherm) holds when the relative pressure (P/P_0) is in the range of 0.05 to 0.30 for pressure P of the adsorbate gas in equilibrium for the volume of gas adsorbed, V_a .

$$\frac{1}{[V_a\{(P_0/P) - 1\}]} = \{(C - 1)/V_m C\} \times (P/P_0) + (1/V_m C) \quad (1)$$

P : Partial vapour pressure of adsorbate gas in equilibrium with the surface at -195.8°C (b.p. of liquid nitrogen), in pascals,

P_0 : Saturated pressure of adsorbate gas, in pascals,

V_a : Volume of gas adsorbed at standard temperature and pressure (STP) [0°C and atmospheric pressure (1.013×10^5 Pa)], in milliliters,

V_m : Volume of gas adsorbed at STP to produce an apparent monolayer on the sample surface, in milliliters,

C : Dimensionless constant that is related to the enthalpy of adsorption of adsorbate gas on the powder sample.

A value of V_a is measured at each of not less than 3 values of P/P_0 . Then the BET value, $1/[V_a\{(P_0/P) - 1\}]$, is plotted against P/P_0 according to equation (1). This plot should yield a straight line usually in the approximate relative pressure range 0.05 to 0.3. The data are considered acceptable if the correlation coefficient, r , of the linear regression is not less than 0.9975; that is, r^2 is not less than 0.995. From the resulting linear plot, the slope, which is equal to $(C - 1)/V_m C$, and the intercept, which is equal to $1/V_m C$, are evaluated by linear regression analysis. From these values, V_m is calculated as $1/(\text{slope} + \text{intercept})$, while C is calculated as $(\text{slope}/\text{intercept}) + 1$. From the value of V_m so determined, the specific surface area, S , in m^2g^{-1} , is calculated by the equation:

$$S = (V_m N a) / (m \times 22400) \quad (2)$$

N : Avogadro constant ($6.022 \times 10^{23} \text{ mol}^{-1}$),

a : Effective cross-sectional area of one adsorbate molecule, in square metres (0.162 nm^2 for nitrogen and 0.195 nm^2 for krypton),

m : Mass of test powder, in grams,

Specific surface area is generally expressed in units of m^2g^{-1} .

SINGLE-POINT MEASUREMENT

Normally, at least 3 measurements of V_a each at different values of P/P_0 are required for the determination of specific surface area by the dynamic flow gas adsorption technique (*Method I*) or by volumetric gas adsorption (*Method II*). However, under certain circumstances described below, it may be acceptable to determine the specific surface area of a powder from a single value of V_a measured at a single value of P/P_0 such as 0.30, using the following equation for calculating V_m :

$$V_m = V_a \{1 - (P/P_0)\} \quad (3)$$

The single-point method may be employed directly for a series of powder samples of a given material for which the material constant C is much greater than unity. Close similarity between the single-point values and multiple-point values suggests that $1/C$ approaches zero. The error on V_m can be reduced by using the multiple-point method to evaluate C for one of the samples of the series on which the material con-

stant C is expected to be large. Then V_m is calculated from the single value of V_a measured at a single value of P/P_0 by the equation:

$$V_m = V_a \{(P_0/P) - 1\} [(1/C) + \{(C - 1)/C\} \times (P/P_0)] \quad (4)$$

SAMPLE PREPARATION

Before the specific surface area of the sample can be determined, it is necessary to remove gases and vapors that may have become physically adsorbed onto the surface during storage and handling. If outgassing is not achieved, the specific surface area may be reduced or may be variable because an intermediate area of the surface is covered with molecules of the previously adsorbed gases or vapors. The outgassing conditions are critical for obtaining the required precision and accuracy of specific surface area measurements on pharmaceuticals because of the sensitivity of the surface of the materials. The outgassing conditions must be demonstrated to yield reproducible BET plots, a constant weight of test powder, and no detectable physical or chemical changes in the test powder.

The outgassing conditions defined by the temperature, pressure and time should be so chosen that the original surface of the solid is reproduced as closely as possible.

Outgassing of many substances is often achieved by applying a vacuum, by purging the sample in a flowing stream of a non-reactive, dry gas, or by applying a desorption-adsorption cycling method. In either case, elevated temperatures are sometimes applied to increase the rate at which the contaminants leave the surface. Caution should be exercised when outgassing powder samples using elevated temperatures to avoid affecting the nature of the surface and the integrity of the sample.

If heating is employed, the recommended temperature and time of outgassing are as low as possible to achieve reproducible measurement of specific surface area in an acceptable time. For outgassing sensitive samples, other outgassing methods such as the desorption-adsorption cycling method may be employed.

The standard technique is the adsorption of nitrogen at liquid nitrogen temperature.

For powders of low specific surface area ($< 0.2 \text{ m}^2\text{g}^{-1}$) the proportion adsorbed is low. In such cases the use of krypton at liquid nitrogen temperature is preferred because the low vapor pressure exerted by this gas greatly reduces error.

The use of larger sample quantities where feasible (equivalent to 1 m^2 or greater total surface area using nitrogen) may compensate for the errors in determining low surface areas.

All gases used must be free from moisture.

Accurately weigh a quantity of the test powder such that the total surface of the sample is at least 1 m^2 when the adsorbate is nitrogen and 0.5 m^2 when the adsorbate is krypton. Lower quantities of sample may be used after appropriate validation.

Adsorption of gas should be measured either by Method I or Method II.

Method I: the dynamic flow method

In the dynamic flow method (see Fig. 3.02-1), the recommended adsorbate gas is dry nitrogen or krypton, while helium is employed as a diluent gas, which is not adsorbed under the recommended conditions. A minimum of 3 mixtures of the appropriate adsorbate gas with helium are required within the P/P_0 range 0.05 to 0.30.

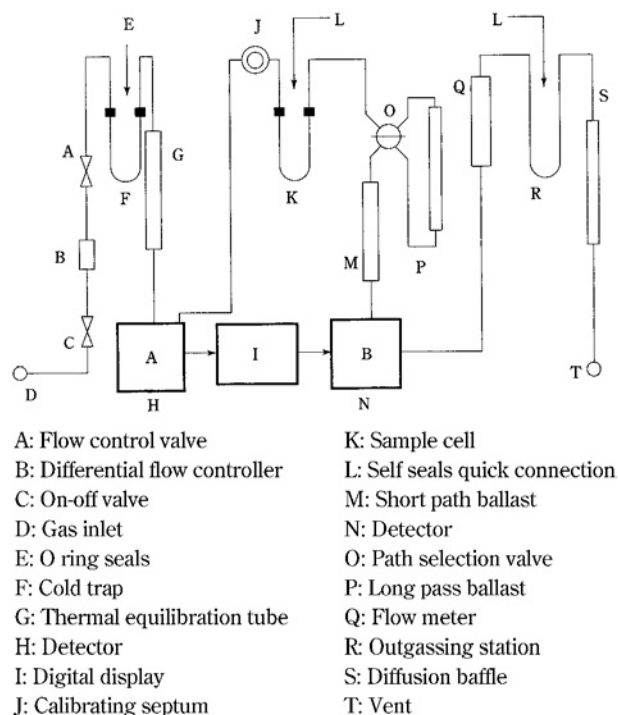


Fig. 3.02-1 Schematic diagram of the dynamic flow method apparatus

The gas detector-integrator should provide a signal that is approximately proportional to the volume of the gas passing through it under defined conditions of temperature and pressure. For this purpose, a thermal conductivity detector with an electronic integrator is one among various suitable types. A minimum of 3 data points within the recommended range of 0.05 to 0.30 for P/P_0 is to be determined.

A known mixture of the gases, usually nitrogen and helium, is passed through a thermal conductivity cell, through the sample again through the thermal conductivity cell and then to a recording potentiometer.

Immerse the sample cell in liquid nitrogen, then the sample adsorbs nitrogen from the mobile phase. This unbalances the thermal conductivity cell, and a pulse is generated on a recorder chart.

Remove from the coolant; this gives a desorption peak equal in area and in the opposite direction to the adsorption peak.

Since this is better defined than the adsorption peak, it is the one used for the determination.

To effect the calibration, inject a known quantity of adsorbate into the system, sufficient to give a peak of similar magnitude to the desorption peak and obtain the proportion of gas volume per unit peak area.

Method II: the volumetric method

In the volumetric method (see Figure 3.02-2), the recommended adsorbate gas is nitrogen is admitted into the evacuated space above the previously outgassed powder sample to give a defined equilibrium pressure, P , of the gas. The use of a diluent gas, such as helium, is therefore unnecessary, although helium may be employed for other purposes, such as to measure the dead volume.

Admit a small amount of dry nitrogen into the sample tube

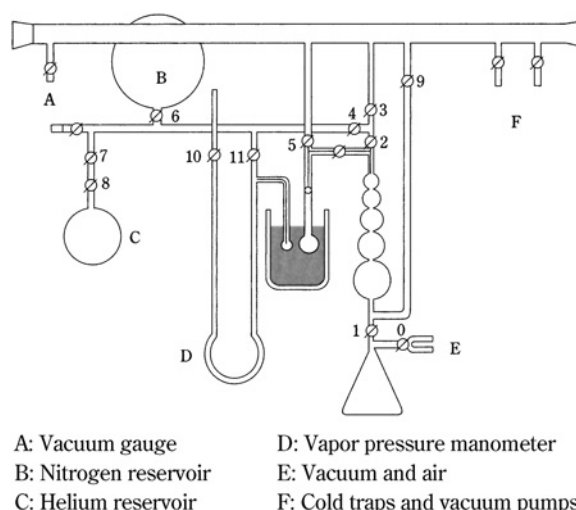


Fig. 3.02-2 Schematic diagram of the volumetric method apparatus

to prevent contamination of the clean surface, remove the sample tube, insert the stopper, and weigh it. Calculate the weight of the sample. Attach the sample tube to the volumetric apparatus. Cautiously evacuate the sample down to the specified pressure (e.g. between 2 Pa and 10 Pa).

If the principle of operation of the instrument requires the determination of the dead volume in the sample tube, this procedure is carried out at this point, followed by evacuation of the sample. Raise a Dewar vessel containing liquid nitrogen up to a defined point on the sample cell. Admit a sufficient volume of adsorbate gas to give the lowest desired relative pressure. Measure the volume adsorbed, V_a . For multipoint measurements, repeat the measurement of V_a at successively higher P/P_0 values. When nitrogen is used as the adsorbate gas, P/P_0 values of 0.10, 0.20, and 0.30 are often suitable.

REFERENCE MATERIALS

Periodically verify the functioning of the apparatus using appropriate reference materials of known surface area, such as α -alumina for specific surface area determination, which should have a specific surface area similar to that the sample to be examined.

3.03 Powder Particle Density Determination

Powder Particle Density Determination is a method to determine particle density of powdered pharmaceutical drugs or raw materials of drugs, and the gas displacement pycnometer is generally used. The powder density by this method is determined with an assumption that the volume of the gas displaced by the powder in a closed system is equal to the volume of the powder. The bulk density at loose packing or the tapped density at tapping express the apparent densities of the powder, since interparticulate void volume of the powder is assumed to contribute a part of the volume of the powder. On the contrary, the pycnometric particle density expresses the powder density nearly equal to the crystal density,

since the volume of the powder, that is deducted with void volume of open pores accessible to gas, is counted.

Powder particle density is expressed in mass per unit volume (kg/m^3), and generally expressed in g/cm^3 .

Apparatus

The schematic diagram of particle density apparatus for gas displacement pycnometric measurement is shown in Fig. 3.03-1. The apparatus consists of a test cell in which the sample is placed, a reference cell and a manometer.

Generally, helium is used as the measurement gas. The apparatus has to be equipped with a system capable of pressuring the test cell to the defined pressure through the manometer.

Calibration of apparatus The volumes of the test cell (V_c) and the reference cell (V_r) must be accurately determined to the nearest 0.001 cm^3 , and to assure accuracy of the results of volume obtained, calibration of the apparatus is carried out as follows using a calibration ball of known volume for particle density measurement. The final pressures (P_f) are determined for the initial empty test cell followed by the test cell placed with the calibration ball for particle density measurement in accordance with the procedures, and V_c and V_r are calculated using the equation described in the section of Procedure. Calculation can be made taking into account that the sample volume (V_s) is zero in the first run.

Procedure

The measurement of the particle density is carried out between 15 and 30°C , and temperature must not vary by more than 2°C during the course of measurement.

Firstly, weigh the mass of the test cell and record it. After weighing out the amount of the sample as described in the individual monograph and placing it in the test cell, seal the cell in the pycnometer. Secondly, introduce the measurement gas (helium) into the test cell, and remove volatile contaminants in the powder. If necessary, keep the sample powder under reduced pressure to remove the volatile contaminants in advance and use it as the test sample for measurement.

Open the valve which connects the reference cell with the test cell, confirm with the manometer that the pressure inside the system is stable, and then read the system reference pressure (P_r). Secondly, close the valve that connects to the two cells, and introduce the measurement gas into the test cell to achieve positive pressure. Confirm with the manometer that the pressure inside the system is stable, and then read the initial pressure (P_i). Open the valve to connect the test cell with the reference cell. After confirming that the indicator of the manometer is stable, read the final pressure (P_f), and calculate the sample volume (V_s) with the following equation.

$$V_s = V_c - \frac{V_r}{\frac{P_i - P_r}{P_f - P_r} - 1}$$

V_r : Reference cell volume (cm^3)

V_c : Test cell volume (cm^3)

V_s : Sample volume (cm^3)

P_i : Initial pressure (kPa)

P_f : Final pressure (kPa)

P_r : Reference pressure (kPa)

Repeat the measurement sequence for the same powder sample until consecutive measurements of the sample volume agree to within 0.5% , and calculate the mean of sample

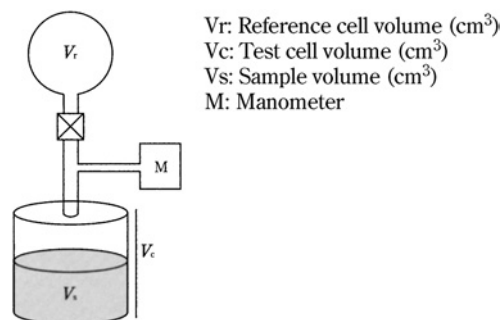


Fig. 3.03-1 Schematic diagram of a gas displacement pycnometer

volumes (V_s). Finally, unload the test cell, weigh the mass of test cell, and calculate the final sample mass by deducting the empty cell mass from the test cell mass. The powder particle density ρ is calculated by the following equation.

$$\rho = m/V_s$$

ρ : Powder particle density (g/cm^3)

m : Final sample mass (g)

V_s : Sample volume (cm^3)

3.04 Particle Size Determination

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\blacklozenge \blacklozenge).

\blacklozenge Particle Size Determination is a method to determine directly or indirectly morphological appearance, shape, size and its distribution of powdered pharmaceutical drugs and excipients to examine their micromeritic properties. Optical microscopy and analytical sieving method may be used depending on the measuring purpose and the properties of test specimen. \blacklozenge

Method 1. Optical Microscopy

\blacklozenge The optical microscopy is used to observe the morphological appearance and shape of individual particle either directly with the naked eye or by using a microscopic photograph, in order to measure the particle size. The particle size distribution can also be determined by this method. It is also possible with this method to measure the size of the individual particle even when different kinds of particles mingle if they are optically distinguishable. Data processing techniques, such as image analysis, can be useful for determining the particle size distribution. \blacklozenge

This method for particle characterization can generally be applied to particles $1 \mu\text{m}$ and greater. The lower limit is imposed by the resolving power of the microscope. The upper limit is less definite and is determined by the increased difficulty associated with the characterization of larger particles. Various alternative techniques are available for particle characterization outside the applicable range of optical microscopy. Optical microscopy is particularly useful for characterizing particles that are not spherical. This method may also serve as a base for the calibration of faster and more routine methods that may be developed.

Apparatus—Use a microscope that is stable and protected from vibration. The microscope magnification (product of the objective magnification, ocular magnification, and additional magnifying components) must be sufficient to allow adequate characterization of the smallest particles to be classified in the test specimen. The greatest numerical aperture of the objective should be sought for each magnification range. Polarizing filters may be used in conjunction with suitable analyzers and retardation plates. Color filters of relatively narrow spectral transmission should be used with achromatic objectives and are preferable with apochromats and are required for appropriate color rendition in photomicrography. Condensers corrected for at least spherical aberration should be used in the microscope substage and with the lamp. The numerical aperture of the substage condenser should match that of the objective under the condition of use; this is affected by the actual aperture of the condenser diaphragm and the presence of immersion oils.

Adjustment—The precise alignment of all elements of the optical system and proper focusing are essential. The focusing of the elements should be done in accordance with the recommendations of the microscope manufacturer. Critical axial alignment is recommended.

Illumination—A requirement for good illumination is a uniform and adjustable intensity of light over the entire field of view; Kohler illumination is preferred. With colored particles, choose the color of the filters used so as to control the contrast and detail of the image.

Visual Characterization—The magnification and numerical aperture should be sufficiently high to allow adequate resolution of the images of the particles to be characterized. Determine the actual magnification using a calibrated stage micrometer to calibrate an ocular micrometer. Errors can be minimized if the magnification is sufficient that the image of the particle is at least 10 ocular divisions. Each objective must be calibrated separately. To calibrate the ocular scale, the stage micrometer scale and the ocular scale should be aligned. In this way, a precise determination of the distance between ocular stage divisions can be made.

♦When the particle size is measured, an ocular micrometer is inserted at the position of the ocular diaphragm, and a calibrated stage micrometer is placed at the center of the microscope stage and fixed in place. The ocular is attached to the lens barrel and adjusted to the focus point of the stage micrometer scale. Then, the distance between the scales of the two micrometers is determined, and the sample size equivalent 1 division of the ocular scale is calculated using the following formula:

The particle size equivalent 1 division on the ocular scale (μm) = Length on the stage micrometer (μm) / Number of scale divisions on the ocular micrometer

The stage micrometer is removed and the test specimen is placed on the microscope stage. After adjusting the focus, the particle sizes are determined from the number of scale divisions read through the ocular. ♦

Several different magnifications may be necessary to characterize materials having a wide particle size distribution.

Photographic Characterization—If particle size is to be determined by photographic methods, take care to ensure that the object is sharply focused at the plane of the photographic emulsion. Determine the actual magnification by photographing a calibrated stage micrometer, using photograph-

ic film of sufficient speed, resolving power, and contrast. Exposure and processing should be identical for photographs of both the test specimen and the determination of magnification. The apparent size of a photographic image is influenced by the exposure, development, and printing processes as well as by the resolving power of the microscope.

Preparation of the Mount—The mounting medium will vary according to the physical properties of the test specimen. Sufficient, but not excessive, contrast between the specimen and the mounting medium is required to ensure adequate detail of the specimen edge. The particles should rest in one plane and be adequately dispersed to distinguish individual particles of interest. Furthermore, the particles must be representative of the distribution of sizes in the material and must not be altered during preparation of the mount. Care should be taken to ensure that this important requirement is met. Selection of the mounting medium must include a consideration of the analyte solubility.

Crystallinity Characterization—The crystallinity of a material may be characterized to determine compliance with the crystallinity requirement where stated in the individual monograph of a drug substance. Unless otherwise specified in the individual monograph, mount a few particles of the specimen in mineral oil on a clean glass slide. Examine the mixture using a polarizing microscope: the particles show birefringence (interference colors) and extinction positions when the microscope stage is revolved.

Limit Test of Particle Size by Microscopy—Weigh a suitable quantity of the powder to be examined (for example, 10 to 100 mg), and suspend it in 10 mL of a suitable medium in which the powder does not dissolve, adding, if necessary, a wetting agent. A homogeneous suspension of particles can be maintained by suspending the particles in a medium of similar or matching density and by providing adequate agitation. Introduce a portion of the homogeneous suspension into a suitable counting cell, and scan under a microscope an area corresponding to not less than 10 μg of the powder to be examined. Count all the particles having a maximum dimension greater than the prescribed size limit. The size limit and the permitted number of particles exceeding the limit are defined for each substance.

Particle Size Characterization—The measurement of particle size varies in complexity depending on the shape of the particle and the number of particles characterized must be sufficient to insure an acceptable level of uncertainty in the measured parameters¹⁾. For spherical particles, size is defined by the diameter. For irregular particles, a variety of definitions of particle size exist. In general, for irregularly shaped particles, characterization of particle size must also include information on the type of diameter measured as well as information on particle shape. Several commonly used measurements of particle size are defined below (see Fig. 3.04-1):

Feret's Diameter—The distance between imaginary parallel lines tangent to a randomly oriented particle and perpendicular to the ocular scale.

Martin's Diameter—The diameter of the particle at the point that divides a randomly oriented particle into two equal projected areas.

Projected area Diameter—The diameter of a circle that has the same projected area as the particle.

Length—The longest dimension from edge to edge of a particle oriented parallel to the ocular scale.

Width—The longest dimension of the particle measured at

Table 3.04-1. Sizes of Standard Sieve Series in Range of Interest

ISO Nominal Aperture			US Sieve No.	Recommended USP Sieves (mesh)	European Sieve No.	Japan Sieve No.
Principal sizes	Supplementary sizes					
R 20/3	R 20	R 40/3				
11.20 mm	11.20 mm 10.00 mm	11.20 mm 9.50 mm			11200	
8.00 mm	9.00 mm 8.00 mm 7.10 mm	8.00 mm 6.70 mm				
5.60 mm	6.30 mm 5.60 mm 5.00 mm	5.60 mm 4.75 mm			5600	3.5 4
4.00 mm	4.50 mm 4.00 mm 3.55 mm	4.00 mm 3.35 mm	5 6	4000	4000	4.7 5.5
2.80 mm	3.15 mm 2.80 mm 2.50 mm	2.80 mm 2.36 mm	7 8	2800	2800	6.5 7.5
2.00 mm	2.24 mm 2.00 mm 1.80 mm	2.00 mm 1.70 mm	10 12	2000	2000	8.6 10
1.40 mm	1.60 mm 1.40 mm 1.25 mm	1.40 mm 1.18 mm	14 16	1400	1400	12 14
1.00 mm	1.12 mm 1.00 mm 900 μm	1.00 mm 850 μm	18 20	1000	1000	16 18
710 μm	800 μm 710 μm 630 μm	710 μm 600 μm	25 30	710	710	22 26
500 μm	560 μm 500 μm 450 μm	500 μm 425 μm	35 40	500	500	30 36
355 μm	400 μm 355 μm 315 μm	355 μm 300 μm	45 50	355	355	42 50
250 μm	280 μm 250 μm 224 μm	250 μm 212 μm	60 70	250	250	60 70
180 μm	200 μm 180 μm 160 μm	180 μm 150 μm	80 100	180	180	83 100
125 μm	140 μm 125 μm 112 μm	125 μm 106 μm	120 140	125	125	119 140
90 μm	100 μm 90 μm 80 μm	90 μm 75 μm	170 200	90	90	166 200
63 μm	71 μm 63 μm 56 μm	63 μm 53 μm	230 270	63	63	235 282
45 μm	50 μm 45 μm 40 μm	45 μm 38 μm	325	45	45	330 391

right angles to the length.

Particle Shape Characterization—For irregularly shaped particles, characterization of particle size must also include information on particle shape. The homogeneity of the pow-

der should be checked using appropriate magnification. The following defines some commonly used descriptors of particle shape (see Fig. 3.04-2):

Acicular—Slender, needle-like particle of similar width

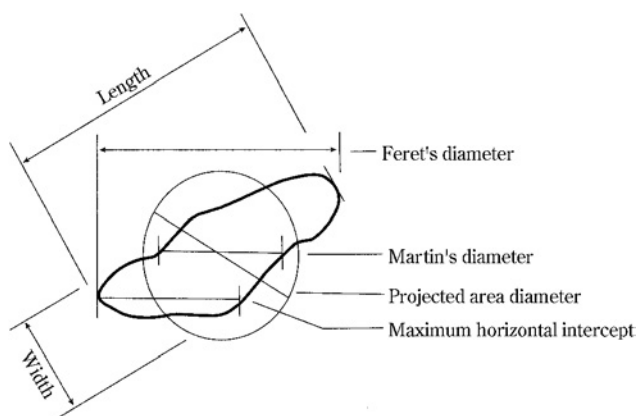


Fig. 3.04-1 Commonly used measurements of particle size

and thickness.

Columnar—Long, thin particle with a width and thickness that are greater than those of an acicular particle.

Flake—Thin, flat particle of similar length and width.

Plate—Flat particles of similar length and width but with greater thickness than flakes.

Lath—Long, thin, and blade-like particle.

Equant—Particles of similar length, width, and thickness; both cubical and spherical particles are included.

General Observations—A particle is generally considered to be the smallest discrete unit. A particle may be a liquid or semisolid droplet; a single crystal or polycrystalline; amorphous or an agglomerate. Particles may be associated. This degree of association may be described by the following terms:

Lamellar—Stacked plates.

Aggregate—Mass of adhered particles.

Agglomerate—Fused or cemented particles.

Conglomerate—Mixture of two or more types of particles.

Spherulite—Radial cluster.

Drusy—Particle covered with tiny particles.

Particle condition may be described by the following terms:

Edges—Angular, rounded, smooth, sharp, fractured.

Optical—Color (using proper color balancing filters), transparent, translucent, opaque.

Defects—Occlusions, inclusions.

Surface characteristics may be described as:

Cracked—Partial split, break, or fissure.

Smooth—Free of irregularities, roughness, or projections.

Porous—Having openings or passageways.

Rough—Bumpy, uneven, not smooth.

Pitted—Small indentations.

Method 2. Analytical Sieving Method

♦The analytical sieving method is a method to estimate the particle size distribution of powdered pharmaceutical drugs by sieving. The particle size determined by this method is shown as the size of a minimum sieve opening through which the particle passes. "Powder" here means a gathering of numerous solid particles. ♦

Sieving is one of the oldest methods of classifying powders and granules by particle size distribution. When using a woven sieve cloth, the sieving will essentially sort the particles

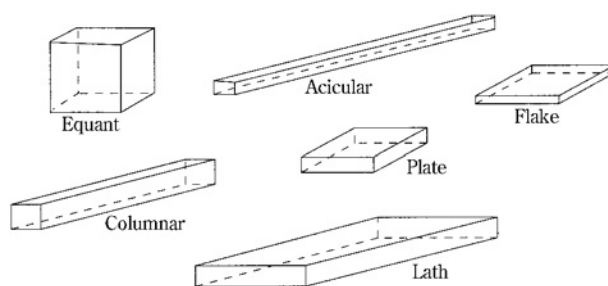


Fig. 3.04-2 Commonly used descriptions of particle shape

by their intermediate size dimension (i.e., breadth or width). Mechanical sieving is most suitable where the majority of the particles are larger than about $75 \mu\text{m}$. For smaller particles, the light weight provides insufficient force during sieving to overcome the surface forces of cohesion and adhesion that cause the particles to stick to each other and to the sieve, and thus cause particles that would be expected to pass through the sieve to be retained. For such materials other means of agitation such as air-jet sieving or sonic sifting may be more appropriate. Nevertheless, sieving can sometimes be used for some powders or granules having median particle sizes smaller than $75 \mu\text{m}$ where the method can be validated. In pharmaceutical terms, sieving is usually the method of choice for classification of the coarser grades of single powders or granules. It is a particularly attractive method in that powders and granules are classified only on the basis of particle size, and in most cases the analysis can be carried out in the dry state.

Among the limitations of sieving method are the need for an appreciable amount of sample (normally at least 25 g, depending on the density of the powder or granule, and the diameter of test sieves) and difficulty in sieving oily or other cohesive powders or granules that tend to clog the sieve openings. The method is essentially a two-dimensional estimate of size because passage through the sieve aperture is frequently more dependent on maximum width and thickness than on length.

This method is intended for estimation of the total particle size distribution of a single material. It is not intended for determination of the proportion of particles passing or retained on one or two sieves.

Estimate the particle size distribution as described under *Dry Sieving Method*, unless otherwise specified in the individual monograph. Where difficulty is experienced in reaching the endpoint (i.e., material does not readily pass through the sieves) or when it is necessary to use the finer end of the sieving range (below $75 \mu\text{m}$), serious consideration should be given to the use of an alternative particle-sizing method.

Sieving should be carried out under conditions that do not cause the test sample to gain or lose moisture. The relative humidity of the environment in which the sieving is carried out should be controlled to prevent moisture uptake or loss by the sample. In the absence of evidence to the contrary, analytical test sieving is normally carried at ambient humidity. Any special conditions that apply to a particular material should be detailed in the individual monograph.

Principles of Analytical Sieving—Analytical test sieves are constructed from a woven-wire mesh, which is of simple

weave that is assumed to give nearly square apertures and is sealed into the base of an open cylindrical container. The basic analytical method involves stacking the sieves on top of one another in ascending degrees of coarseness, and then placing the test powder on the top sieve.

The nest of sieves is subjected to a standardized period of agitation, and then the weight of material retained on each sieve is accurately determined. The test gives the weight percentage of powder in each sieve size range.

This sieving process for estimating the particle size distribution of a single pharmaceutical powder is generally intended for use where at least 80% of the particles are larger than 75 μm . The size parameter involved in determining particle size distribution by analytical sieving is the length of the side of the minimum square aperture through which the particle will pass.

TEST SIEVES

Test sieves suitable for pharmacopoeial tests conform to the most current edition of International Organisation for Standardization (ISO) Specification ISO 3310-1; Test sieves—Technical requirements and testing (see Table 3.04-1). Unless otherwise specified in the monograph, use those ISO sieves listed in the Table as recommended in the particular region.

Sieves are selected to cover the entire range of particle sizes present in the test specimen. A nest of sieves having a $\sqrt{2}$ progression of the area of the sieve openings is recommended. The nest of sieves is assembled with the coarsest screen at the top and the finest at the bottom. Use micrometers or millimeters in denoting test sieve openings. [Note—Mesh numbers are provided in the table for conversion purposes only.] Test sieves are made from stainless steel or, less preferably, from brass or other suitable non-reactive wire.

Calibration and recalibration of test sieves is in accordance with the most current edition of ISO 3310-1²⁾. Sieves should be carefully examined for gross distortions and fractures, especially at their screen frame joints, before use. Sieves may be calibrated optically to estimate the average opening size, and opening variability, of the sieve mesh. Alternatively, for the valuation of the effective opening of test sieves in the size range of 212 to 850 μm , Standard Glass Spheres are available. Unless otherwise specified in the individual monograph, perform the sieve analysis at controlled room temperature and at ambient relative humidity.

Cleaning Test Sieves—Ideally, test sieves should be cleaned using only an air jet or a liquid stream. If some apertures remain blocked by test particles, careful gentle brushing may be used as a last resort.

Test Specimen—If the test specimen weight is not given in the monograph for a particular material, use a test specimen having a weight between 25 and 100 g, depending on the bulk density of the material, and test sieves having a 200 mm diameter. For 76 mm sieves the amount of material that can be accommodated is approximately 1/7th that which can be accommodated on a 200 mm sieve. Determine the most appropriate weight for a given material by test sieving accurately weighed specimens of different weights, such as 25, 50, and 100 g, for the same time period on a mechanical shaker. [Note—If the test results are similar for the 25-g and 50-g specimens, but the 100-g specimen shows a lower percentage through the finest sieve, the 100-g specimen size is too large.] Where only a specimen of 10 to 25 g is available, smaller di-

ameter test sieves conforming to the same mesh specifications may be substituted, but the endpoint must be re-determined. The use of test samples having a smaller mass (e.g. down to 5 g) may be needed. For materials with low apparent particle density, or for materials mainly comprising particles with a highly iso-diametrical shape, specimen weights below 5 g for a 200 mm screen may be necessary to avoid excessive blocking of the sieve. During validation of a particular sieve analysis method, it is expected that the problem of sieve blocking will have been addressed.

If the test material is prone to picking up or losing significant amounts of water with varying humidity, the test must be carried out in an appropriately controlled environment. Similarly, if the test material is known to develop an electrostatic charge, careful observation must be made to ensure that such charging is not influencing the analysis. An antistatic agent, such as colloidal silicon dioxide and/or aluminum oxide, may be added at a 0.5 percent (m/m) level to minimize this effect. If both of the above effects cannot be eliminated, an alternative particle-sizing technique must be selected.

Agitation Methods—Several different sieve and powder agitation devices are commercially available, all of which may be used to perform sieve analyses. However, the different methods of agitation may give different results for sieve analyses and endpoint determinations because of the different types and magnitude of the forces acting on the individual particles under test. Methods using mechanical agitation or electromagnetic agitation, and that can include either a vertical oscillation or a horizontal circular motion, or tapping or a combination of both tapping and horizontal circular motion are available. Entrainment of the particles in an air stream may also be used. The results must indicate which agitation method was used and the agitation parameters used (if they can be varied), since changes in the agitation conditions will give different results for the sieve analysis and endpoint determinations, and may be sufficiently different to give a failing result under some circumstances.

Endpoint Determination—The test sieving analysis is complete when the weight on any of the test sieves does not change by more than 5% or 0.1 g (10% in the case of 76 mm sieves) of the previous weight on that sieve. If less than 5% of the total specimen weight is present on a given sieve, the endpoint for that sieve is increased to a weight change of not more than 20% of the previous weight on that sieve.

If more than 50% of the total specimen weight is found on any one sieve, unless this is indicated in the monograph, the test should be repeated, but with the addition to the sieve nest of a more coarse sieve intermediate between that carrying the excessive weight and the next coarsest sieve in the original nest, i.e., addition of the ISO series sieve omitted from the nest of sieves.

SIEVING METHODS

Mechanical agitation

Dry Sieving Method—Tare each test sieve to the nearest 0.1 g. Place an accurately weighed quantity of test specimen on the top (coarsest) sieve, and replace the lid. Agitate the nest of sieves for 5 minutes. Then carefully remove each from the nest without loss of material. ♦ If there is some fine powder on the down surface of each sieve, take it off by the brush gently, and combine it with the sieve fraction retained on each next down sieve. ♦ Reweigh each sieve, and determine

the weight of material on each sieve. Determine the weight of material in the collecting pan in a similar manner. Reassemble the nest of sieves, and agitate for 5 minutes. Remove and weigh each sieve as previously described. Repeat these steps until the endpoint criteria are met (see *Endpoint Determination* under *Test Sieves*). Upon completion of the analysis, reconcile the weights of material. Total losses must not exceed 5% of the weight of the original test specimen.

Repeat the analysis with a fresh specimen, but using a single sieving time equal to that of the combined times used above. Confirm that this sieving time conforms to the requirements for endpoint determination. When this endpoint has been validated for a specific material, then a single fixed time of sieving may be used for future analyses, providing the particle size distribution falls within normal variation.

If there is evidence that the particles retained on any sieve are aggregates rather than single particles, the use of mechanical dry sieving is unlikely to give good reproducibility, a different particle size analysis method should be used.

Air Entrainment Methods

Air Jet and Sonic Shifter Sieving—Different types of commercial equipment that use a moving air current are available for sieving. A system that uses a single sieve at a time is referred to as *air jet* sieving. It uses the same general sieving methodology as that described under the *Dry Sieving Method*, but with a standardized air jet replacing the normal agitation mechanism. It requires sequential analyses on individual sieves starting with the finest sieve to obtain a particle size distribution. Air jet sieving often includes the use of finer test sieves than used in ordinary dry sieving. This technique is more suitable where only oversize or undersize fractions are needed.

In the *sonic sifting* method, a nest of sieves is used, and the test specimen is carried in a vertically oscillating column of air that lifts the specimen and then carries it back against the mesh openings at a given number of pulses per minute. It may be necessary to lower the sample amount to 5 g, when sonic shifting is employed.

The air jet sieving and sonic sieving methods may be useful for powders or granules when mechanical sieving techniques are incapable of giving a meaningful analysis.

These methods are highly dependent upon proper dispersion of the powder in the air current. This requirement may be hard to achieve if the method is used at the lower end of the sieving range (i.e., below 75 μm), when the particles tend to be more cohesive, and especially if there is any tendency for the material to develop an electrostatic charge. For the above reasons endpoint determination is particularly critical, and it is very important to confirm that the oversize material comprises single particles and is not composed of aggregates.

INTERPRETATION

The raw data must include the weight of test specimen, the total sieving time, and the precise sieving methodology and the set values for any variable parameters, in addition to the weights retained on the individual sieves and in the pan. It may be convenient to convert the raw data into a cumulative weight distribution, and if it is desired to express the distribution in terms of a cumulative weight undersize, the range of sieves used should include a sieve through which all the material passes. If there is evidence on any of the test sieves that the material remaining on it is composed of aggregates formed during the sieving process, the analysis is invalid.

¹Additional information on particle size measurement, sample size, and data analysis is available, for example, in ISO 9276.

²International Organization for Standardization (ISO) Specification ISO 3310-1; Test sieves-Technical requirements and testing

4. Biological Tests/Biochemical Tests/Microbial Tests

4.01 Bacterial Endotoxins Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\blacklozenge \blacklozenge).

Bacterial Endotoxins Test is a test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using a lysate reagent prepared from blood corpuscle extracts of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). There are two types of techniques for this test: the gel-clot techniques, which are based on gel formation by the reaction of the lysate TS with endotoxins, and the photometric techniques, which are based on endotoxin-induced optical changes of the lysate TS. The latter include turbidimetric techniques, which are based on the change in lysate TS turbidity during gel formation, and chromogenic techniques, which are based on the development of color after cleavage of a synthetic peptide-chromogen complex.

Proceed by any one of these techniques for the test. In the event of doubt or dispute, the final decision is made based on the gel-clot techniques, unless otherwise indicated.

The test is carried out in a manner that avoids endotoxin contamination.

Apparatus

Depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process. Commonly used minimum time and temperature settings are 30 minutes at 250°C. If employing plastic apparatus, such as multi-well plates and tips for micropipettes, use only that which has been shown to be free of detectable endotoxin and which does not interfere with the test.

Preparation of Standard Endotoxin Stock Solution

\blacklozenge Prepare Standard Endotoxin Stock Solution by dissolving Endotoxin 10000 Reference Standard or Endotoxin 100 Reference Standard in water for bacterial endotoxins test (BET). \blacklozenge Endotoxin is expressed in Endotoxin Units (EU). One EU is equal to one International Unit (IU) of endotoxin.

Preparation of Standard Endotoxin Solution

After mixing Standard Endotoxin Stock Solution thoroughly, prepare appropriate serial dilutions of Standard Endotoxin Solution, using water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

Preparation of sample solutions

Unless otherwise specified, prepare sample solutions by dissolving or diluting drugs, using water for BET. \blacklozenge Sample solutions for containers for medicines should be prepared according to other specified procedures. \blacklozenge If necessary, adjust

the pH of the solution to be examined so that the pH of the mixture of the lysate TS and sample solution falls within the specified pH range for the lysate reagent to be used. This usually applies to a sample solution with a pH in the range of 6.0 to 8.0. TSs or solutions used for adjustment of pH may be prepared using water for BET, and then stored in containers free of detectable endotoxin.

Determination of Maximum Valid Dilution

The Maximum Valid Dilution (MVD) is the maximum allowable dilution of a sample solution at which the endotoxin limit can be determined.

Determine the MVD from the following equation:

$$\text{MVD} = \frac{\text{Endotoxin limit} \times \text{Concentration of sample solution}}{\lambda}$$

Endotoxin limit:

The endotoxin limit for injections, defined on the basis of dose, equals K/M , where K is a minimum pyrogenic dose of endotoxin per kg body mass (EU/kg), and M is equal to the maximum dose of product per kg of body mass in a single hour period.

Concentration of sample solution:

mg/mL in the case of endotoxin limit specified by mass (EU/mg)

♦mEq/mL in the case of endotoxin limit specified by equivalent (EU/mEq)♦

Units/mL in the case of endotoxin limit specified by biological unit (EU/Unit)

mL/mL in the case of endotoxin limit specified by volume (EU/mL)

λ : the labeled lysate reagent sensitivity in the gel-clot techniques (EU/mL) or the lowest point used (EU/mL) in the standard regression curve of the turbidimetric or chromogenic techniques

Gel-clot techniques

The gel-clot techniques detect or quantify endotoxins based on clotting of the lysate TS in the presence of endotoxin. To ensure both the precision and validity of the test, perform the tests for confirming the labeled lysate reagent sensitivity and for interfering factors as described under Preparatory testing.

(1) Preparatory testing

(i) Test for confirmation of labeled lysate reagent sensitivity

The labeled sensitivity of lysate reagent is defined as the lowest concentration of endotoxin that is needed to cause the lysate TS to clot under the conditions specified for the lysate reagent to be used.

The test for confirmation of the labeled lysate reagent sensitivity is to be carried out when each new lot of lysate reagent is used or when there is any change in the experimental conditions which may affect the outcome of the test. Perform the test by the following procedures.

Prepare standard solutions having four concentrations equivalent to 2λ , λ , 0.5λ and 0.25λ by diluting the Standard Endotoxin Stock Solution with water for BET. Prepare the lysate TS by dissolving the lysate reagent with water for BET or a suitable buffer. Mix a volume of the lysate TS with an equal volume of one of the standard solutions (usually, 0.1 mL aliquots) in each test tube. When single test vials or ampoules containing lyophilized lysate reagent are used, add

solutions directly to the vial or ampoule.

Keep the tubes (or containers such as vials or ampoules) containing the reaction mixture usually at $37 \pm 1^\circ\text{C}$ for 60 ± 2 minutes, avoiding vibration. To test the integrity of the gel after incubation, invert each tube or container through approximately 180° in one smooth motion. If a firm gel has formed that remains in place upon inversion, record the result as positive. A result is negative if either a firm gel is not formed, or if a fragile gel has formed but flows out upon inversion.

Making the standard solutions of four concentrations one set, test four replicates of the set.

The test is not valid unless 0.25λ of the standard solution shows a negative result in each set of tests. If the test is not valid, repeat the test after verifying the test conditions.

The endpoint is the last positive test in the series of decreasing concentrations of endotoxin. Calculate the geometric mean endpoint concentration using the following formula:

Geometric Mean Endpoint Concentration = $\text{antilog}(\Sigma e/f)$

Σe = the sum of the log endpoint concentrations of the dilution series used

f = the number of replicates

If the geometric mean endpoint concentration is not less than 0.5λ and not more than 2.0λ , the labeled sensitivity is confirmed.

(ii) Test for interfering factors

This test is performed to check for the presence of enhancing or inhibiting factors for the reaction in sample solutions.

Following Table 4.01-1, prepare solutions A and B using a sample solution under test, and solutions C and D using water for BET. Test solutions A and B and solutions C and D in quadruplicate and in duplicate, respectively. Concerning the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

The geometric mean endpoint concentrations of B and C solutions are determined by using the formula described in (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

This test must be repeated when there is any change in the experimental conditions which may affect the outcome of the test.

Table 4.01-1

Solution	Concentration of added endotoxin in each solution/ Solution to which endotoxin is added	Diluent	Dilution factor	Concentration of added endotoxin after dilution	Number of replicates
A	0/Sample solution	—	—	—	4
B	2λ /Sample solution	Sample solution	1	2λ	4
			2	1λ	
			4	0.5λ	
			8	0.25λ	
C	2λ /Water for BET	Water for BET	1	2λ	2
			2	1λ	
			4	0.5λ	
			8	0.25λ	
D	0/Water for BET	—	—	—	2

The test is valid if solutions A and D show no reaction and the result for solution C confirms the labeled sensitivity.

If the geometric mean endpoint concentration of solution B is not less than 0.5λ and not greater than 2.0λ , the sample solution being examined does not contain interfering factors and complies with the test for interfering factors. Otherwise the sample solution interferes with the test.

If the sample under test does not comply with the test at a dilution less than the MVD, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment.

(2) Limit test

Based on the formation of a firm gel in the presence of endotoxin at above labeled lysate reagent sensitivity, this method tests whether a sample solution contains endotoxin not greater than the endotoxin limit.

(i) Procedure

Prepare solutions A, B, C and D according to Table 4.01-2. Making these four solutions one set, test two replicates of the set.

In preparing solutions A and B, use the sample solutions complying with (ii) Test for interfering factors of (1) Preparatory testing. Concerning the test conditions including the incubation temperature, incubation time, and procedure for the confirmation of gel formation, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

Table 4.01-2

Solution	Concentration of added endotoxin in each solution/Solution to which endotoxin is added	Number of replicates
A	0/Sample solution	2
B	2λ /Sample solution	2
C	2λ /Water for BET	2
D	0/Water for BET	2

(ii) Interpretation

The test is valid when both replicates of solutions B and C are positive and those of solution D are negative.

The sample meets the endotoxin limit requirement of the test when a negative result is found for both replicates of solution A.

Repeat the test in duplicate when the test results are positive for one test but negative for the other one. The sample meets the endotoxin limit requirement of the test when a negative result is found for both replicates of solution A in the repeat test.

The sample does not meet the endotoxin limit requirement of the test when a positive result is found for both replicates of the solution A at a dilution equal to the MVD. If the test is positive for the sample at a dilution less than the MVD, the test may be performed at a dilution not greater than the MVD.

(3) Assay

The test measures endotoxin concentrations of sample solutions by titration to an endpoint of gel formation.

(i) Procedure

Prepare solutions A, B, C and D according to Table

4.01-3. Making these four solutions one set, test two replicates of the set. When preparing solutions A and B, use sample solutions complying with (ii) Test for interfering factors of (1) Preparatory testing. Concerning the test conditions, follow the procedure under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

Table 4.01-3

Solution	Concentration of added endotoxin in each solution/Solution to which endotoxin is added	Diluent	Dilution factor*	Concentration of added endotoxin after dilution	Number of replicates
A	0/Sample solution	Water for BET	1 2 4 8	— — — —	2
B	2λ /Sample solution	—	1	2λ	2
C	2λ /Water for BET	Water for BET	1 2 4 8	2λ 1λ 0.5λ 0.25λ	2
D	0/Water for BET	—	—	—	2

* The dilution range of the dilution series of solution A may be changed as appropriate, but not exceeding the MVD.

(ii) Calculation and interpretation

The test is valid when the following three conditions are met: (a) both replicates of the negative control solution D are negative, (b) both replicates of the positive product control solution B are positive and (c) the geometric mean endpoint concentration of solution C is in the range of 0.5λ to 2λ .

The endpoint is defined as the maximum dilution showing the last positive test in the dilution series of solution A, and the endotoxin concentration of solution A is calculated by multiplying the endpoint dilution factor by λ .

Calculate the geometric mean endotoxin concentration of the two replicates, using the formula given under (i) Test for confirmation of labeled lysate reagent sensitivity of (1) Preparatory testing.

If none of the dilutions of solution A is positive, report the endotoxin concentration solution A as less than $\lambda \times$ the lowest dilution factor of solution A.

If all dilutions are positive, the endotoxin concentration of solution A is reported as equal to or greater than the greatest dilution factor of solution A multiplied by λ .

Calculate the endotoxin concentration (in EU per mL, in EU per mg or mEq or in EU per Unit) of the sample, based on the mean endotoxin concentration of solution A. The sample complies with the Bacterial Endotoxins Test <4.01> if the endotoxin concentration of the sample meets the requirement for the endotoxin limit (in EU per mL, in EU per mg or mEq or in EU per Unit) specified in the individual monograph.

Photometric techniques

(1) Turbidimetric technique

This technique measures the endotoxin concentrations of sample solutions based on the measurement of turbidity change accompanying gel formation of the lysate TS. This technique is classified as either endpoint-turbidimetric or kinetic-turbidimetric.

The endpoint-turbidimetric technique is based on the

quantitative relationship between the concentration of endotoxins and the turbidity of the reaction mixture at a specified reaction time.

The kinetic-turbidimetric technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined turbidity of the reaction mixture or the rate of turbidity development.

The test is usually carried out at $37 \pm 1^\circ\text{C}$, and turbidity is expressed in terms of either absorbance or transmission.

(2) Chromogenic technique

This technique measures the endotoxin concentrations of sample solutions based on the measurement of chromophore released from a synthetic chromogenic substrate by the reaction of endotoxins with the lysate TS. This technique is classified as either endpoint-chromogenic or kinetic-chromogenic.

The endpoint-chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and the release of chromophore at the end of an incubation period.

The kinetic-chromogenic technique is based on the quantitative relationship between the concentration of endotoxins and either the time needed to reach a predetermined absorbance (or transmittance) of the reaction mixture or the rate of color development.

The test is usually carried out at $37 \pm 1^\circ\text{C}$.

(3) Preparatory testing

To assure the precision and validity of the turbidimetric or chromogenic techniques, perform both Test for assurance of criteria for the standard curve and Test for interfering factors, as indicated below.

(i) Test for assurance of criteria for the standard curve

♦The test must be carried out for each lot of lysate reagent. ♦

Using the Standard Endotoxin Solution, prepare at least three endotoxin concentrations to generate the standard curve within the range of endotoxin concentrations indicated by the instructions for the lysate reagent used. Perform the test using at least three replicates of each standard endotoxin concentration according to the optimal conditions for the lysate reagent used (with regard to volume ratios, incubation time, temperature, pH, etc.). If the desired range is greater than two logs, additional standards should be included to bracket each log increase in the range of the standard curve.

The absolute value of the correlation coefficient, $|r|$, must be greater than or equal to 0.980 for the range of endotoxin concentrations set up.

If the test is not valid, repeat the test after verifying the test conditions.

The test for assurance of criteria for the standard curve must be repeated when any condition changes, which is likely to influence the result of the test.

(ii) Test for interfering factors

Prepare solutions A, B, C and D according to Table 4.01-4. Perform the test on these solutions following the optimal conditions for the lysate reagent used (with regard to volume of sample solution and lysate TS, volume ratio of sample solution to lysate TS, incubation time, etc.).

The test for interfering factors must be repeated when any condition changes, which is likely to influence the result of the test.

Table 4.01-4

Solution	Concentration of added endotoxin in each solution	Solution to which endotoxin is added	Number of test tubes or wells
A	0	Sample solution* ¹	Not less than 2
B	Middle concentration of the standard curve* ²	Sample solution* ¹	Not less than 2
C	At least 3 concentrations* ³	Water for BET	Each not less than 2
D	0	Water for BET	Not less than 2

*¹ The sample solution may be diluted not to exceed the MVD.

*² Add the Standard Endotoxin Solution to the sample solution to make an endotoxin concentration at or near the middle of the standard curve.

*³ The concentrations used in the test for the (i) Assurance of criteria for the standard curve of (3) Preparatory testing.

♦The test is not valid unless the following conditions are met.

- 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
- 2: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent employed, or it is less than the endotoxin detection limit of the lysate reagent employed. ♦

Calculate the recovery of the endotoxin added to solution B from the concentration found in solution B after subtracting the endotoxin concentration found in solution A.

When the recovery of the endotoxin added to solution B is within 50% to 200%, the sample solution under test is considered to be free of interfering factors.

♦When the endotoxin recovery is out of the specified range, the sample solution under test is considered to contain interfering factors. If the sample under test does not comply with the test, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the sample solution or diluted sample solution not to exceed the MVD may be eliminated by suitable treatment, such as filtration, neutralization, dialysis or heat treatment. ♦

(4) Assay

(i) Procedure

Prepare solutions A, B, C and D according to Table 4.01-4, and follow the procedure described in (ii) Test for interfering factors of (3) Preparatory testing.

(ii) Calculation of endotoxin concentration

Calculate the endotoxin concentration of solution A using the standard curve generated with solution C. The test is not valid unless all the following requirements are met.

- 1: The absolute value of the correlation coefficient of the standard curve generated using solution C is greater than or equal to 0.980.
- 2: The endotoxin recovery, calculated from the concentration found in solution B after subtracting the concentration of endotoxin found in solution A, is within the range of 50% to 200%.
- 3: The result with solution D does not exceed the limit of the blank value required in the description of the lysate reagent employed, ♦or it is less than the endotoxin detection limit of the lysate reagent employed. ♦

(iii) Interpretation

The sample complies with the Bacterial Endotoxins Test if the endotoxin concentration of the sample calculated from the endotoxin concentration of solution A meets the requirement of the endotoxin limit (in EU per mL, in EU per mg or

mEq or in EU per Unit) specified in the individual monograph.

4.02 Microbial Assay for Antibiotics

Microbial Assay for Antibiotics is a method to determine the antimicrobial potency of antibiotics based on their antimicrobial activities. There are three methods for this test: the cylinder-plate, perforated plate, and turbidimetric methods. The former two are based on the measurement of the size of the zones of microbial growth inhibition in a nutrient agar medium, and the turbidimetric method is based on the measurement of the inhibition of turbidity development in a fluid medium with microbial growth. Unless otherwise specified in the individual monograph, tests specified to be carried out by the cylinder-plate method may be conducted under the same test conditions using the perforated plate method instead. If necessary, first sterilize water, isotonic sodium chloride solution, buffer solutions, reagents, test solutions and essential parts of measuring instruments and appliances to be used for the test. In performing the test, precautions must be taken to prevent biohazard.

I. Cylinder-plate method

The cylinder-plate method is a method to determine the antimicrobial potency of the antibiotic to be tested, and is based on the measurement of the size of the zone of growth inhibition of a test organism by the use of cylinder-agar plates.

1. Test organisms

Use the test organism specified in the individual monograph.

2. Culture media

Unless otherwise specified, use media with the following compositions. When 'peptone' is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. In the case of the medium for *Bacillus subtilis* ATCC 6633, adjust the pH using ammonia TS, potassium hydroxide TS or 1 mol/L hydrochloric acid TS. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

(1) Agar media for seed and base layer

1) Medium for test organism *Bacillus subtilis* ATCC 6633

i. Peptone	5.0 g
Meat extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

ii. Peptone	5.0 g
Meat extract	3.0 g
Trisodium citrate dihydrate	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the

solution so that it will be 6.5 to 6.6 after sterilization.

2) Medium for test organism *Saccharomyces cerevisiae* ATCC 9763

Glucose	10.0 g
Peptone	9.4 g
Meat extract	2.4 g
Yeast extract	4.7 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

3) Medium for other organisms

i. Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

iii. Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Agar media for transferring test organisms

1) Medium for test organism *Saccharomyces cerevisiae* ATCC 9763

Glucose	15.0 g
Peptone	5.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.0 to 6.2 after sterilization.

2) Medium for other organisms

i. Glucose	1.0 g
Meat peptone	6.0 g
Casein peptone	4.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

ii. Peptone	10.0 g
Meat extract	5.0 g
Sodium chloride	2.5 g
Agar	15.0 g

Water 1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

3. Preparation of agar slant or plate media

Unless otherwise specified, dispense approximately 9 mL of melted agar medium in each test tube (approximately 16 mm in inside diameter), and make them as slant media, or dispense approximately 20 mL of melted agar medium in each Petri dish (approximately 90 mm in inside diameter), and make them as plate media.

4. Preparation of stock suspensions of test spores or organisms

Unless otherwise specified, prepare stock suspensions of test spore or organism cultures as follows. Check the aspects of the test spores or organisms as occasion demands.

(1) Preparation of a stock spore suspension of test organism *Bacillus subtilis* ATCC 6633

Inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the test organisms specified in 2 (2) 2) i. Incubate at 32 to 37°C for 16 to 24 hours. Inoculate the subcultured test organism onto a suitable volume of slant or plate of the agar medium (described above), which was prepared for transferring the test organisms specified in 2 (2) 2) ii. Then incubate at 32 to 37°C for not less than 1 week to prepare spores. Suspend the spores in isotonic sodium chloride solution, heat at 65°C for 30 minutes, and then centrifuge. Wash the spore sediment three times with isotonic sodium chloride solution by means of centrifugation. Re-suspend the spore sediment in water or isotonic sodium chloride solution, and heat again at 65°C for 30 minutes to prepare the stock spore suspension. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock spore suspension at a temperature not exceeding 5°C, and use within 6 months. If the stock spore suspension shows a clear and definite zone of growth inhibition in an antibiotics potency test using adequate antibiotics, it may be used for further 6 months.

(2) Preparation of a stock suspension of the test organism *Saccharomyces cerevisiae* ATCC 9763

Inoculate test organism onto the slant or plate agar medium which has been prepared for transferring test organism specified in 2 (2) 1). Incubate at 25 to 26°C for 40 to 48 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate at 25 to 26°C for 40 to 48 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 30 days.

(3) Preparation of a stock suspension of other test organisms

Inoculate the test organism onto the slant or the plate of the agar medium which has been prepared for transferring the test organisms specified in 2 (2) 2) i. Incubate the inoculated slant at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Inoculate the subcultured test organism onto another slant or plate agar medium (described above), and incubate the slant at 32 to 37°C

for 16 to 24 hours. Scrape away and suspend the resulting growth from the agar surface in isotonic sodium chloride solution, and use this as a stock suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands. Store the stock suspensions of the test organisms at a temperature not exceeding 5°C, and use within 5 days.

5. Preparation of agar base layer plates

Unless otherwise specified, dispense 20 mL of the melted agar medium for the base layer into each Petri dish, and in the case of a large dish, dispense a quantity of the agar medium to form a uniform layer 2 to 3 mm thick. Distribute the agar evenly in each dish on a flat, level surface, and allow it to harden.

6. Preparation of seeded agar layers

Unless otherwise specified, determine the volume of the stock suspension of the spore or the test organism with which the employed standard solution shows a clear and definite zone of growth inhibition. Prepare the seeded agar layer by mixing thoroughly the previously determined volume of stock suspension of spore or test organism with agar medium for the seed layer kept at 48 to 51°C. Usually, the rate of a stock spore suspension and a stock suspension of the test organism to add to the agar medium for the seed layer are 0.1 to 1.0 vol% and 0.5 to 2.0 vol%, respectively.

7. Preparation of cylinder-agar plates

Dispense 4 to 6 mL of the seeded agar layer, which is specified in the individual monograph, on an agar base layer plate in a Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Place 4 cylinders on an agar plate in a Petri dish so that the individual cylinders are equidistant from the center of the plate and equally spaced from one another (the cylinders are set on the circumference of a circle of 25 to 28 mm radius). When large dish plates are used, place cylinders on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cylinders on each large dish plate is considered to be equivalent to one Petri dish plate.

Use stainless steel cylinders with the following dimensions: outside diameter 7.9 to 8.1 mm; inside diameter 5.9 to 6.1 mm; length 9.9 to 10.1 mm. The cylinders should not interfere with the test. Prepare the cylinder-agar plates before use.

8. Standard solutions

Use both a standard solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

9. Sample solutions

Use both a sample solution of high concentration and one of low concentration, as specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

10. Procedure

Unless otherwise specified, use 5 cylinder-agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cylinders for one assay set

should be equal to that defined when using Petri dishes. Apply the standard solution of high concentration and that of low concentration to a pair of cylinders set opposite each other on each plate. Apply the high and low concentration sample solutions to the remaining 2 cylinders. The same volume of these solutions must be added to each cylinder. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

11. Estimation of potency

The following correlation between the potency (P) of solution in a cylinder and the diameter (d) of zone of inhibition is established.

$$d = \alpha \log P + \beta$$

where, α and β are constants.

If necessary, ascertain the values in the above equation.

Based on this equation, estimate the potency of the sample solutions by application of the following equation:

$$\begin{aligned} \text{Amount (potency) of sample} \\ = A \times \text{Potency of } S_H \text{ per mL} \times \text{Dilution factor of } U_H \end{aligned}$$

where:

$$\log A = \frac{IV}{W}$$

$$I = \log (\text{potency of } S_H / \text{potency of } S_L)$$

$$V = \sum U_H + \sum U_L - \sum S_H - \sum S_L$$

$$W = \sum U_H + \sum S_H - \sum U_L - \sum S_L$$

The sum of the diameter (mm) of the inhibitory zone measured in each plate is designated as follows:

$$\text{for standard solution of high concentration (S}_H\text{)} = \sum S_H$$

$$\text{for standard solution of low concentration (S}_L\text{)} = \sum S_L$$

$$\text{for sample solution of high concentration (U}_H\text{)} = \sum U_H$$

$$\text{for sample solution of low concentration (U}_L\text{)} = \sum U_L$$

II. Perforated plate method

The perforated plate method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the size of the zone of growth inhibition of a test organism by the use of perforated agar plates.

This method is carried out by the use of perforated agar plates in lieu of cylinder-agar plates used in Cylinder-plate method.

Proceed as directed below, but comply with the requirements of Cylinder-plate method, such as test organisms, media, preparation of agar slant or plate media, preparation of stock suspensions of spores or test organisms, preparation of agar base layer plates, preparation of seeded agar layers, standard solutions, sample solutions, and estimation of potency.

1. Preparation of perforated agar plates

Dispense 4 to 6 mL of the seeded agar layer specified in the individual monograph on each agar base layer plate of the Petri dish. In the case of large dishes, dispense a quantity of the agar medium to form a uniform layer 1.5 to 2.5 mm thick, and spread evenly over the surface before hardening. After coagulating the agar, allow the plate to stand under a clean atmosphere to exhale moisture vapor of the inside of Petri or large dishes and water on the agar surface. Using a

suitable tool, prepare 4 circular cavities having a diameter of 7.9 to 8.1 mm on a Petri dish agar plate so that the individual cavities are equidistant from the center of the plate. The cavities spaced equally from one another on the circumference of a circle with radius 25 to 28 mm, and are deep enough to reach the bottom of dish. When large dish plates are used, prepare the circular cavities on each plate according to the method of preparation for Petri dish agar plates. A set of 4 cavities on each large dish plate is considered to be equivalent to one Petri dish plate.

Prepare the perforated agar plates before use.

2. Procedure

Unless otherwise specified, use 5 perforated agar plates as one assay set when Petri dishes are employed. When large dishes are employed, the number of cavities for one assay set should be equal to that defined when using Petri dishes. Apply the high and low concentration standard solutions to a pair of cavities prepared opposite each other on each plate, and apply the high and low concentration sample solutions to the remaining 2 cavities. The same volume of these solutions must be added to each cavity. Incubate the plates at 32 to 37°C for 16 to 20 hours. Using a suitable measuring tool, measure the diameters of the circular inhibition zones with a precision that can discriminate differences of at least 0.25 mm. Each procedure should be performed quickly under clean laboratory conditions.

III. Turbidimetric method

The turbidimetric method is a method to determine the antimicrobial potency of an antibiotic, based on the measurement of the inhibition of growth of a microbial culture in a fluid medium. The inhibition of growth of a test organism is photometrically measured as changes in turbidity of the microbial culture.

1. Test organisms

Use the test organism specified in the individual monograph.

2. Culture media

Unless otherwise specified, use media with the following compositions. When peptone is indicated as an ingredient of a medium, either meat peptone or casein peptone is applicable. Use sodium hydroxide TS or 1 mol/L hydrochloric acid TS to adjust the pH of the medium to obtain the specified value after sterilization. A different medium to the one specified for each test organism may be used if it has both a similar composition and an equal or better growth efficiency of the test organism in comparison with the specified medium. Unless otherwise specified, sterilize the media to be used in an autoclave.

(1) Agar media for transferring test organisms

Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	2.5 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.5 to 6.6 after sterilization.

(2) Liquid media for suspending test organisms

Glucose	1.0 g
Peptone	5.0 g

Meat extract	1.5 g
Yeast extract	1.5 g
Sodium chloride	3.5 g
Potassium dihydrogen phosphate	1.32 g
Disodium hydrogen phosphate*	3.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.0 to 7.1 after sterilization.

*Dipotassium hydrogen phosphate (3.68 g) may be used in lieu of disodium hydrogen phosphate (3.0 g).

3. Preparation of agar slant or plate media

Unless otherwise specified, proceed as directed in Preparation of agar slant or plate media under Cylinder-plate method.

4. Preparation of stock suspensions of test organisms

Unless otherwise specified, inoculate the test organism onto the slant or plate of the agar medium which was prepared for transferring the specified test organism. Incubate the inoculated medium at 32 to 37°C for 16 to 24 hours. The subculture should be performed at least three times. Check the aspects of the test spores or organisms as occasion demands. Inoculate the subcultured test organism onto another slant or plate of the agar medium (described above), and incubate the slant at 32 to 37°C for 16 to 24 hours. After incubation, suspend the test organism in the liquid medium for suspending the test organism, and use as the suspension of the test organism. The concentration of the test organism is confirmed with the turbidity or absorbance, as occasion demands.

5. Standard solutions

Use the standard solutions specified in the individual monograph. Unless otherwise specified, prepare the standard solutions before use.

6. Sample solutions

Use the sample solutions specified in the individual monograph. Unless otherwise specified, prepare the sample solutions before use.

7. Procedure

Unless otherwise specified, proceed as follows:

Distribute 1.0 mL of each concentration of the standard solution, the sample solution, and water used as a control, into each set composed of 3 test tubes (about 14 mm in inside diameter and about 13 cm in length). Add 9.0 mL of the suspension of the test organism to each tube, and then incubate in a water bath maintained at 35 to 37°C for 3 to 4 hours. After incubation, add 0.5 mL of dilute formaldehyde (1 in 3) to each tube, and read each transmittance or absorbance at a wavelength of 530 nm.

8. Estimation of potency

Average the transmittance or absorbance values of each concentration of the standard solution, the sample solution and water used as a control, respectively. Generate the standard curve based on the average values of transmittance or absorbance of each concentration of the standard solution, and estimate the potency of the sample solution from its average value of transmittance or absorbance using the obtained standard curve.

If the standard dilutions of five concentrations in geometrical progression are used, calculate the L and H values from the following equations. Plot point L and point H on graph

paper and construct a straight line for the standard curve.

$$L = \frac{(3a + 2b + c - e)}{5}$$

$$H = \frac{(3e + 2d + c - a)}{5}$$

where:

L = calculated value of transmittance or absorbance for the lowest concentration of the standard curve.

H = calculated value of transmittance or absorbance for the highest concentration of the standard curve.

a, b, c, d, e = average transmittance or absorbance values for each standard dilution, where a is the value from the lowest concentration standard solution, b, c and d are the values from each geometrically increased concentration standard solution, respectively, and e is the value from the highest concentration standard solution.

4.03 Digestion Test

Digestion Test is a test to measure the activity of digestive enzymes, as crude materials or preparations, on starch, protein and fat.

(1) Assay for Starch Digestive Activity

The assay for starch digestive activity is performed through the measurement of starch saccharifying activity, dextrinizing activity, and liquefying activity.

(i) Measurement of starch saccharifying activity

The starch saccharifying activity can be obtained by measuring an increase of reducing activity owing to the hydrolysis of the glucoside linkages when amylase acts on the starch. Under the conditions described in Procedure, one starch saccharifying activity unit is the amount of enzyme that catalyzes the increase of reducing activity equivalent to 1 mg of glucose per minute.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the reducing activity increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.4 to 0.8 starch saccharifying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

Use potato starch TS for measuring the starch digestive activity. If necessary, add 10 mL of buffer or salts solution specified in the monograph, instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

Procedure

Pipet 10 mL of the substrate solution, stand at $37 \pm 0.5^\circ\text{C}$ for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at $37 \pm 0.5^\circ\text{C}$ for exactly 10 minutes, add exactly 2 mL of alkaline tartrate solution of the Fehling's TS for amylolytic activity test, and shake immediately. Then, add exactly 2 mL of copper solution of the Fehling's TS for amylolytic activity test, shake gently, heat the solution in a water bath for exactly 15

minutes, and then immediately cool to below 25°C. Then, add exactly 2 mL of concentrated potassium iodide TS and 2 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> the released iodine with 0.05 mol/L sodium thiosulfate VS to the disappearance of the blue color produced by addition of 1 to 2 drops of soluble starch TS (*a* mL). Separately, pipet 10 mL of water instead of the substrate solution and titrate <2.50> in the same manner (*b* mL).

Starch saccharifying activity (unit/g)

$$= \text{amount (mg) of glucose} \times \frac{1}{10} \times \frac{1}{W}$$

Amount (mg) of glucose = (*b* - *a*) × 1.6

W: Amount (g) of sample in 1 mL of sample solution

(ii) Measurement of starch dextrinizing activity

The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component (amylose) in starch when amylase acts on the starch. Under the conditions described in Procedure, one starch dextrinizing activity unit is the amount of enzyme required to reduce the coloration of potato starch by iodine by 10% per minute.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water or a buffer or salts solution specified in the monograph so that the coloration of starch by iodine decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.2 to 0.5 starch dextrinizing activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

Prepare the substrate solution in the same manner as the substrate solution in the measurement of starch saccharifying activity.

Procedure

Pipet 10 mL of the substrate solution, stand at 37 ± 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Allow this solution to stand at 37 ± 0.5°C for exactly 10 minutes. Pipet 1 mL of this solution, add it to 10 mL of 0.1 mol/L hydrochloric acid TS, and shake immediately. Pipet 0.5 mL of this solution, add exactly 10 mL of 0.0002 mol/L iodine TS, and shake. Determine the absorbance A_T of this solution at the wavelength of 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Separately, using 1 mL of water instead of the sample solution, determine the absorbance A_B in the same manner.

Starch dextrinizing activity (unit/g)

$$= \frac{(A_B - A_T)}{A_B} \times \frac{1}{W}$$

W: Amount (g) of sample in 1 mL of sample solution

(iii) Measurement of starch liquefying activity

The starch liquefying activity can be obtained by measuring a decrease in the viscosity of starch solution resulting from the hydrolysis of molecules when amylase acts on the starch. Under the conditions described in Procedure, one starch liquefying activity unit is the amount of enzyme required to reduce the viscosity of the substrate solution equivalent to 1 g of potato starch from 200% to 100% of that

of the 50% sucrose standard solution.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the viscosity decreases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 0.15 to 0.25 starch liquefying activity unit/mL. Filter if necessary.

Preparation of Substrate Solution

Weigh accurately about 1 g of potato starch, and measure the loss of drying at 105°C for 2 hours. Weigh exactly potato starch equivalent to 15.00 g calculated on the dried basis, add 300 mL of water, then add gradually 25 mL of 2 mol/L sodium hydroxide TS under thorough shaking, until the mixture forms a paste. Heat the mixture in a water bath for 10 minutes, shaking it occasionally. After cooling, neutralize the mixture with 2 mol/L hydrochloric acid TS, and add 50 mL of the buffer solution specified in the monograph and water to make exactly 500 g. Prepare before use.

Preparation of 50% Standard Sucrose Solution

Dissolve 50.0 g of sucrose in 50.0 mL of water.

Procedure

Put 50 mL of the 50% standard sucrose solution in a 100-mL conical flask, and allow it to stand in a thermostat at 37 ± 0.5°C for 15 minutes. Fix a viscometer shown in Fig. 4.03-1 so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. After slowly pulling up the 50% standard sucrose solution by suction to the middle of the upper bulb of the viscometer, let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t_1 seconds). Take exactly 50 g of the substrate solution in another 100-mL conical flask, and stand it in another thermostat at 37 ± 0.5°C for 20 minutes. Add exactly 1 mL of the sample solution to it, and shake the flask immediately. Fix a viscometer vertically so that its lower end almost touches the bottom of the flask and that the water in the thermostat circulates around the outer cylinder of the viscometer. Occasionally pull the reaction solution up by suction to the middle of the upper bulb slowly, then let it flow down by gravity, measuring the time taken for the solution to fall from the upper to the lower indicators (t seconds).

Repeat this operation until t becomes shorter than t_1 . At each measurement, record the time (T' seconds) from the moment that the sample solution is added to the moment that the solution surface in the flask passes the upper indicator. ($T' + t/2$) is the reaction time (T) corresponding to t . Draw a curve for both t and T . Obtain T_1 and T_2 that correspond to t_1 and ($2 \times t_1$) by interpolation.

Starch liquefying activity (unit/g)

$$= \frac{60}{(T_1 - T_2)} \times \frac{1.5}{W}$$

W: Amount (g) of sample in 1 mL of sample solution

(2) Assay for Protein Digestive Activity

The protein digestive activity can be obtained by the colorimetric measurement, making use of Folin's reaction, of the amount of acid-soluble low-molecular products, which is increased owing to the hydrolysis of the peptide linkages when protease acts on casein. One protein digestive activity unit is

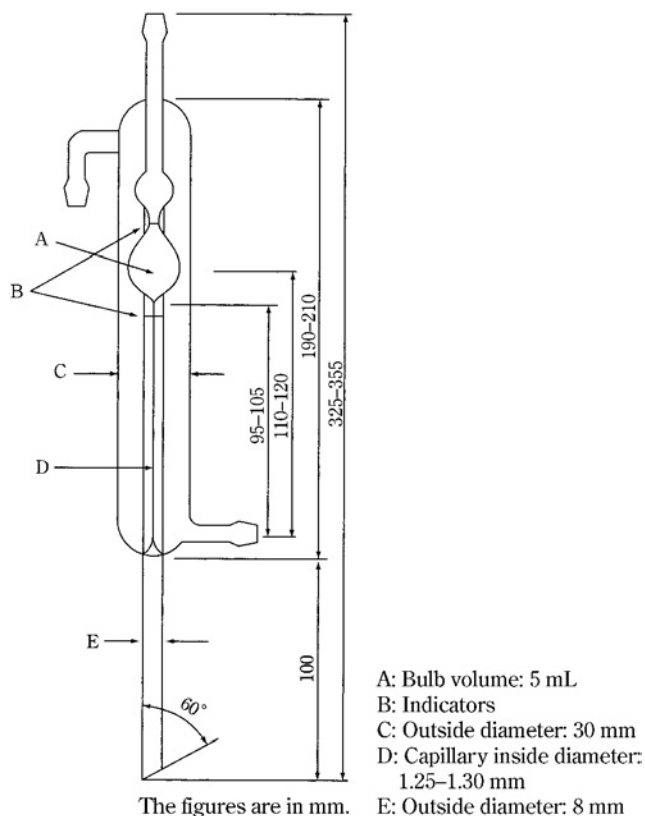


Fig. 4.03-1

the amount of enzymes that produces Folin's TS-colorable substance equivalent to 1 μg of tyrosine per minute under the conditions described in Procedure.

Preparation of Sample Solution

Dissolve the sample in an appropriate amount of water, or a buffer or salts solution specified in the monograph so that the amount of non-protein, Folin's TS-colorable substances increase in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 15 to 30 protein digestive activity unit/mL.

Tyrosine Calibration Curve

Weigh exactly 50 mg of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, and dissolve in 0.2 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 1 mL, 2 mL, 3 mL and 4 mL of this solution separately, and add 0.2 mol/L hydrochloric acid TS to each solution to make them exactly 100 mL. Pipet 2 mL of each solution, and add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to each solution, shake immediately, then stand them at 37 \pm 0.5°C for 30 minutes. Determine the absorbances, A_1 , A_2 , A_3 and A_4 , of these solutions at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with exactly 2 mL of 0.2 mol/L hydrochloric acid TS in the same manner as the blank. Then, draw a calibration curve with the absorbances, A_1 , A_2 , A_3 and A_4 as the ordinate, and with the amount (μg) of tyrosine in 2 mL of each solution as the abscissa. Obtain the amount (μg) of tyrosine for the absorbance 1.

Preparation of Substrate Solution

Substrate solution 1: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 12 mL of lactic acid TS and 150 mL of water, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Substrate solution 2: Weigh accurately about 1 g of milk casein, and measure the loss on drying at 105°C for 2 hours. Weigh exactly an amount of milk casein equivalent to 1.20 g calculated on the dried basis, add 160 mL of 0.05 mol/L disodium hydrogenphosphate TS, and warm to dissolve in a water bath. After cooling in running water, adjust to the pH specified in the monograph with the 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 200 mL. Prepare before use.

Preparation of Precipitation Reagent

Trichloroacetic acid TS A: Dissolve 7.20 g of trichloroacetic acid in water to make 100 mL.

Trichloroacetic acid TS B: Dissolve 1.80 g of trichloroacetic acid, 1.80 g of anhydrous sodium acetate and 5.5 mL of 6 mol/L acetic acid TS in water to make 100 mL.

Procedure

Pipet 5 mL of the substrate solution specified in the monograph, stand at 37 \pm 0.5°C for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. After standing this solution at 37 \pm 0.5°C for exactly 10 minutes, add exactly 5 mL of trichloroacetic acid TS A or B as specified in the monograph, shake, stand it at 37 \pm 0.5°C for 30 minutes, and then filter. Discard the first 3 mL of the filtrate, exactly measure the subsequent 2 mL of the filtrate, add exactly 5 mL of 0.55 mol/L sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 3) to the solution, shake well, and stand it at 37 \pm 0.5°C for 30 minutes. Determine the absorbance A_T of this solution at 660 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS A or B to the solution as specified in the monograph, and shake. To this solution add exactly 5 mL of the substrate solution specified in the monograph, shake immediately, and stand it at 37 \pm 0.5°C for 30 minutes. Follow the same procedure for the sample solution, and determine the absorbance A_B at 660 nm.

Protein digestive activity (unit/g)

$$= (A_T - A_B) \times F \times \frac{11}{2} \times \frac{1}{10} \times \frac{1}{W}$$

W : Amount (g) of sample in 1 mL of sample solution

F : Amount (μg) of tyrosine for absorbance 1 determined from Tyrosine Calibration Curve

(3) Assay for Fat Digestive Activity

The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from the hydrolysis of the ester linkage, when lipase acts on olive oil. One fat digestive activity unit is the amount of enzymes that produces 1 μmole of fatty acid per minute under the conditions described in Procedure.

Preparation of Sample Solution

Dissolve or suspend the sample in an appropriate amount of cold water, or a buffer or salts solution specified in the monograph so that the amount of fatty acid increases in proportion to the concentration of the sample solution, when measuring under the conditions described in Procedure. The concentration is normally 1 to 5 fat digestive activity unit/mL.

Preparation of Substrate Solution

Take 200 to 300 mL of a mixture of emulsifier and olive oil (3:1) in a blender (see Fig. 4.03-2), and emulsify it at 12,000 to 16,000 revolutions per minute for 10 minutes, while cooling the solution to a temperature below 10°C. Stand this solution in a cool place for 1 hour, and make sure before use that the oil does not separate.

Preparation of Emulsifier

Dissolve 20 g of polyvinyl alcohol specified in the monograph in 800 mL of water by heating between 75°C and 80°C for 1 hour while stirring. After cooling, filter the solution if necessary, and add water to make exactly 1000 mL.

Procedure

Pipet 5 mL of the substrate solution and 4 mL of the buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing the mixture at $37 \pm 0.5^\circ\text{C}$ for 10 minutes, add exactly 1 mL of the sample solution, and shake immediately. Stand this solution at $37 \pm 0.5^\circ\text{C}$ for exactly 20 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Then add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, add 10 mL of a mixture of ethanol (95) and acetone (1:1), and shake. Titrate $\langle 2.50 \rangle$ the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (b mL) (indicator: 2 to 3 drops of phenolphthalein TS). Separately, pipet 5 mL of the substrate solution and 4 mL of buffer solution specified in the monograph, transfer them to a conical flask, and shake. After standing it at $37 \pm 0.5^\circ\text{C}$ for 10 minutes, add 10 mL of a mixture of ethanol (95) and acetone (1:1), then add exactly 1 mL of the sample solution, and shake. Add exactly 10 mL of 0.05 mol/L sodium hydroxide VS, and titrate $\langle 2.50 \rangle$ in the same manner (a mL).

Fat digestive activity (unit/g)

$$= 50 \times (a - b) \times \frac{1}{20} \times \frac{1}{W}$$

W : Amount (g) of sample in 1 mL of sample solution

4.04 Pyrogen Test

Pyrogen Test is a method to test the existence of pyrogens by using rabbits.

Test animals

Use healthy mature rabbits, each weighing not less than 1.5 kg, which have not lost body mass when kept on a constant diet for not less than one week. House the rabbits individually in an area free from disturbances likely to excite them. Keep the temperature of the area constant between 20°C and 27°C for at least 48 hours before and throughout the test. Before using a rabbit that has not previously been used for a pyrogen test, condition it 1 to 3 days prior to the test by con-

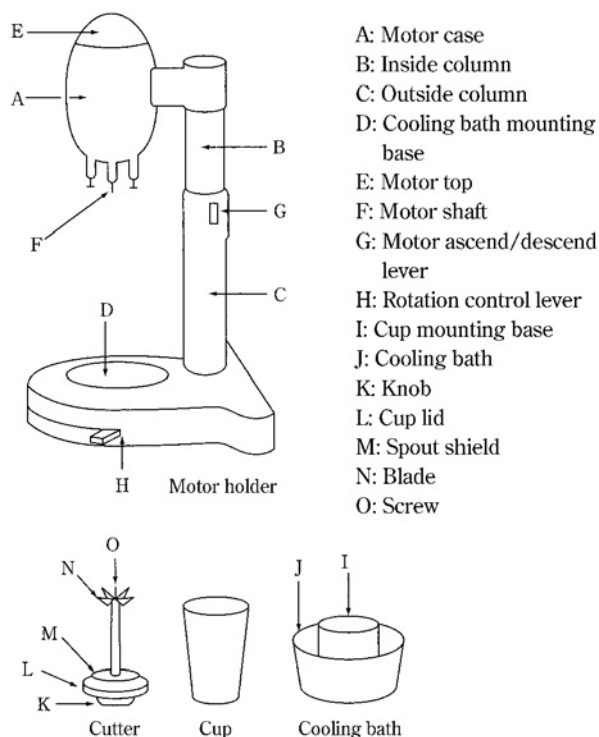


Fig. 4.03-2 Blender

ducting a sham test omitting the injection. Do not use a rabbit for pyrogen tests more frequently than once every 48 hours, or after it has been given a test sample that was adjudged pyrogen-positive or that contained an antigen present commonly in the test sample to be examined.

Apparatus, instruments

(1) Thermometer—Use a rectal thermometer or temperature-measuring apparatus with an accuracy of $\pm 0.1^\circ\text{C}$ or less.

(2) Syringe and injection needle—Depyrogenate the syringes and needles in a hot-air oven using a validated process, usually by heating at 250°C for not less than 30 minutes. Sterilized syringes with needles are also available provided that they have been validated to assure that they are free of detectable pyrogens and do not interfere with the test.

Test procedures

(1) Quantity of injection—Unless otherwise specified, inject 10 mL of the sample per kg of body mass of each rabbit.

(2) Procedure—Perform the test in a separate area at an environmental temperature similar to that of the room wherein the animals were housed and free from disturbances likely to excite them. Withhold food from the rabbits for several hours before the first record of the temperature and throughout the testing period. The test animals are usually restrained with loosely fitting neck stocks that allow the rabbits to assume a natural resting posture. Determine the temperature of each rabbit by inserting the thermometer or temperature-measuring probe into the rectum of the test animal to a constant depth within the range of 60 mm to 90 mm. The “control temperature” of each rabbit is the mean of two temperature readings recorded for that rabbit at an interval of 30 min in the 40 min immediately preceding the injection of the sample to be examined. Rabbits showing a

temperature variation greater than 0.2°C between the two successive temperature readings or rabbits having an initial temperature higher than 39.8°C are withdrawn from the test.

Warm the test solution to a temperature of $37 \pm 2^\circ\text{C}$ before injection, and inject the solution slowly into the marginal vein of the ear of each rabbit over a period not exceeding 10 min. Hypotonic test sample may be made isotonic by the addition of pyrogen-free sodium chloride. Record the temperature of each rabbit during a period of 3 hours after the injection, taking the measurements at intervals of not more than 30 min. The difference between the control temperature and the maximum temperature of each rabbit is taken to be the rise in body temperature. Consider any temperature decreases as zero rise.

Interpretation of results

The test is carried out on a group of three rabbits and the result is judged on the basis of the sum of the three temperature rises. Repeat if necessary on further groups of three rabbits to a total of three groups, depending on the results obtained. If the summed response of the first group does not exceed 1.3°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 2.5°C, the sample is judged to be pyrogen-positive. If the summed response exceed 1.3°C but does not exceed 2.5°C, repeat the test on another group of three rabbits. If the summed response of the first and second group does not exceed 3.0°C, the sample is judged to be pyrogen-negative. If the summed response of the 6 rabbits exceeds 4.2°C, the sample is judged to be pyrogen-positive. If the summed response exceeds 3.0°C but does not exceed 4.2°C, repeat the test on one more group of three rabbits. If the summed response of the 9 rabbits does not exceed 5.0°C, the sample is judged to be pyrogen-negative. If the summed response exceeds 5.0°C, the sample is judged to be pyrogen-positive.

When the test sample is judged to be pyrogen-negative, the sample passes the pyrogen test.

4.05 Microbial Limit Test

This chapter provides tests for the qualitative and quantitative estimation of viable microorganisms present in pharmaceutical articles. It includes tests for total viable count (bacteria and fungi) and specified microbial species (*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Microbial limit test must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test. When test specimens have antimicrobial activity, or contain antimicrobial substances, any such antimicrobial properties must be eliminated by means of procedures such as dilution, filtration, neutralization or inactivation. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

1. Total viable aerobic count

This test determines the mesophilic bacteria and fungi which grow under aerobic conditions. Psychrophilic, thermophilic, basophilic and anaerobic bacteria, and microor-

ganisms which require specific ingredients for growth, may give a negative result, even if a significant number exists in the test specimens. The test may be carried out using one of the following 4 methods, i.e., membrane filtration method, pour plate method, spread plate method or serial dilution method (most probable number method). Use an appropriate method depending on the purpose. An automated method may be used for the test presented here, provided it has been properly validated as giving equivalent or better results. Different culture media and temperature are required for the growth of bacteria and fungi (molds and yeasts). Serial dilution method is applicable only to the enumeration of bacteria.

Preparation of the test solution

Phosphate Buffer (pH 7.2), Buffered Sodium Chloride-Peptone Solution or fluid medium used for the test is used to dissolve or dilute the test specimen. Unless otherwise specified, 10 g or 10 mL of the test specimen is used for the test, but another quantity or volume may be used according to the nature of the test specimen. The pH of the solution is adjusted to between 6 and 8. The test solution must be used within an hour after preparation.

Fluid specimens or soluble solids: Take 10 g or 10 mL of the test specimen, mix with the buffer or fluid medium specified to make 100 mL, and use this as the test fluid. A fluid specimen containing insoluble materials must be shaken well, just prior to mixing, to effect fine suspension.

Insoluble solids: Take 10 g or 10 mL of the test specimen, reduce the substance to a fine powder, suspend it in the buffer or fluid medium specified to make 100 mL, and use this as the test fluid. A larger volume of the buffer or fluid medium than indicated may be used for the suspension, depending on the nature of the test specimen. The suspension may be dispersed well using, if necessary, a mechanical blender. A suitable surface-active agent (such as 0.1 w/v% polysorbate 80) may be added to aid dissolution.

Fatty products: For water-immiscible fluids, ointments, creams, waxes, and lotions which consist mainly of lipid, take 10 g or 10 mL of the test specimen, emulsify it in the buffer or fluid medium specified with the aid of a suitable surface-active agent such as polysorbate 20 or 80 to make 100 mL, and use this as the test fluid. An emulsion may be made by warming to a temperature not exceeding 45°C, but do not maintain this temperature for more than 30 minutes.

Test procedures

(1) Membrane Filtration Method

This method is applicable especially to specimens which contain antimicrobial substances. Use membrane filters of appropriate materials, having a normal pore size not greater than 0.45 μm . Filter discs about 50 mm in diameter are recommended, but filters of a different diameter may also be used. Filters, the filtration apparatus, media, etc., should be sterilized well. Usually, take 20 mL of the test fluid (containing 2 g of test specimen), transfer 10 mL of the solution to each of two membrane filters, and filter. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. After the filtration of the test fluid, wash each membrane by filtering through it three or more times with a suitable liquid such as Buffered Sodium Chloride-Peptone Solution, Phosphate Buffer, or the fluid medium to be used. The volume of the washings to be used is approximately 100 mL each, but in case filter disc is significantly different

from 50 mm in diameter, the volume may be adjusted according to the size of the filter. For fatty substances, the washings may contain a suitable surface-active agent such as polysorbate 80. Put one of the membrane filters, intended primarily for the enumeration of bacteria, on the surface of a plate of Soybean-Casein Digest Agar and the other, intended primarily for the enumeration of fungi, on the surface of a plate of one of Sabouraud Glucose Agar, Potato Dextrose Agar, or GP Agar Medium (each contains antibiotics). After incubation of the plates at least for 5 days, between 30°C and 35°C in the test for the detection of bacteria and between 20°C and 25°C in the test for fungi, count the number of colonies that are formed. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

(2) Pour Plate Method

Use petri dishes 9 to 10 cm in diameter. Use at least two petri dishes for each dilution. Pipet 1 mL of the test fluid or its diluted solution onto each petri dish aseptically. Promptly add to each dish 15 to 20 mL of sterilized agar medium that has previously been melted and kept below 45°C, and mix. Primarily for the detection of bacteria, use Soybean-Casein Digest Agar Medium, and, primarily for the detection of fungi, use one of Sabouraud Glucose Agar, Potato Dextrose Agar, and GP Agar Medium (each contains antibiotics). After the agar solidifies, incubate the plates for at least 5 days, between 30°C and 35°C for bacteria and between 20°C and 25°C for fungi. If too many colonies are observed, dilute the fluid as described above so that a colony count of not more than 300 per plate may be expected in the case of bacteria, and not more than 100 per plate in the case of fungi. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

(3) Spread Plate Method

On the solidified and dried surface of the agar medium, pipet 0.05 to 0.2 mL of the test fluid and spread it on the surface with a spreader. The diameter of petri dishes, the kind and volume of the medium to be used, the temperature and time of incubation, and the method for calculation of total viable count are the same as described in the Pour Plate Method section.

(4) Serial Dilution Method (Most Probable Number Method)

Prepare a series of 12 tubes each containing 9 to 10 mL of Fluid Soybean-Casein Digest Medium. Use three tubes for each dilution. To each of the first three tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. To the last three tubes add 1 mL of the diluent as a control. Incubate the tubes between 30°C and 35°C for not less than 5 days. The control tubes should show no microbial growth. If the reading of the results is difficult or uncertain, transfer about 0.1 mL to a liquid or solid medium and read the results after a further period of incubation between 30°C and 35°C for 24 to 72 hours. Determine the most probable number of microorganisms per g or mL of the specimen from Table 4.05-1.

Table 4.05-1. Most probable number of microorganisms

Number of tubes in which microbial growth is observed for each quantity of the specimen			Most probable number of microorganisms per g or per mL
0.1 g or 0.1 mL per tube	0.01 g or 0.01 mL per tube	1 mg or 1 µL per tube	
3	3	3	> 1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

If, for the first column (containing 0.1 g or 0.1 mL of specimen), the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per mL is likely to be less than 100.

Effectiveness of culture media and confirmation of antimicrobial substances

Use microorganisms of the following strains or their equivalent. Grow them in Fluid Soybean-Casein Digest Medium between 30°C and 35°C for bacteria and between 20°C and 25°C for *Candida albicans*.

Escherichia coli, such as ATCC 8739, NCIMB 8545, NBRC 3972

Bacillus subtilis, such as ATCC 6633, NCIMB 8054, NBRC 3134, JCM 2499

Staphylococcus aureus, such as ATCC 6538, NCIMB 8625, NBRC 13276

Candida albicans, such as ATCC 2091, ATCC 10231, NBRC 1594, JCM 2085

Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, or Phosphate Buffer to prepare test suspensions containing about 50 to 200 CFU per mL. Growth-promoting qualities are tested by inoculating 1 mL of each microorganism into each medium. The test media are satisfactory if clear evidence of growth appears in all inoculated media after incubation at indicated temperature for 5 days. When a count of the test organisms with a test specimen differs by more than a factor of 5 from that without the test specimen, any such effect must be eliminated by dilution, filtration, neutralization or inactivation. To confirm the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total viable count method using sterile Buffered Sodium Chloride-Peptone Solution or Phosphate Buffer as the control.

2. Test for the detection of specified microorganisms

Escherichia coli, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are included as the target strains of the test. These four species of microorganisms are im-

portant for the evaluation of microbial contamination not only in the finished products, but also in the bulk or intermediate of the production process, and are representative of the microorganisms which should not exist in these materials.

Preparation of the test fluid

If necessary, refer to the paragraph on Preparation of the Test Solution in Total viable aerobic count. When test specimens are dissolved in or diluted with a fluid medium, use the medium designated in each test, unless otherwise specified.

Test procedure

(1) *Escherichia coli*

To 10 g or 10 mL of the test specimen add a volume of Fluid Lactose Medium to make 100 mL, and incubate between 30°C and 35°C for 24 to 72 hours. Examine the medium for growth, and if growth is present, mix by gentle shaking, take a portion by means of an inoculating loop, and streak it on the surface of MacConkey Agar Medium. Incubate between 30°C and 35°C for 18 to 24 hours. If brick-red colonies of Gram-negative rods surrounded by a reddish precipitation zone are not found, the specimen meets the requirements of the test for absence of *Escherichia coli*. If colonies matching the above description are found, transfer the suspect colonies individually to the surface of EMB Agar Medium, and incubate between 30°C and 35°C for 18 to 24 hours. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen and a blue-black appearance under transmitted light, the specimen meets the requirements of the test for the absence of *Escherichia coli*. Confirm any suspect colonies on the plate by means of the IMViC test (Indole production test, Methyl red reaction test, and Voges-Proskauer test, and Citrate utilization test), and the colonies which exhibit the pattern of either [+ + - -] or [- + - -] are judged as *Escherichia coli*. Rapid detection kits for *Escherichia coli* may also be used.

(2) *Salmonella*

As in the case of the detection of *Escherichia coli*, to 10 g or 10 mL of the test specimen add a volume of Fluid Lactose Medium to make 100 mL, and incubate between 30°C and 35°C for 24 to 72 hours. Examine the medium for growth, and if growth is present, mix by gentle shaking, pipet 1 mL portions, respectively, into 10 mL of Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium, and incubate for 12 to 24 hours. 10 mL of Fluid Selenite-Cystine Medium may be replaced by the same volume of Fluid Rappaport Medium. After the incubation, streak portions from both the fluid media on the surface of at least two of Brilliant Green Agar Medium, XLD Agar Medium, and Bismuth Sulfite Agar Medium, and incubate between 30°C and 35°C for 24 to 48 hours. Upon examination, if none of the colonies conforms to the description given in Table 4.05-2, the specimen meets the requirements of the test for the absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 4.05-2 are found, transfer suspect colonies individually, by means of an inoculating wire, to a slant of TSI Agar Medium using both surface and deep inoculation. Incubate between 35°C and 37°C for 18 to 24 hours. The presence of genus *Salmonella* is confirmed if, in the deep culture but not in the surface culture, there is a change of color from red to yellow and usually a formation of gas with or without production of hydrogen sulfide. Precise identification and typing of genus *Salmonella* may be carried out by using appropriate biochemical and serological tests addition-

ally including an identification kit.

Table 4.05-2. Morphologic characteristics of *Salmonella* species on selective agar media

Medium	Description of colony
Brilliant Green Agar Medium	Small, transparent and colorless, or opaque, pink or white (often surrounded by a pink to red zone)
XLD Agar Medium	Red, with or without black centers
Bismuth Sulfite Agar Medium	Black or green

(3) *Pseudomonas aeruginosa*

To 10 g or 10 mL of the specimen add Fluid Soybean-Casein Digest Medium, or another suitable fluid medium without antimicrobial activity, to make 100 mL. Fluid Lactose Medium is not suitable. Incubate between 30°C and 35°C for 24 to 48 hours. If, upon examination, growth is present, use an inoculating loop to streak a portion of the medium on the surface of Cetrimide Agar Medium or NAC Agar Medium and incubate between 30°C and 35°C for 24 to 48 hours. If no growth of microorganisms is detected, the specimen passes the test. If growth of colonies of Gram-negative rods with a greenish fluorescence occurs, streak suspect colonies from the agar surface of Cetrimide Agar Medium on the agar surfaces of *Pseudomonas* Agar Medium for Detection of Fluorescein and *Pseudomonas* Agar Medium for Detection of Pyocyanin, and incubate between 30°C and 35°C for 24 to 72 hours. The production of yellowish fluorescence on the surface of the former medium shows the production of fluorescein, and the production of blue fluorescence on the latter medium indicates the production of pyocyanin. Confirm any suspect colonies as *Pseudomonas aeruginosa* by means of the Oxidase test. Transfer each of the suspect colonies to filter paper that has previously been impregnated with *N,N*-dimethyl-*p*-phenylenediammonium dichloride. If the colony changes to purple within 5 to 10 seconds, the oxidase test is judged as positive. If the oxidase test is judged to be negative, the specimen meets the requirements of the test for the absence of *Pseudomonas aeruginosa*. The presence of *Pseudomonas aeruginosa* may also be confirmed by using appropriate biochemical tests including an identification kit.

(4) *Staphylococcus aureus*

To 10 g or 10 mL of the specimen add Fluid Soybean-Casein Digest Medium, or another suitable fluid medium without antimicrobial activity to make 100 mL. Incubate the fluid containing the specimen between 30°C and 35°C for 24 to 48 hours. If, upon examination, growth is present, use an inoculating loop to streak a portion of the medium on the surface of one of the Vogel-Johnson Agar Medium, Baird-Parker Agar Medium, or Mannitol-Salt Agar Medium, and incubate between 30°C and 35°C for 24 to 48 hours. Upon examination, if no colonies of Gram-positive cocci having the characteristics listed in Table 4.05-3 are found, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*. Confirm any suspect colonies as *Staphylococcus aureus* by means of the coagulase test. With the aid of an inoculating loop, transfer suspect colonies to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a thermostat at 37 ± 1°C. Examine the coagulation after 3 hours and subsequently at suitable inter-

vals up to 24 hours. Test positive and negative controls simultaneously. If no coagulation is observed, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*.

Table 4.05-3. Morphologic characteristics of *Staphylococcus aureus* on selective agar media

Medium	Colonial characteristics
Vogel-Johnson Agar Medium	Black surrounded by yellow zone
Baird-Parker Agar Medium	Black, shiny, surrounded by clear zones
Mannitol-Salt Agar Medium	Yellow colonies with yellow zones

Effectiveness of culture media and confirmation of antimicrobial substances

Grow the test strains listed in Table 4.05-4 in the media indicated between 30°C and 35°C for 18 to 24 hours. Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, Phosphate Buffer, or medium indicated for each bacterial strain to make test suspensions containing about 1000 CFU per mL. As occasion demands, using a mixture of 0.1 mL of each suspension of *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* containing about 1000 CFU, the validity of the medium and the presence of antimicrobial substances are tested in the presence or absence of the specimen.

Table 4.05-4. Bacterial strains and media used for confirmation of the effectiveness of culture medium and validity of the test for specified microorganisms

Microorganisms	Strain number	Media
<i>Escherichia coli</i>	ATCC 8739, NCIMB 8545, NBRC 3972 or equivalent strains	Fluid Lactose Medium
<i>Salmonella</i>	No strain number is recommended*	Fluid Lactose Medium
<i>Pseudomonas aeruginosa</i>	ATCC 9027, NCIMB 8626, NBRC 13275 or equivalent strains	Fluid Soybean-Casein Digest Medium
<i>Staphylococcus aureus</i>	ATCC 6538, NCIMB 8625, NBRC 13276 or equivalent strains	Fluid Soybean-Casein Digest Medium

**Salmonella* strains of weak or no pathogenicity may be used. *Salmonella* Typhi may not be used.

Retest

For the purpose of confirming a doubtful result, a retest is conducted using 25 g or 25 mL of test specimen. Proceed as directed under Test procedure, but make allowance for the larger specimen size, for example by adjusting the volume of the medium.

3. Buffer solution and media

Buffer solution and media used for the microbial limit test are described below. Other media may be used if they have similar nutritive ingredients, and selective and growth-promoting properties for the microorganisms to be tested.

(1) Buffer solution

(i) Phosphate Buffer (pH 7.2)

Stock Solution: Dissolve 34 g of potassium dihydrogen-

phosphate in about 500 mL of water. Adjust to pH 7.1 to 7.3 by the addition of 175 mL of sodium hydroxide TS, add water to make 1000 mL, and use this solution as the stock solution. After sterilization by heating in an autoclave, store under refrigeration. For use, dilute the Stock Solution with water in the ratio of 1 to 800, and sterilize at 121°C for 15 to 20 minutes.

(ii) Buffered Sodium Chloride-Peptone Solution (pH 7.0)

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate dodecahydrate	18.23 g
Sodium chloride	4.30 g
Peptone	1.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.1. Polysorbate 20 or 80 (0.1 to 1.0 w/v%) may be added.

(2) Media

(i) Soybean-Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.3.

(ii) Fluid Soybean-Casein Digest Medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

(iii) Sabouraud Glucose Agar Medium with Antibiotics

Peptones (animal tissue and casein)	10.0 g
Glucose	40.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(iv) Potato Dextrose Agar Medium with Antibiotics

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(v) GP (Glucose-peptone) Agar Medium with Antibiotics

Glucose	20.0 g
Yeast extract	2.0 g

Magnesium sulfate heptahydrate	0.5 g
Peptone	5.0 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.6 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(vi) Fluid Lactose Medium

Meat extract	3.0 g
Gelatin peptone	5.0 g
Lactose monohydrate	5.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. After sterilization, cool immediately.

(vii) MacConkey Agar Medium

Gelatin peptone	17.0 g
Casein peptone	1.5 g
Animal tissue peptone	1.5 g
Lactose monohydrate	10.0 g
Sodium desoxycholate	1.5 g
Sodium chloride	5.0 g
Agar	13.5 g
Neutral red	0.03 g
Crystal violet	1.0 mg
Water	1000 mL

Mix all the components, boil for 1 minute, mix, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.3.

(viii) EMB (Eosin-Methylene Blue) Agar Medium

Gelatin peptone	10.0 g
Dipotassium hydrogen phosphate	2.0 g
Lactose monohydrate	10.0 g
Agar	15.0 g
Eosin Y	0.40 g
Methylene blue	0.065 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.3.

(ix) Fluid Selenite-Cystine Medium

Gelatin peptone	5.0 g
Lactose monohydrate	4.0 g
Trisodium phosphate dodecahydrate	10.0 g
Sodium selenite	4.0 g
L-Cystine	0.010 g
Water	1000 mL

Mix all the components, and heat to effect solution. Final pH: 6.8 – 7.2. Do not sterilize.

(x) Fluid Tetrathionate Medium

Casein peptone	2.5 g
Animal tissue peptone	2.5 g
Sodium desoxycholate	1.0 g
Calcium carbonate	10.0 g
Sodium thiosulfate pentahydrate	30.0 g
Water	1000 mL

Heat the solution of solids to boiling. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 mL of water. Then add 10 mL of a so-

lution of brilliant green (1 in 1000), and mix. Do not heat the medium after adding the brilliant green solution.

(xi) Fluid Rappaport Medium

Soybean peptone	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate	1.6 g
Malachite green oxalate	0.12 g
Magnesium chloride hexahydrate	40.0 g
Water	1000 mL

Dissolve malachite green oxalate and magnesium chloride hexahydrate, and the remaining solids separately in the water, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. For the use, mix the both solutions after sterilization. Final pH: 5.4 – 5.8.

(xii) Brilliant Green Agar Medium

Peptones (animal tissue and casein)	10.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g
Phenol red	0.080 g
Brilliant green	0.0125 g
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute. Sterilize just prior to use by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. Cool to about 50°C and pour to petri dishes.

(xiii) XLD (Xylose-Lysine-Desoxycholate) Agar Medium

D-Xylose	3.5 g
L-Lysine monohydrochloride	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	0.080 g
Sodium desoxycholate	2.5 g
Sodium thiosulfate pentahydrate	6.8 g
Ammonium iron (III) citrate	0.80 g
Agar	13.5 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.2 – 7.6. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

(xiv) Bismuth Sulfite Agar Medium

Meat extract	5.0 g
Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Glucose	5.0 g
Trisodium phosphate dodecahydrate	4.0 g
Iron (II) sulfate heptahydrate	0.30 g
Bismuth sulfite indicator	8.0 g
Brilliant green	0.025 g
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.4 – 7.8. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

(xv) TSI (Triple Sugar Iron) Agar Medium

Casein peptone	10.0 g
Animal tissue peptone	10.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g

Glucose	1.0 g
Ammonium iron (II) sulfate hexahydrate	0.20 g
Sodium chloride	5.0 g
Sodium thiosulfate pentahydrate	0.20 g
Phenol red	0.025 g
Agar	13.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. Distribute in small tubes and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5. Use as a slant agar medium. The medium containing 3 g of meat extract or yeast extract additionally, or the medium containing ammonium iron (III) citrate instead of ammonium iron (II) sulfate hexahydrate may be used.

(xvi) Cetrimide Agar Medium

Gelatin peptone	20.0 g
Magnesium chloride hexahydrate	3.0 g
Potassium sulfate	10 g
Cetrimide	0.30 g
Glycerin	10 mL
Agar	13.6 g
Water	1000 mL

Dissolve all solid components in the water, and add the glycerin. Heat, with frequent agitation, boil for 1 minute, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.0 – 7.4.

(xvii) NAC Agar Medium

Peptone	20.0 g
Dipotassium hydrogen phosphate	0.3 g
Magnesium sulfate heptahydrate	0.2 g
Cetrimide	0.2 g
Nalidixic acid	0.015 g
Agar	15.0 g
Water	1000 mL

Final pH: 7.2 – 7.6. Do not sterilize. Dissolve by warming.

(xviii) Pseudomonas Agar Medium for Detection of Fluorescein

Casein peptone	10.0 g
Animal tissue peptone	10.0 g
Dipotassium hydrogen phosphate	1.5 g
Magnesium sulfate heptahydrate	1.5 g
Glycerin	10 mL
Agar	15.0 g
Water	1000 mL

Dissolve all the solid components in the water, and add the glycerin. Heat, with frequent agitation, boil for 1 minute, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.0 – 7.4.

(xix) Pseudomonas Agar Medium for Detection of Pyocyanin

Gelatin peptone	20.0 g
Magnesium chloride hexahydrate	3.0 g
Potassium sulfate	10.0 g
Glycerin	10 mL
Agar	15.0 g
Water	1000 mL

Dissolve all the solid components in the water, and add the glycerin. Heat, with frequent agitation, boil for 1 minute, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.0 – 7.4.

(xx) Vogel-Johnson Agar Medium

Casein peptone	10.0 g
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Yeast extract	5.0 g
D-Mannitol	10.0 g
Dipotassium hydrogen phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	16.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute to effect solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 – 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xxi) Baird-Parker Agar Medium

Casein peptone	10.0 g
Meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Agar	20.0 g
Water	950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 – 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxii) Mannitol-Salt Agar Medium

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Phenol red	0.025 g
Agar	15.0 g
Water	1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 – 7.6.

4.06 Sterility Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Test for sterility is the method to establish the presence or absence of viable microorganisms (bacteria and fungi) using the defined culturing method. Unless otherwise specified, the test is carried out by I. Membrane filtration method or II. Direct inoculation method. Water, reagents, test solutions, equipment, materials and all other requisites for the test should be pre-sterilized. The test for sterility is carried out under aseptic conditions. In order to achieve such conditions, the test environment has to be adapted to the way in which the sterility test is performed. The precautions taken to avoid contamination are such that they do not affect any microorganisms which are to be revealed in the test. The working

conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

Media and rinsing fluids

Fluid thioglycolate medium, soybean-casein digest medium are used, unless otherwise specified. ♦When it is difficult to use fluid thioglycolate medium due to turbidity or viscosity of samples, alternative thioglycolate medium can be used, provided it is heated on a water bath just prior to use and incubated under anaerobic conditions. ♦ Other products of suitable quality yielding similar formulations may be used according to the indications on the label.

(1) Fluid thioglycolate medium

L-Cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose, monohydrate/anhydrate	5.5/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycolate or Thioglycolic acid	0.5 g 0.3 mL
Resazurin sodium solution (1 in 1000), freshly prepared	1.0 mL
Water	1000 mL

(pH after sterilization 7.1 ± 0.2)

Mix the L-cystine, agar, sodium chloride, glucose, water-soluble yeast extract and pancreatic digest of casein with the water, and heat until solution is effected. Dissolve the sodium thioglycolate or thioglycolic acid in the solution and, if necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.1 ± 0.2 . If filtration is necessary, heat the solution again without boiling and filter while hot through moistened filter paper. Add the resazurin sodium solution, mix and place the medium in suitable vessels which provide a ratio of surface to depth of medium such that not more than the upper half of the medium has undergone a color change indicative of oxygen uptake at the end of the incubation period. Sterilize using a validated process. Store the medium at a temperature between 2 – 25°C. If more than the upper one-third of the medium has acquired a pink color, the medium may be restored once by heating the containers in a water-bath or in free-flowing steam until the pink color disappears and cooling quickly, taking care to prevent the introduction of non-sterile air into the container.

♦(2) Alternative thioglycolate medium

L-Cystine	0.5 g
Sodium chloride	2.5 g
Glucose, monohydrate/anhydrate	5.5/5.0 g
Yeast extract (water-soluble)	5.0 g
Pancreatic digest of casein	15.0 g
Sodium thioglycolate or Thioglycolic acid	0.5 g 0.3 mL
Water	1000 mL

(pH after sterilization 7.1 ± 0.2)

The methods for preparation follow those of fluid thioglycolate medium. ♦

(3) Soybean-casein digest medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose, monohydrate/anhydrous	2.5/2.3 g

Water	1000 mL
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(pH after sterilization 7.3 ± 0.2)

Mix all the ingredients and heat until solution is effected. If necessary, add sodium hydroxide TS so that, after sterilization, the solution will have a pH of 7.3 ± 0.2 . Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2 – 25°C in a sterile container.

(4) Rinsing fluids

Meat or casein peptone	1.0 g
Water	1000 mL

(pH after sterilization 7.1 ± 0.2)

Dissolve animal tissue or casein peptone in water and adjust the pH of the solution so that, after sterilization, it will show 7.1 ± 0.2 . Filter, if necessary, to clarify, distribute into suitable vessels and sterilize using a validated process. Store at a temperature between 2 – 25°C in a sterile container.

To rinsing fluid to be used for antibiotics or pharmaceutical products containing an antimicrobial agent, a suitable neutralizer or inactive agent at concentration shown to be appropriate in the validation of the test can be added. To rinsing fluid to be used for oils, oily solutions, ointments or creams, suitable emulsifying agent at a concentration shown to be appropriate in the validation of the test, for example polysorbate 80 at a concentration of 10 g/L can be added.

Suitability of media

The media used comply with the following tests, carried out before or in parallel with the test on the product to be examined.

(1) Sterility of media

Confirm the sterility of each sterilized batch of medium by incubating a portion of the media at the specified incubation temperature for 14 days. No growth of microorganisms occurs.

(2) Growth promotion test

Test each batch of ready-prepared medium and each batch (lot) of medium prepared either from dehydrated medium or from ingredients*. Inoculate a small number (not more than 100 CFU) of microorganism listed in Table 4.06-1 ♦ or other strains considered to be equivalent to these strains ♦ in containers of each medium. Each of the test organisms should show clearly visible growth in all inoculated media within 3 days for bacteria and within 5 days for fungi.

Effective period of media

♦If prepared media are stored in unsealed containers, they can be used for one month, provided that they are tested for growth promotion within two weeks of the time of use and that color indicator requirements are met. If stored in tight containers, the media can be used for one year, provided that they are tested for growth promotion within 3 months of the time of use and that the color indicator requirements are met. ♦

Validation test

The validation may be performed simultaneously with the Test for sterility of the product to be examined in the following cases.

a) When the test for sterility has to be carried out on a new product.

♦*In appropriate cases periodic testing of the different batches prepared from the same lot of dehydrated medium is acceptable. ♦

Table 4.06-1. Microorganisms for growth promotion test and the validation test

Medium	Test microorganisms	Incubation conditions
Fluid thioglycolate medium	<i>Staphylococcus aureus</i> (ATCC 6538, NBRC 13276, CIP 4.83, NCTC 10788, NCIMB 9518) <i>Pseudomonas aeruginosa</i> (ATCC 9027, NBRC 13275, NCIMB 8626, CIP 82.118) <i>Clostridium sporogenes</i> (ATCC 19404, CIP 79.3, NCTC 532, or ATCC 11437, NBRC 14293)	Aerobic
♦Alternative thioglycolate medium	<i>Clostridium sporogenes</i> (ATCC 19404, CIP 79.3, NCTC 532, or ATCC 11437, NBRC 14293)	Anaerobic♦
Soybean-casein digest medium	<i>Bacillus subtilis</i> (ATCC 6633, NBRC 3134, CIP 52.62, NCIMB 8054) <i>Candida albicans</i> (ATCC 10231, NBRC 1594, IP 48.72, NCPF 3179) <i>Aspergillus niger</i> (ATCC 16404, NBRC 9455, IP 1431.83, IMI 149007)	Aerobic

Seed lot culture maintenance techniques (seed-lot systems) are used so that the viable microorganisms used for inoculation are not more than five passages removed from the original master seed-lot.

b) Whenever there is a change in the experimental conditions of the test.

Carry out the test as described below under *Test for sterility of the product to be examined* using exactly the same methods except for the following modifications.

Membrane filtration After transferring the content of the container or containers to be tested to the membrane add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.

Direct inoculation After transferring the contents of the container or containers to be tested to the culture medium add an inoculum of a small number of viable micro-organisms (not more than 100 CFU) to the medium.

In both cases use the same micro-organisms as those described above under *Growth promotion test*. Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days. If clearly visible growth of micro-organisms is obtained after the incubation, visually comparable to that in the control vessel without product, either the product possesses no antimicrobial activity under the conditions of the test or such activity has been satisfactorily eliminated. The test for sterility may then be carried out without further modification. If clearly visible growth is not obtained in the presence of the product to be tested, visually comparable to that in the control vessels without product, the product possesses antimicrobial activity that has not been satisfactorily eliminated

under the conditions of the test. Modify the conditions in order to eliminate the antimicrobial activity and repeat the validation test.

In the membrane filtration, the antimicrobial activity should be suppressed by suitable means such as replacement of the membrane filters with less adsorptive ones, increase of the amount of rinsing fluid, or addition of a suitable inactivating agent to the rinsing fluid. Do not exceed a washing cycle of 5 times ♦100 mL♦ per filter, even if during validation it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity.

In the direct inoculation, use a suitable inactivating agent which does not affect the growth of microorganisms or increase the volume of medium irrespective of the prescription in II-2 so that no antimicrobial activity remains.

Test for sterility of the products to be examined

Number of articles to be tested

Items to be used for the test are taken from the lot according to an appropriate sampling plan prepared by referring to the numbers specified in Table 4.06-2.

Testing methods

The test may be carried out using the technique of membrane filtration or by direct inoculation of the culture media with the product to be examined. Appropriate negative controls are included. The technique of membrane filtration is used whenever the nature of the product permits, that is, for filterable aqueous preparations, for alcoholic or oily preparations and for preparations miscible with or soluble in aqueous or oily solvents provided these solvents do not have an antimicrobial effect in the conditions of the test.

I. Membrane filtration

By this method, a test article is filtered through a membrane filter, and the filter is rinsed and incubated by being transferred to a medium or by adding a medium to the filtration apparatus. Use membrane filter made from suitable material having a nominal pore size of 0.45 µm or smaller. Use a filter funnel sterilizable by the moist heat method or other methods and free from any leakage or back flow when filtration is performed with the membrane in place. The technique described below assumes that membranes about 50 mm in diameter will be used. If filters of a different diameter are used the volumes of the dilutions and the washings should be adjusted accordingly.

I-1. Preparation of sample solution

- Liquid medicine: Use as it is, as the sample solution.
- ♦Solid medicine: In the case of a solid medicine, to be administered after dissolving or suspending, the sample solution is prepared with the provided solvent, isotonic sodium chloride solution or water to give the concentration of use.♦
- Oils and oily solutions: Oils and oily solutions of sufficiently low viscosity may be filtered without dilution through a dry membrane. Viscous oils may be diluted as necessary with a suitable sterile diluent such as isopropyl myristate shown not to have antimicrobial activity in the conditions of the test.
- Ointments and creams: Ointments in a fatty base and emulsions of the water-in-oil type may be diluted by using sterile isopropyl myristate that has previously been filtered through a sterilizing membrane filter or by using other solvents not affecting the growth of microorganisms. Heat

Table 4.06-2. ♦Number of items to be taken from the lot.♦

Number of items in the lot	Minimum number of items to be tested for each medium* ¹
Injections Not more than 100 containers	10% or 4 containers, whichever is greater
More than 100 but not more than 500 containers	10 containers
More than 500 containers	2% or 20 containers, whichever is less
♦For large-volume products (more than 100 mL) of more than 500 containers.♦	♦2% or 10 containers, whichever is less.♦
Ophthalmic and other non-injectable products Not more than 200 containers	5% or 2 containers, whichever is greater
More than 200 containers If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral use	10 containers
Bulk solid products* ² Up to 4 containers More than 5 containers but not more than 50 containers More than 50 containers	Each container 20% or 4 containers, whichever is greater 2% or 10 containers, whichever is greater
<i>Antibiotic Solids</i> Pharmacy bulk packages (≥ 5 g) ^{*3} ♦Pharmacy bulk packages (< 5 g).♦	6 containers ♦20 containers.♦

*¹If the contents of one container are enough to inoculate the two media, this column gives the number of containers needed for both the media together.

*²Bulk solid product is a sterile bulk powder product to be able to prepare plural injections.

*³Pharmacy bulk package is an antibiotic solid to be able to prepare plural injections. It should be dispensed in an infusion set at a time after dissolving under a clean atmosphere.

the sample preparation, if necessary, to not more than 40°C. In exceptional cases it may be necessary to heat to not more than 44°C.

I-2. Quantities of sample solution to be tested

Use for each medium not less than quantity of the product prescribed in Table 4.06-3, unless otherwise specified. If the contents of one container are insufficient to inoculate the two media, twice or more containers shown in Table 4.06-2 are used. When using the technique of membrane filtration, use, whenever possible, the whole contents of the container, but not less than the quantities indicated in Table 4.06-3, diluting where necessary to about 100 mL with a suitable sterile rinsing fluid.

I-3. Procedures

Usually complete the filtration of the sample solution with one or two separate filter funnels. Transfer the contents of the container or containers to be tested to the membrane or

Table 4.06-3. Minimum quantity to be used for each medium

Quantity per container	Minimum quantity to be used for each medium
Liquids	
Less than 1 mL	The whole contents of each container
1 – 40 mL	Half the contents of each container but not less than 1 mL
Greater than 40 mL and not greater than 100 mL	20 mL
Greater than 100 mL	10% of the contents of the container but not less than 20 mL
Antibiotic liquids	1 mL
Other preparations soluble in water or in isopropyl myristate	The whole contents of each container to provide not less than 200 mg
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200 mg
Solids	
Less than 50 mg	The whole contents of each container
50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
300 mg – 5 g	150 mg
Greater than 5 g	500 mg

membranes. If the sample solution is not readily filterable, it may be further diluted with rinsing fluid and thereafter filtered. Rinse the membrane(s) with each 100-mL of rinsing fluid per filter for established cycles in the validation test. Provided the sample does not have antimicrobial activity, the rinsing procedure can be omitted. Employ either of the two methods described below for incubation of the membrane(s). Use the same volume of each medium as in the validation test.

(1) The processed membrane is aseptically transferred from the apparatus and cut into two equal parts, or half the volume of sample solution is filtered into an entire membrane. Transfer each half of the cut membrane, or each whole membrane into the medium.

(2) After filtration of sample solution into the apparatus to which the membrane filters are fitted, each medium is added to the apparatus itself.

II. Direct inoculation of the culture medium

This is the method by which the entire content or a portion of the content of a sample container is transferred directly to the culture medium and incubated. Usually, this method is applied for medicines to which the membrane filtration method cannot be applied or for which the application of the direct transfer method, rather than the membrane filtration method, is rational.

♦For products containing a mercurial preservative that cannot be tested by the membrane-filtration method, fluid thioglycolate medium incubated at 20 – 25°C may be used instead of Soybean-casein digest medium.♦

II-1. Preparation of sample solution

Usually, proceed as directed for the membrane filtration method. In the case of an insoluble medicine, the product is suspended or crushed in a suitable manner and used as a sample.

- a) Oily liquids. Use media to which have been added a suitable emulsifying agent at a concentration shown to be appropriate in the validation of the test, for example, polysorbate 80 at a concentration of 10 g/L.
- b) Ointments and creams. Prepare by diluting to about 1 in 10 by emulsifying with the chosen emulsifying agent in a suitable sterile diluent such as a 1 g/L neutral solution of meat or casein peptone. Transfer the diluted product to a medium not containing an emulsifying agent.

II-2. Quantities of sample solution to be tested

Transfer the quantity of the preparation to be examined prescribed in Table 4.06-3, by using pipette, syringe or other suitable inoculation devices, directly into the culture medium so that the volume of the product is not more than 10% of the volume of the medium, unless otherwise prescribed. Shake cultures containing oily products gently each observation day. However when thioglycolate medium is used for the detection of anaerobic microorganisms keep shaking or mixing to a minimum in order to maintain anaerobic conditions.

Cultivation and observation

Fluid thioglycolate medium ♦ and Alternative thioglycolate medium ♦ are to be incubated at 30 – 35°C and Soybean-casein digest medium is to be incubated at 20 – 25°C for not less than 14 days. Observe the cultures several times during the incubation period. If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be readily determined by visual examination, 14 days after the beginning of incubation transfer ♦ suitable portions of ♦ the medium to fresh vessels of the same medium and then incubate the original and transfer vessels for not less than 4 days.

Observation and interpretation of results

If no evidence of microbial growth is found, the product to be examined complies with the test for sterility. If evidence of microbial growth is found the product examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. ♦ However, provided that the test itself was inadequate, the test is repeated. ♦ If no evidence of microbial growth is found in the repeat test the product complies with the Sterility Test. If microbial growth is found in the repeat test the product does not comply with the Sterility Test.

5. Tests for Crude Drugs

5.01 Crude Drugs Test

Crude Drugs Test is applied to the crude drugs mentioned in the General Rules for Crude Drugs.

Sampling

Unless otherwise specified, sample should be taken by the

following methods. If necessary, preserve the samples in tight containers.

(1) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(2) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly.

(3) When the mass of each single piece of the crude drugs is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly.

Preparation of the test sample for analysis

Preparations are to be made by mixing the sample well. Powdered drugs should be used as they are, and in the case of unpowdered drugs, unless otherwise specified, grind the sample into powder. If the sample cannot be ground into powder, reduce it as finely as possible, spread it out in a thin layer, and withdraw a typical portion for analysis. If necessary, preserve the test sample in a tight container.

Microscopic examination**(1) Apparatus**

Use an optical microscope with objectives of 10 and 40 magnifications, and an ocular of 10 magnifications.

(2) Preparation for microscopic examination

(i) Section: To a section on a slide glass add 1 to 2 drops of a mounting agent, and put a cover glass on it, taking precaution against inclusion of bubbles. Usually use a section 10 to 20 μm in thickness.

(ii) Powder: Place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of a swelling agent, stir well with a small rod preventing inclusion of bubbles, and allow to stand for a while to swell the sample. Add 1 drop of the mounting agent, and put a cover glass on it so that the tissue sections spread evenly without overlapping each other, taking precaution against inclusion of bubbles. In the case where the tissue sections are opaque, place about 1 mg of powdered sample on a slide glass, add 1 to 2 drops of chloral hydrate TS, heat to make the tissues clear while stirring with a small glass rod to prevent boiling. After cooling, add 1 drop of mounting agent, and put a cover glass on it in the same manner as above.

Unless otherwise specified, use a mixture of glycerin and water (1:1) or a mixture of water, ethanol (95) and glycerin (1:1:1) as the mounting agent and swelling agent.

(3) Observation of components in the Description

In each monograph, description is usually given of the outer portion and the inner portion of a section in this order, followed by a specification of cell contents. Observation should be made in the same order. In the case of a powdered sample, description is given of a characteristic component or a matter present in large amount, rarely existing matter, and cell contents in this order. Observation should be made in the same order.

Purity

(1) Foreign matter—Unless otherwise specified, weigh 25 to 500 g of the sample, spread out in a thin layer, and separate the foreign matter by inspecting with the naked eye or with the use of a magnifying glass of 10 magnifications. Weigh, and determine the percentage of foreign matter.

(2) Total BHC's and total DDT's—Sodium chloride, anhydrous sodium sulfate and synthetic magnesium silicate for

column chromatography used in this procedure are used after drying by heating at about 130°C for more than 12 hours and cooling in a desiccator (silica gel). Chromatographic column is prepared as follows: Place 20 g of synthetic magnesium silicate for column chromatography in a 200-mL flask, add 50 mL of hexane for Purity of crude drug, shake vigorously, and immediately pour the mixture into a chromatographic tube about 2 cm in inside diameter and about 30 cm in length. Drip until the depth of hexane layer at the upper part is about 5 cm, introduce 8 g of anhydrous sodium sulfate from the top, and further drip until a small quantity of hexane is left at the upper part.

Weigh accurately about 5 g of pulverized sample, place in a glass-stoppered centrifuge tube, add 30 mL of a mixture of acetone for Purity of crude drug and water (5:2), stopper tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the same procedure twice with the residue using two 30-mL portions of the mixture of acetone for Purity of crude drug and water (5:2). Combine all the supernatant liquids, and concentrate under reduced pressure at a temperature not higher than 40°C until the order of acetone is faint. Transfer the concentrated solution to a separator containing 100 mL of sodium chloride TS, and shake twice with two 50-mL portions of hexane for Purity of crude drug for 5 minutes each. Combine the hexane layers, transfer to a separator containing 50 mL of sodium chloride TS, and shake for 5 minutes. Take the hexane layer, dry with 30 g of anhydrous sodium sulfate, and filter. Wash the residue on the filter paper with 20 mL of hexane for Purity of crude drug. Combine the filtrate and the washings, and concentrate under reduced pressure at a temperature not higher than 40°C to about 5 mL. Transfer this solution to the chromatographic column and allow to pass with 300 mL of a mixture of hexane for Purity of crude drug and diethyl ether for Purity of crude drug (17:3) at a rate of not more than 5 mL per minute. After concentrating the eluate under reduced pressure at a temperature not higher than 40°C, add hexane for Purity of crude drug to make exactly 5 mL. Transfer this solution to a glass-stoppered test tube, add 1 mL of sulfuric acid, and shake carefully. Take 4 mL of the upper layer, transfer to a separate glass-stoppered test tube, add 2 mL of water, and shake gently. Take 3 mL of the upper layer so obtained, transfer to a glass-stoppered centrifuge tube, dry with 1 g of anhydrous sodium sulfate, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg each of α -BHC, β -BHC, γ -BHC, δ -BHC, o,p' -DDT, p,p' -DDT, p,p' -DDD and p,p' -DDE, dissolve in 5 mL of acetone for Purity of crude drug, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 10 mL of this solution, and add hexane for Purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for Purity of crude drug to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and the standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas corresponding to α -BHC, β -BHC, γ -BHC, δ -BHC, o,p' -DDT, p,p' -DDT, p,p' -DDD and p,p' -DDE, A_{TA} and A_{SA} ; A_{TB} and A_{SB} ; A_{TC} and A_{SC} ; A_{TD} and A_{SD} ; A_{TE} and A_{SE} ; A_{TF} and A_{SF} ; A_{TG} and A_{SG} ; A_{TH} and A_{SH} . Calculate the content of each of α -BHC, β -BHC, γ -BHC, δ -BHC, o,p' -DDT, p,p' -DDT, p,p' -DDD and p,p' -DDE by means of the following equations.

$$\begin{aligned} \text{Content (ppm) of } \alpha\text{-BHC} \\ = \frac{\text{amount (g) of } \alpha\text{-BHC}}{W} \times \frac{A_{TA}}{A_{SA}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } \beta\text{-BHC} \\ = \frac{\text{amount (g) of } \beta\text{-BHC}}{W} \times \frac{A_{TB}}{A_{SB}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } \gamma\text{-BHC} \\ = \frac{\text{amount (g) of } \gamma\text{-BHC}}{W} \times \frac{A_{TC}}{A_{SC}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } \delta\text{-BHC} \\ = \frac{\text{amount (g) of } \delta\text{-BHC}}{W} \times \frac{A_{TD}}{A_{SD}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } o,p'\text{-DDT} \\ = \frac{\text{amount (g) of } o,p'\text{-DDT}}{W} \times \frac{A_{TE}}{A_{SE}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } p,p'\text{-DDT} \\ = \frac{\text{amount (g) of } p,p'\text{-DDT}}{W} \times \frac{A_{TF}}{A_{SF}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } p,p'\text{-DDD} \\ = \frac{\text{amount (g) of } p,p'\text{-DDD}}{W} \times \frac{A_{TG}}{A_{SG}} \times 50 \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of } p,p'\text{-DDE} \\ = \frac{\text{amount (g) of } p,p'\text{-DDE}}{W} \times \frac{A_{TH}}{A_{SH}} \times 50 \end{aligned}$$

W : Amount (g) of pulverized sample

$$\begin{aligned} \text{Content (ppm) of total BHC's} \\ = \text{content (ppm) of } \alpha\text{-BHC} + \text{content (ppm) of } \beta\text{-BHC} \\ + \text{content (ppm) of } \gamma\text{-BHC} + \text{content (ppm) of } \delta\text{-BHC} \end{aligned}$$

$$\begin{aligned} \text{Content (ppm) of total DDT's} \\ = \text{content (ppm) of } o,p'\text{-DDT} + \text{content (ppm) of } p,p'\text{-DDT} \\ + \text{content (ppm) of } p,p'\text{-DDD} + \text{content (ppm) of } p,p'\text{-DDE} \end{aligned}$$

Operating conditions—

Detector: An electron capture detector

Sample injection system: A splitless injection system

Column: A fused silica capillary column about 0.3 mm in inside diameter and about 30 m in length, coated the inside wall with 7% cyanopropyl-7% phenylmethylsilicone polymer for gas chromatography in a thickness of 0.25 to 1.0 μ m.

Column temperature: Maintain the temperature at 60°C for 2 minutes after injection, program to increase the temperature at a rate of 10°C per minute to 200°C, and then program to increase the temperature at a rate of 2°C per minute to 260°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention times of the objective compounds are between 10 and 30 minutes.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add hexane to make exactly 10 mL. Confirm that the peak area of each objective compound obtained with 1 μ L of this solution is equivalent to 5 to 15% of that of cor-

responding compound with 1 μL of the standard solution.

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, the peaks of the object compounds separate completely each other.

System repeatability: Repeat the test 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of each object compound is not more than 10%.

Loss on drying

Unless otherwise specified, transfer 2 to 6 g of the test sample for analysis to a tared weighing bottle, and weigh accurately. Dry at 105°C for 5 hours, allow to cool in a desiccator (silica gel), and weigh accurately. Continue the drying at 105°C, and weigh accurately at 1-hour intervals. When the mass of the sample becomes constant, the loss of mass represents the percentage of loss on drying (%). When the period of time for drying is specified, weigh accurately after drying for the period of time specified, and determine the loss on drying (%).

Total ash

Ignite previously a crucible of platinum, quartz or porcelain between 500°C and 550°C for 1 hour. Cool, and weigh accurately the crucible. Unless otherwise specified, weigh accurately 2 to 4 g of the test sample for analysis in this crucible, take off the lid or keep it open a little if necessary, heat the crucible at a low temperature at first, then gradually heat to a temperature between 500°C and 550°C, ignite to incinerate the residue for more than 4 hours until no carbonized substance remains in the ash, cool, and weigh accurately the ash. Incinerate repeatedly to constant mass, cool, weigh accurately, and determine the amount (%) of total ash. If a carbonized substance remains and a constant mass cannot be obtained in the above-mentioned method, extract the charred mass with hot water, collect the insoluble residue on filter paper for assay, and incinerate the residue and filter paper until no carbonized substance remains in the ash. Then add the filtrate, evaporate it to dryness, and incinerate. Cool, weigh accurately, and determine the mass (%) of the total ash. If a carbon-free ash cannot be obtained even in this way, moisten the ash with a small amount of ethanol (95), break up the ash with a glass rod, wash the rod with a small amount of ethanol (95), evaporate carefully, and determine the mass of the total ash as described above. A desiccator (silica gel) is used for cooling.

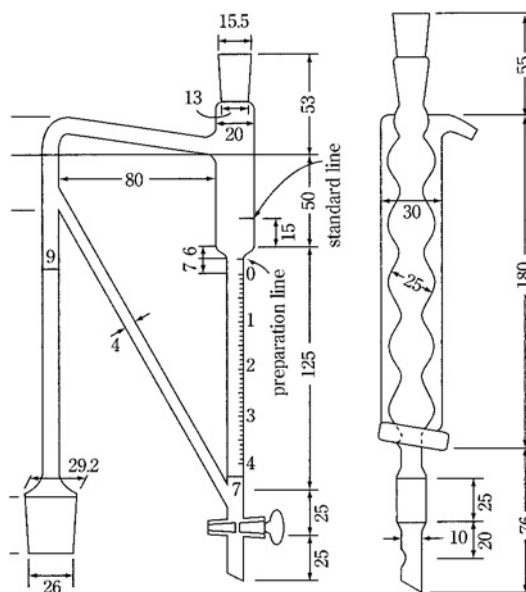
Acid-insoluble ash

Add carefully 25 mL of dilute hydrochloric acid to the total ash, boil gently for 5 minutes, collect the insoluble matter on filter paper for assay, and wash thoroughly with hot water. Dry the residue together with the filter paper, and ignite to incinerate in a tared crucible of platinum, quartz or porcelain for 3 hours. Cool in a desiccator (silica gel), weigh, and determine the amount (%) of acid-insoluble ash. When the amount determined exceeds the limit specified, incinerate repeatedly to a constant mass.

Extract content

The test for the extract content in crude drugs is performed as directed in the following methods:

(1) **Dilute ethanol-soluble extract**—Unless otherwise specified, weigh accurately about 2.3 g of the sample for analysis, extract with 70 mL of dilute ethanol in a suitable flask



The figures are in mm.

Fig. 5.01-1

Fig. 5.01-2

with occasional shaking for 5 hours, and allow to stand for 16 to 20 hours. Filter, and wash the flask and residue with small portions of dilute ethanol until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness, dry at 105°C for 4 hours, and cool in a desiccator (silica gel). Weigh accurately the amount, multiply it by 2, and determine the amount of dilute ethanol-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

(2) **Water-soluble extract**—Proceed as directed in (1), using water instead of dilute ethanol, weigh accurately the amount, multiply by 2, and determine the amount of water-soluble extract. Calculate the extract content (%) with respect to the amount of the sample on the dried basis, obtained under the loss on drying.

(3) **Diethyl ether-soluble extract**—Unless otherwise specified, dry the test sample for analysis in a desiccator (silica gel) for 48 hours, weigh accurately about 2 g of it, and place in a suitable flask. Add 70 mL of diethyl ether, attach a reflux condenser to the flask, and boil gently on a water bath for 4 hours. Cool, filter, and wash the flask and the residue with small portions of diethyl ether until the filtrate measures 100 mL. Evaporate a 50 mL aliquot of the filtrate to dryness on a water bath, dry in a desiccator (silica gel) for 24 hours, weigh accurately the amount, multiply it by 2, determine the amount of diethyl ether-soluble extract, and calculate the extract content (%).

Essential oil content

The test of essential oil content in crude drugs is performed as directed in the following method:

Essential oil determination: Weigh the quantity of the test sample for analysis directed in the monograph in a 1-L hard glass-stoppered flask, and add from 5 to 10 times as much water as the drug. Set up an apparatus for essential oil determination (Fig. 5.01-1), inserting a reflux condenser (Fig.

5.01-2) in the upper mouth of it, and heat the content of the flask in an oil bath between 130°C and 150°C to boiling. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2.0 mL of xylene is added to the graduated tube. Unless otherwise specified, continue boiling for 5 hours, allow to stand for some time, and open the stopper of the apparatus. Draw off the water slowly until the surface of the oil layer corresponds to the preparation line, and allow it to stand for more than 1 hour at ordinary temperature. Then lower the surface of the oil layer to the zero line, and read the volume (mL) of the oil at ordinary temperature. Subtract the volume (mL) of xylene from the volume of the total oil.

5.02 Microbial Limit Test for Crude Drugs

This chapter provides tests for the qualitative and quantitative estimation of viable microorganisms present in crude drugs. It includes tests for total viable count (aerobic bacteria and fungi) and specified microbial species (Enterobacteria and other gram-negative bacteria, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*). Microbial limit test must be carried out under conditions designed to avoid accidental microbial contamination of the preparation during the test. When test specimens have antimicrobial activity, or contain antimicrobial substances, any such antimicrobial properties must be eliminated by means of procedures such as dilution, filtration, neutralization or inactivation. For the test, use a mixture of several portions selected at random from the bulk or from the contents of a sufficient number of containers. If test specimens are diluted with fluid medium, the test should be performed quickly. In performing the test, precautions must be taken to prevent biohazard.

1. Total viable aerobic count

This test determines mesophilic aerobic bacteria and fungi (molds and yeasts) which grow under aerobic conditions. Psychrophilic, thermophilic, basophilic, and anaerobic bacteria, and microorganisms which require specific ingredients for growth, may give a negative result, even if a significant number exists in the test specimens. The test may be carried out using one of the following 4 methods, i.e., pour plate method, spread plate method, serial dilution method (most probable number method) or membrane filtration method. Use an appropriate method depending on the purpose. An automated method may be used for the test presented here, provided it has been properly validated as giving equivalent or better results. Different culture media and temperature are required for the growth of bacteria and fungi (molds and yeasts). The serial dilution method is applicable only to the enumeration of bacteria.

Sampling and Preparation of the test specimens

Unless otherwise specified, samples should be taken by the following methods.

(1) When crude drugs to be sampled are small-sized, cut or powdered, 50 to 250 g of sample should be taken after mixing thoroughly.

(2) When crude drugs to be sampled are large-sized, 250 to 500 g of sample should be taken after mixing thoroughly

and cutting.

(3) When the mass of each single piece of the crude drug is not less than 100 g, not less than 5 pieces should be taken for a sample, or not less than 500 g of the sample should be taken after cutting to a suitable size and mixing thoroughly. If necessary, cut more for use.

(4) When crude drugs to be sampled are in the form of a solution or a preparation, the sample should be taken after mixing thoroughly.

(5) An insoluble solid should be taken after reducing the substance to a moderately fine powder.

Preparation of the test fluid

Phosphate Buffer, pH 7.2, Buffered Sodium Chloride-Peptone Solution, pH 7.0 or fluid medium used for the test is used to suspend or dilute the test specimen. Unless otherwise specified, usually take 10 g or 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A test specimen as a suspension must be shaken for 10 minutes. If necessary, for crude drugs to which microorganisms might adhere, repeat the same method and use this as the test fluid. A different quantity or volume may be used if the nature of the test specimen requires it. The pH of the test fluid is adjusted to between 6 and 8. The test fluid must be used within an hour after preparation.

Fluid specimen: Take 10 mL of the test specimen, and suspend or dissolve it in 90 mL of the buffer or fluid medium specified. A different quantity or volume may be used if the nature of the test specimen requires it.

Insoluble solids: Pulverize the substance to a moderately fine powder, take 10 g of the test specimen, and suspend it in 90 mL of the buffer or fluid medium specified. A different quantity or a larger volume of buffer and fluid medium than indicated may be used for the suspension, if the nature of the test specimen requires it. The suspension may be dispersed well using, if necessary, a mechanical blender. A suitable surface active agent (such as 0.1 w/v% Polysorbate 80) may be added to aid dissolution.

Test procedures

(1) Pour Plate Method

Use petri dishes 9 to 10 cm in diameter. Use at least two petri dishes for each dilution. Pipet 1 mL of the test fluid or its diluted solution onto each petri dish aseptically. Promptly add to each dish 15 to 20 mL of sterilized agar medium that has previously been melted and kept below 45°C, and mix. Primarily for the detection of aerobic microbes, use Soybean-Casein Digest Agar Medium. For specimens that consist of fragments of crude drugs, or to control the growth of fungi, 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) TS for aerobic bacterial strains and amphotericin B TS as an antimycotic may be added to the agar. Just prior to use, add 2.5–5 mL of TTC TS or 2 mL of amphotericin B TS per liter of sterile medium and mix. Primarily for the detection of fungi, use one of Sabouraud Glucose Agar with antibiotics, Potato Dextrose Agar with antibiotics, and GP Agar Medium with antibiotics. For an agar medium that is suffused with fungi, Rose Bengal TS may be added to the agar. Add 5 mL of Rose Bengal TS per liter of agar medium, mix and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. After the agar solidifies, incubate the plates for at least 5 days at between 30°C and 35°C for aerobic bacteria, and between 20°C and 25°C for fungi. If too many colonies are observed, dilute the fluid as described above so that a colony count of not

more than 300 per plate may be expected in the case of aerobic bacteria, and not more than 100 per plate in the case of fungi. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

(2) Spread Plate Method

On the solidified and dried surface of the agar medium, pipet 0.05 to 0.2 mL of the test fluid and spread it on the surface with a spreader. The diameter of petri dishes, the kind and volume of the medium to be used, TS to be added, temperature and time of incubation, and the method for calculation of total viable count are the same as described in the Pour Plate Method section.

(3) Serial Dilution Method (Most Probable Number Method)

Prepare tubes each containing 9 to 10 mL of Fluid Soybean-Casein Digest Medium. To each of the first three tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. If necessary, dilute further. To the last three tubes add 1 mL of the diluent as a control. Incubate the tubes between 30°C and 35°C for not less than 5 days. The control tubes should show no microbial growth. If the reading of the results is difficult or uncertain, transfer about 0.1 mL to a liquid or solid medium and read the results after a further period of incubation between 30°C and 35°C for 24 to 72 hours. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

Table 5.02-1. Most probable number of microorganisms

Number of tubes in which microbial growth is observed for each quantity of the specimen			Most probable number of microorganisms per g or per mL
0.1 g or 0.1 mL per tube	0.01 g or 0.01 mL per tube	1 mg or 1 μ L per tube	
3	3	3	> 1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

If, for the first column (0.1 g or 0.1 mL of specimen), the number of tubes showing microbial growth is two or less, the most probable number of microorganisms per g or per mL is likely to be less than 100.

(4) Membrane Filtration Method

This method employs membrane filters of appropriate materials, having a normal pore size not greater than 0.45

μ m. Filter discs about 50 mm in diameter are recommended, but filters of a different diameter may also be used. Filters, the filtration apparatus, media, etc., should be well sterilized. Usually, take 20 mL of the test fluid (containing 2 g of test specimen), transfer 10 mL of the solution to each of two membrane filters, and filter. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. After the filtration of the test fluid, wash each membrane by filtering through it three or more times with a suitable liquid such as Buffered Sodium Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or the fluid medium to be used. The volume of the washing to be used is approximately 100 mL each time, but if the filter disc is not about 50 mm in diameter, the volume may be adjusted according to the size of the filter. For fatty substances, the washings may contain a suitable surface-active agent such as Polysorbate 80. Put one of the membrane filters, intended primarily for the enumeration of aerobic bacteria, on the surface of a plate of Soybean-Casein Digest Agar and the other, intended primarily for the enumeration of fungi, on the surface of a plate of one of Sabouraud Glucose Agar with antibiotics, Potato Dextrose Agar with antibiotics, and GP Agar Medium with antibiotics. After incubation of the plates for at least 5 days, at between 30°C and 35°C in the test for the detection of aerobic bacteria and between 20°C and 25°C in the test for fungi, count the number of colonies that are formed. If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

Effectiveness of culture media and confirmation of antimicrobial substances

Use microorganisms of the following strains or their equivalent. Grow them in Fluid Soybean-Casein Digest Medium between 30°C and 35°C for aerobic bacteria and between 20°C and 25°C for *Candida albicans*.

<i>Escherichia coli</i> ,	NBRC 3972, ATCC 8739, NCIMB 8545, etc.
<i>Bacillus subtilis</i> ,	NBRC 3134, ATCC 6633, NCIMB 8054, etc.
<i>Staphylococcus aureus</i> ,	NBRC 13276, ATCC 6538, NCIMB 9518, etc.
<i>Candida albicans</i> ,	NBRC 1393, NBRC 1594, ATCC 2091, ATCC 10231, etc.

Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, pH 7.0, or Phosphate Buffer, pH 7.2 to prepare test suspensions containing 50 to 200 cfu per mL. Growth-promoting qualities are tested by inoculating 1 mL of each microorganism into each medium. The test media are satisfactory if clear evidence of growth appears in all inoculated media after incubation at the indicated temperature for 5 days. When a count of test organisms with a test specimen is less than 1/5 of that without the test specimen, any such effect must be eliminated by dilution, filtration, neutralization or inactivation. To confirm the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total viable count method using sterile Buffered Sodium Chloride-Peptone Solution, pH 7.0 or Phosphate Buffer, pH 7.2 as the control.

2. Test for the detection of specified microorganisms

Enterobacteria and certain other gram-negative bacteria, *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*, are included as target strains of the test.

Sampling and Preparation of the test specimens

Refer to the paragraph on Sampling and Preparation of the Test Solution in Total viable aerobic count.

Preparation of the test fluid

If necessary, refer to the paragraph on Preparation of the Test Fluid in Total viable aerobic count. When test specimens are prepared, use the medium designated in each test, unless otherwise specified. If necessary, eliminate antimicrobial substances so as to permit growth of the inocula, and adjust the quantity of test specimen or increase the volume of medium to suitable values.

Test Procedure

(1) Enterobacteria and certain other gram-negative bacteria

(i) Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Transfer 10 mL to 90 mL of Enterobacteria enrichment broth Mossel and incubate at between 35°C and 37°C for 18 to 24 hours. Mix by gently shaking the container, take a portion by means of an inoculating loop, and streak it on the surface of Crystal violet, Neutral red, Bile Agar with glucose. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, the specimen may contain Enterobacteria and certain other gram-negative bacteria.

(ii) Quantitative evaluation

If Enterobacteria and certain other gram-negative bacteria are found, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Transfer 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen) to a tube containing 9 mL of the fluid, and mix. Next, transfer 1 mL of the test fluid (containing 0.01 g or 0.01 mL of specimen) to a tube containing 9 mL of the fluid, and mix. Furthermore, transfer 1 mL of the test fluid (containing 1 mg or 1 μ L of specimen) to a tube containing 9 mL of the fluid, and mix. Incubate the tubes at between 35°C and 37°C for 18 to 24 hours, take a portion by means of an inoculating loop, and streak it on the surface of Crystal violet, Neutral red, Bile Agar with glucose. Incubate at between 35°C and 37°C for 18 to 24 hours. If red or reddish colonies are found, this constitutes a positive result. Note the smallest quantity of the product which gives a positive result and the largest quantity that gives a negative result. Determine from Table 5.02-2 the probable number of microorganisms.

Table 5.02-2. Most probable number of microorganisms

Results for each quantity of product			Probable number of microorganisms (cfu) per g or per mL
0.1 g or 0.1 mL	0.01 g or 0.01 mL	1 mg or 1 μ L	
+	+	+	more than 10 ³
+	+	-	less than 10 ³ and more than 10 ²
+	-	-	less than 10 ² and more than 10 ¹
-	-	-	less than 10 ¹

(2) *Escherichia coli*

(i) Detection of bacteria

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to make a suspension or solution. Transfer 1 mL to a fermentation tube containing 9 to 10 mL of EC

broth and incubate the tube at 44.5 \pm 0.2°C for 24 \pm 2 hours in a water bath. If gas bubbles are not found, the specimen meets the requirements of the test for absence of *Escherichia coli*. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between 30°C and 35°C for 18 to 24 hours. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen and a blue-black appearance under transmitted light, the specimen meets the requirements of the test for absence of *Escherichia coli*. Confirm any suspect colonies on the plate by means of the IMViC test. (Indole production test, Methyl red reaction test, Voges-Proskauer test, and Citrate utilization test); colonies which exhibit the pattern of either [++--] or [-+--] are judged as *Escherichia coli*. Rapid detection kits for *Escherichia coli* may also be used.

(ii) Quantitative evaluation

If *Escherichia coli* is found, prepare tubes each containing 9 to 10 mL of EC broth. Use three tubes for each dilution. To 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium and suspend or dissolve. To each of the first three fermentation tubes add 1 mL of the test fluid (containing 0.1 g or 0.1 mL of specimen), resulting in 1 in 10 dilution. To the next three fermentation tubes add 1 mL of a 1 in 10 dilution of the fluid, resulting in 1 in 100 dilution. To the next three fermentation tubes add 1 mL of a 1 in 100 dilution of the fluid, resulting in 1 in 1000 dilution. To the last three fermentation tubes add 1 mL of the diluent as a control. Incubate the tubes at 44.5 \pm 0.2°C for 24 \pm 2 hours in a water bath. If gas bubbles are found, take a portion by means of an inoculating loop, and streak it on the surface of EMB Agar Medium. Incubate at between 30°C to 35°C for 18 to 24 hours. Upon examination, colonies of Gram-negative organisms show both a characteristic metallic sheen and a blue-black appearance under transmitted light. Determine the most probable number of microorganisms per g or per mL of the specimen from Table 5.02-1.

(3) *Salmonella*

As in the case of the detection of *Escherichia coli*, to 10 g or 10 mL of the test specimen add 90 mL of Fluid Lactose Medium to form a suspension or solution. Incubate at between 30°C to 35°C for 24 to 72 hours. Examine the medium for growth, and if growth is apparent, mix by gentle shaking, then pipet 1 mL portions, respectively, into 10 mL of Fluid Selenite-Cystine Medium and Fluid Tetrathionate Medium, and incubate for 12 to 24 hours. 10 mL of Fluid Selenite-Cystine Medium may be replaced by the same volume of Fluid Rappaport Medium. After the incubation, streak portions from both the fluid media on the surface of at least two of Brilliant Green Agar Medium, XLD Agar Medium, and Bismuth Sulphite Agar Medium, and incubate at between 30°C and 35°C for 24 to 48 hours. Upon examination, if none of the colonies conforms to the description given in Table 5.02-3, the specimen meets the requirements of the test for the absence of the genus *Salmonella*. If colonies of Gram-negative rods matching the description in Table 5.02-3 are found, transfer suspect colonies individually, by means of an inoculating wire, to a slant of TSI Agar Medium using both surface and deep inoculation. Incubate at between 35°C and 37°C for 18 to 24 hours. The presence of genus *Salmonella* is confirmed if, in the deep culture but not in the surface culture, there is a change of color from red to yellow and usually formation of gas with or without production of hydrogen sul-

fide. Precise identification and typing of genus *Salmonella* may be carried out by using appropriate biochemical and serological tests additionally, including an identification kit.

Table 5.02-3. Morphologic characteristics of *Salmonella* species on selective agar media

Medium	Description of colony
Brilliant Green Agar Medium	Small, transparent and colorless, or opaque, pink or white (often surrounded by a pink to red zone)
XLD Agar Medium	Red, with or without a black center
Bismuth Sulfite Agar Medium	Black or green

(4) *Staphylococcus aureus*

To 10 g or 10 mL of the test specimen add 90 mL of Fluid Soybean-Casein Digest Medium, or another suitable fluid medium without antimicrobial activity, to form a suspension or solution. Incubate the fluid containing the specimen at between 30°C and 35°C for 24 to 48 hours, and pipet 1 mL into 9 mL of Fluid Soybean-Casein Digest Medium with 7.5% sodium chloride. If, upon examination, growth is apparent, use an inoculating loop to streak a portion of the medium on the surface of one of Vogel-Johnson Agar Medium, Baird-Parker Agar Medium, or Mannitol-Salt Agar Medium, and incubate at between 30°C and 35°C for 24 to 48 hours. Upon examination, if no colonies of Gram-positive rods having the characteristics listed in Table 5.02-4 are found, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*. Confirm any suspect colonies as *Staphylococcus aureus* by means of the coagulase test. With the aid of an inoculating loop, transfer suspect colonies to individual tubes, each containing 0.5 mL of mammalian, preferably rabbit or horse, plasma with or without suitable additives. Incubate in a thermostat at 37 ± 1°C. Examine the coagulation after 3 hours and subsequently at suitable intervals up to 24 hours. Test positive and negative controls simultaneously. If no coagulation is observed, the specimen meets the requirements of the test for the absence of *Staphylococcus aureus*.

Table 5.02-4. Morphologic characteristics of *Staphylococcus aureus* on selective agar media

Medium	Colonial characteristics
Vogel-Johnson Agar Medium	Black surrounded by a yellow zone
Baird-Parker Agar Medium	Black, shiny, surrounded by a clear zone
Mannitol-Salt Agar Medium	Yellow colonies surrounded by a yellow zone

Effectiveness of culture media and confirmation of antimicrobial substances

Grow the test strains listed in Table 5.02-5 in the media indicated at between 30°C and 35°C for 18 to 24 hours. Dilute portions of each of the cultures using Buffered Sodium Chloride-Peptone Solution, pH 7.0, Phosphate Buffer, pH 7.2, or medium indicated for each bacterial strain to make test suspensions containing about 1000 cfu per mL. As occasion demands, using a mixture of 0.1 mL of each suspension of *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* con-

taining about 1000 cfu, test the validity of the medium and the presence of antimicrobial substances in the presence or absence of the specimen.

Table 5.02-5. Bacteria strains and media used for confirmation of the effectiveness of culture medium and validity of the test for specified microorganisms

Microorganism	Strain number	Media
<i>Escherichia coli</i>	NBRC 3972, ATCC 8739, NCIMB 8545 or equivalent strains	Fluid Lactose Medium
<i>Salmonella</i>	No strain number is recommended*	Fluid Lactose Medium
<i>Staphylococcus aureus</i>	NBRC 13276, ATCC 6538, NCIMB 9518 or equivalent strains	Fluid Soybean-Casein Digest Medium

**Salmonella* strains of weak or no pathogenicity may be used. *Salmonella typhi* may not be used.

Retest

For the purpose of confirming a doubtful result, a retest is conducted using a test specimen 2.5 times larger than the first test specimen. Proceed as directed under Test procedure, but make allowance for the larger specimen size, for example by adjusting the volume of the medium.

3. Buffer solution, media and test solution (TS)

Buffer solution, media and TS used for the microbial limit test are described below. Other media may be used if they have similar nutritive ingredients, and selective and growth-promoting properties for the microorganisms to be tested.

(1) Buffer solution

(i) Phosphate Buffer, pH 7.2

Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate in about 500 mL of water. Adjust to pH 7.1 to 7.3 by the addition of 175 mL of sodium hydroxide TS, add water to make 1000 mL, and use this solution as the stock solution. After sterilization by heating in an autoclave, store under refrigeration. For use, dilute the Stock Solution with water in the ratio of 1 to 800, and sterilize at 121°C for 15 to 20 minutes.

(ii) Buffered Sodium Chloride-Peptone Solution, pH 7.0

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate dodecahydrate	18.23 g
Sodium chloride	4.30 g
Peptone	1.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.1. Polysorbate 20 or 80 (0.1 to 1.0 w/v%) may be added.

(2) Media

(i) Soybean-Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.3.

(ii) Fluid Soybean-Casein Digest Medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

(iii) Sabouraud Glucose Agar Medium with Antibiotics

Peptone (animal tissue and casein)	10.0 g
Glucose	40.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(iv) Potato Dextrose Agar Medium with Antibiotics

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.4 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(v) GP (Glucose-peptone) Agar Medium with Antibiotics

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g
Peptone	5.0 g
Potassium dihydrogen phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 5.6 – 5.8. Just prior to use, add 0.10 g of benzylpenicillin potassium and 0.10 g of tetracycline per liter of medium as sterile solutions or, alternatively, add 50 mg of chloramphenicol per liter of medium.

(vi) Fluid Lactose Medium

Meat extract	3.0 g
Gelatin peptone	5.0 g
Lactose monohydrate	5.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. After sterilization, cool immediately.

(vii) Fluid EC Medium

Peptone	20.0 g
Lactose monohydrate	5.0 g
Bile salts	1.5 g
Dipotassium hydrogen phosphate	4.0 g
Potassium dihydrogen phosphate	1.5 g
Sodium chloride	5.0 g
Water	1000 mL

Mix all the components, and sterilize by heating in an au-

toclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.8 – 7.0. After sterilization cool immediately. If gas remains in durham tube after cool, do not use the tube.

(viii) EMB (Eosin-Methylene Blue) Agar Medium

Gelatin peptone	10.0 g
Dipotassium hydrogen phosphate	2.0 g
Lactose monohydrate	10.0 g
Agar	15.0 g
Eosin	0.40 g
Methylene blue	0.065 g
Water	1000 mL

Mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.9 – 7.3.

(ix) Fluid Enterobacteria Enrichment Broth Mossel Medium

Gelatin peptone	10.0 g
Glucose	5.0 g
Bile salts	20.0 g
Potassium dihydrogen phosphate	2.0 g
Disodium hydrogen phosphate dodecahydrate	8.0 g
Brilliant green	0.015 g
Water	1000 mL

Mix all the components, boil at 100°C for 30 minutes, and cool immediately. pH after boiling: 7.0 – 7.4.

(x) VRB (Violet/Red/Bile) Agar with glucose

Yeast extract	3.0 g
Gelatin peptone	7.0 g
Bile salts	1.5 g
Lactose monohydrate	10.0 g
Sodium chloride	5.0 g
Glucose	10.0 g
Agar	15.0 g
Neutral red	0.030 g
Crystal violet	0.002 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.2 – 7.6. Do not sterilize in an autoclave.

(xi) Fluid Selenite-Cystine Medium

Gelatin peptone	5.0 g
Lactose monohydrate	4.0 g
Trisodium phosphate dodecahydrate	10.0 g
Sodium selenite	4.0 g
L-Cystine	0.010 g
Water	1000 mL

Mix all the components, and heat to dissolve. Final pH: 6.8 – 7.2. Do not sterilize.

(xii) Fluid Tetrathionate Medium

Casein peptone	2.5 g
Animal tissue peptone	2.5 g
Sodium desoxycholate	1.0 g
Calcium carbonate	10.0 g
Sodium thiosulfate pentahydrate	30.0 g
Water	1000 mL

Heat the solution of solids to boiling. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 mL of water. Then add 10 mL of a solution of brilliant green (1 in 1000), and mix. Do not heat the medium after adding the brilliant green solution.

(xiii) Fluid Rappaport Medium

Soybean peptone	5.0 g
Sodium chloride	8.0 g

Potassium dihydrogen phosphate	1.6 g
Malachite green oxalate	0.12 g
Magnesium chloride hexahydrate	40.0 g
Water	1000 mL

Dissolve malachite green oxalate and magnesium chloride hexahydrate, and the remaining solids separately in the water, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. For the use, mix the both solutions after sterilization. Final pH: 5.4 – 5.8.

(xiv) Brilliant Green Agar Medium

Peptones (animal tissue and casein)	10.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g
Phenol red	0.080 g
Brilliant green	0.0125 g
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute. Sterilize just prior to use by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 6.7 – 7.1. Cool to about 50°C and pour to petri dishes.

(xv) XLD (Xylose-Lysine-Desoxycholate) Agar Medium

D-Xylose	3.5 g
L-Lysine monohydrochloride	5.0 g
Lactose monohydrate	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	0.080 g
Sodium desoxycholate	2.5 g
Sodium thiosulfate pentahydrate	6.8 g
Ammonium iron (III) citrate	0.80 g
Agar	13.5 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.2 – 7.6. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

(xvi) Bismuth Sulfite Agar Medium

Meat extract	5.0 g
Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Glucose	5.0 g
Trisodium phosphate dodecahydrate	4.0 g
Iron (II) sulfate heptahydrate	0.30 g
Bismuth sulfite indicator	8.0 g
Brilliant green	0.025 g
Agar	20.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. pH after boiling: 7.4 – 7.8. Do not sterilize in an autoclave or overheat. Cool to about 50°C and pour to petri dishes.

(xvii) TSI (Triple Sugar Iron) Agar Medium

Casein peptone	10.0 g
Animal tissue peptone	10.0 g
Lactose monohydrate	10.0 g
Sucrose	10.0 g
Glucose	1.0 g
Ammonium iron (II) sulfate hexahydrate	0.20 g
Sodium chloride	5.0 g
Sodium thiosulfate pentahydrate	0.20 g

Phenol red	0.025 g
Agar	13.0 g
Water	1000 mL

Mix all the components, and boil to effect solution. Distribute in small tubes and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5. Use as a slant agar medium. The medium containing 3 g of meat extract or yeast extract additionally, or the medium containing ammonium iron (III) citrate instead of ammonium iron (II) sulfate hexahydrate may be used.

(xviii) Fluid Soybean-Casein Digest Medium with 7.5% Sodium Chloride

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	75.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 g
Water	1000 mL

Add the sodium chloride 70.0 g for (ii) Fluid Soybean-Casein Digest Medium (containing 5 g of sodium chloride), mix all the components, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.1 – 7.5.

(xix) Vogel-Johnson Agar Medium

Casein peptone	10.0 g
Yeast extract	5.0 g
D-Mannitol	10.0 g
Dipotassium hydrogen phosphate	5.0 g
Lithium chloride	5.0 g
Glycine	10.0 g
Phenol red	0.025 g
Agar	16.0 g
Water	1000 mL

Mix all the components, and boil for 1 minute to make solution. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 7.0 – 7.4. To this solution add 20 mL of sterile potassium tellurite solution (1 in 100), and mix.

(xx) Baird-Parker Agar Medium

Casein peptone	10.0 g
Meat extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Agar	20.0 g
Water	950 mL

Mix all the components. Heat the mixture with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes, and cool to between 45°C and 50°C. pH after sterilization: 6.6 – 7.0. To this solution add 10 mL of sterile potassium tellurite solution (1 in 100) and 50 mL of egg-yolk emulsion. Mix gently, and pour into petri dishes. Prepare the egg-yolk emulsion by mixing egg-yolk and sterile saline with the ratio of about 30% to 70%.

(xxi) Mannitol-Salt Agar Medium

Casein peptone	5.0 g
Animal tissue peptone	5.0 g
Meat extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Phenol red	0.025 g

Agar	15.0 g
Water	1000 mL

Mix all the components. Heat with frequent agitation, and boil for 1 minute. Sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. pH after sterilization: 7.2 – 7.6.

(3) Reagents·Test solutions

Amphotericin B powder Amphotericin B added sodium deoxycholic acid, sterilized by γ -ray.

Amphotericin B TS Dissolve 22.5 mg of amphotericin B powder in 9 mL of sterile purified water.

Bile salts Yellow-brown powder made from dried bile of animal, consist of sodium taurocholic acid and sodium glycocholic acid, and containing not less than 45% of cholic acid. pH of 5% solution: 5.5 – 7.5.

Rose bengal $C_{20}H_2Cl_4I_4Na_2O_5$ [Special class] Red-brown powder, purple-red solution in water.

Rose bengal TS Dissolve 1 g of rose bengal in water to make 100 mL.

TTC TS (2,3,5-Triphenyl-2H-tetrazolium chloride TS)

Dissolve 0.8 g of 2,3,5-triphenyl-2H-tetrazolium chlorid in water to make 100 mL, distribute in small tubes, and sterilize by heating in an autoclave at 121°C for 15 to 20 minutes. Store in light-resistant containers.

(4) Preparation

Preparation of agar medium with TTC

Just prior to use, add 2.5 to 5 mL of TTC TS per liter of sterile agar medium and mix.

Preparation of agar medium with amphotericin B

Just prior to use, add 2 mL of amphotericin B TS in a liter of agar medium, previously sterilized in an autoclave at 121°C for 15 to 20 minutes, and mix.

Preparation of agar medium with rose bengal

Add 5 mL of rose bengal TS in a liter of agar medium, and mix. Sterilize in an autoclave at 121°C for 15 to 20 minutes.

6. Tests for Preparations in Ophthalmic Ointments

6.01 Test for Metal Particles in Ophthalmic Ointments

Test of Metal Particles in Ophthalmic Ointments is a method to test the existence of foreign metal particles in the ophthalmic ointments described in General Rules for Preparations.

Preparation of test sample

The test should be carried out in a clean place. Take 10 ophthalmic ointments to be tested, and extrude the contents as completely as practicable into separate flat-bottomed petri dishes 60 mm in diameter when the amount of the content is 5 g or less. Weigh 5 g of the contents when the amount of the content is more than 5 g, and proceed in the same manner as described above. Cover the dishes, and heat between 85°C and 110°C for 2 hours to dissolve bases. Allow the samples to cool to room temperature without agitation to solidify the contents.

Procedure

Invert each dish on the stage of a suitable microscope previously adjusted to provide more than 40 times magnifications and equipped with an eyepiece micrometer disk. Each dish is illuminated from above 45° relative to the plane of the dish. Examine the entire bottom of each dish for metal particles, and record the total number of particles, measuring 50 μ m or more in any dimension.

Note: Use petri dishes with a clean bottom and free from foams and scratches, and if possible, the walls are at right angles with the bottom.

6.02 Uniformity of Dosage Units

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (\blacklozenge \blacklozenge).

The term “Uniformity of dosage unit” is defined as the degree of uniformity in the amount of the drug substance among dosage units. Therefore, the requirements of this chapter apply to each drug substance being comprised in dosage units containing one or more drug substances, unless otherwise specified elsewhere in this Pharmacopoeia.

To ensure the consistency of dosage units, each unit in a batch should have a drug substance content within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of a drug substance in each dosage unit. The Uniformity of Dosage Units specification is not intended to apply to suspensions, emulsions, or gels in unit-dose containers intended for external, cutaneous administration.

The uniformity of dosage units can be demonstrated by either of two methods, *Content uniformity* or *Mass variation* (see Table 6.02-1.). The test for *Content Uniformity* of preparations presented in dosage units is based on the assay of the individual contents of drug substance(s) of a number of dosage units to determine whether the individual contents are within the limits set. The *Content Uniformity* method may be applied in all cases.

The test for *Mass Variation* is applicable for the following dosage forms:

- (1) solutions enclosed in unit-dose containers and into soft capsules \blacklozenge in which all components are perfectly dissolved; \blacklozenge
- (2) solids (including powders, granules and sterile solids) that are packaged in single-unit containers and contain no active or inactive added substances;
- (3) solids (including sterile solids) that are packaged in single-unit containers, with or without active or inactive added substances, that have been prepared from true solutions and freeze-dried in the final containers and are labeled to indicate this method of preparation; and
- (4) hard capsules, uncoated tablets, or film-coated tablets, containing 25 mg or more of a drug substance comprising 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, \blacklozenge or in the case of film-coated tablets, the pre-coated tablets, \blacklozenge except that uniformity of other drug substances present in lesser proportions is demonstrated by meeting *Content Uniformity* requirements.

The test for *Content Uniformity* is required for all dosage forms not meeting the above conditions for the *Mass Varia-*

Table 6.02-1. Application of Content Uniformity (CU) and Mass Variation (MV) Test for Dosage Forms

Dosage Forms	Type	Sub-type	Dose and ratio of drug substance	
			≥ 25 mg & ≥ 25%	< 25 mg or < 25%
Tablets	uncoated		MV	CU
	coated	Film	MV	CU
		others	CU	CU
Capsules	hard		MV	CU
	soft	Sus., eml., gel	CU	CU
		solutions	MV	MV
Solids in single unit containers ♦(divided forms, lyophilized forms, et al.)◆	Single component		MV	MV
	Multiple components	Solution freeze-dried in final container	MV	MV
		others	CU	CU
Solutions enclosed in unit-dose containers			MV	MV
Others			CU	CU

Sus.: suspension; eml.: emulsion;

tion test. Alternatively, products listed in item (4) above that do not meet the 25 mg/25% threshold limit may be tested for uniformity of dosage units by *Mass Variation* instead of the *Content Uniformity* test if the concentration relative standard deviation (RSD) of the drug substance in the final dosage units is not more than 2%, based on process validation data and development data, and if there has been regulatory approval of such a change. The concentration RSD is the RSD of the concentration per dosage unit (w/w or w/v), where concentration per dosage unit equals the assay result per dosage unit divided by the individual dosage unit weight. See the RSD formula in Table 6.02-2.

CONTENT UNIFORMITY

Select not less than 30 units, and proceed as follows for the dosage form designated.

Where different procedures are used for assay of the preparation and for the content uniformity test, it may be necessary to establish a correction factor to be applied to the results of the latter.

Solid dosage forms—Assay 10 units individually using an appropriate analytical method. Calculate the acceptance value (see Table 6.02-2).

Liquid dosage forms—Carry out the assay on the amount of well-mixed material that is removed from an individual container in conditions of normal use and express the results as delivered dose. Calculate the acceptance value (see Table 6.02-2.).

Calculation of Acceptance Value

Calculate the acceptance value by the formula:

$$|M - \bar{X}| + ks,$$

in which the terms are as defined in Table 6.02-2.

MASS VARIATION

Mass Variation is carried out based on the assumption that the concentration (mass of drug substance per mass of dosage unit) is uniform in a lot.

Carry out an assay for the drug substance(s) on a represen-

tative sample of the batch using an appropriate analytical method. This value is result *A*, expressed as % of label claim (see *Calculation of the Acceptance Value*). Select not less than 30 dosage units, and proceed as follows for the dosage form designated.

Uncoated or film-coated Tablets. Accurately weigh 10 tablets individually. Calculate the content, expressed as % of label claim, of each tablet from the mass of the individual tablets and the result of the assay. Calculate the acceptance value.

Hard Capsules. Accurately weigh 10 capsules individually, taking care to preserve the identity of each capsule. Remove the contents of each capsule by suitable means. Accurately weigh the emptied shells individually, and calculate for each capsule the net mass of its contents by subtracting the mass of the shell from the respective gross mass. Calculate the drug substance content of each capsule from the mass of the individual capsules and the result of the assay. Calculate the acceptance value.

Soft Capsules. Accurately weigh the 10 intact capsules individually to obtain their gross masses, taking care to preserve the identity of each capsule. Then cut open the capsules by means of a suitable clean, dry cutting instrument such as scissors or a sharp open blade, and remove the contents by washing with a suitable solvent. Allow the occluded solvent to evaporate from the shells at room temperature over a period of about 30 min, taking precautions to avoid uptake or loss of moisture. Weigh the individual shells, and calculate the net contents. Calculate the drug substance content in each capsule from the mass of product removed from the individual capsules and the result of the assay. Calculate the acceptance value.

Solid dosage forms other than tablets and capsules—Proceed as directed for *Hard Capsules*, treating each dosage unit as described therein. Calculate the acceptance value.

Liquid dosage forms—Accurately weigh the amount of liquid that is removed from each of 10 individual containers in

Table 6.02-2.

Variable	Definition	Conditions	Value
\bar{X}	mean of individual contents (x_1, x_2, \dots, x_n) expressed as a percentage of the label claim		
x_1, x_2, \dots, x_n	individual contents of the dosage units tested, expressed as a percentage of the label claim		
n	sample size (number of dosage units in a sample)		
k	acceptability constant	If $n = 10$, then	2.4
		If $n = 30$, then	2.0
s	sample standard deviation		$\sqrt{\frac{\sum_{i=1}^n (x_i - \bar{X})^2}{n-1}}$
<i>RSD</i>	relative standard deviation (the sample standard deviation expressed as a percentage of the mean)		$\frac{100s}{\bar{X}}$
<i>M</i> (case 1) To be applied when $T \leq 101.5$	reference value	If $98.5\% \leq \bar{X} \leq 101.5\%$, then	$M = \bar{X}$ ($AV = ks$)
		If $\bar{X} < 98.5\%$, then	$M = 98.5\%$ ($AV = 98.5 - \bar{X} + ks$)
		If $\bar{X} > 101.5\%$, then	$M = 101.5\%$ ($AV = \bar{X} - 101.5 + ks$)
<i>M</i> (case 2) To be applied when $T > 101.5$	reference value	If $98.5\% \leq \bar{X} \leq T$, then	$M = \bar{X}$ ($AV = ks$)
		If $\bar{X} < 98.5\%$, then	$M = 98.5\%$ ($AV = 98.5 - \bar{X} + ks$)
		If $\bar{X} > T$, then	$M = T\%$ ($AV = \bar{X} - T + ks$)
Acceptance Value (<i>AV</i>)			general formula: $ M - \bar{X} + ks$ [Calculations are specified above for the different cases.]
<i>L1</i>	maximum allowed acceptance value		$L1 = 15.0$ unless otherwise specified.
<i>L2</i>	maximum allowed range for deviation of each dosage unit tested from the calculated value of <i>M</i>	On the low side, no dosage unit result can be less than $0.75M$ while on the high side, no dosage unit result can be greater than $1.25M$ (This is based on an <i>L2</i> value of 25.0.)	$L2 = 25.0$ unless otherwise specified.
<i>T</i>	target test sample amount at time of manufacture. Unless otherwise specified in the individual monograph, <i>T</i> is 100.0%.		

conditions of normal use. Calculate the drug substance content in each container from the mass of product removed from the individual containers and the result of the assay. Calculate the acceptance value.

Calculation of Acceptance Value

Calculate the acceptance value as shown in *Content Uniformity*, except that ♦the value of \bar{X} is replaced with A , and that ♦ the individual contents of the dosage units are replaced with the individual estimated contents defined below.

$x_1, x_2 \dots x_n$ = individual estimated contents of the dosage units tested, where

$$x_i = w_i \times \frac{A}{\bar{W}}$$

$w_1, w_2 \dots w_n$ = individual masses of the dosage units tested,

A = content of drug substance (% of label claim) obtained using an appropriate analytical method.

\bar{W} = mean of individual masses ($w_1, w_2 \dots, w_n$).

CRITERIA

Apply the following criteria, unless otherwise specified.

Solid and Liquid Dosage Forms—The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to $L1\%$. If the acceptance value is greater than $L1\%$, test the next 20 dosage units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to $L1\%$ and no individual content of the dosage unit is less than $(1 - L2 \times 0.01)M$ nor more than $(1 + L2 \times 0.01)M$ in *Calculation of Acceptance Value* under *Content Uniformity* or under *Mass Variation*. Unless otherwise specified, $L1$ is 15.0 and $L2$ is 25.0.

6.03 Particle Size Distribution Test for Preparations

Particle Size Distribution Test for Preparations is a method to determine the particle size distribution of the granules and powders described in General Rules for Preparations.

Procedure

(1) Granules

The test is performed employing No. 10 (1700 μm), No. 12 (1400 μm), and No. 42 (355 μm) sieves with the inside diameter of 75 mm.

Weigh accurately 20.0 g of granules to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

(2) Powders

The test is performed employing No. 18 (850 μm), No. 30 (500 μm), and No. 200 (75 μm) sieves with the inside diameter of 75 mm.

Weigh accurately 10.0 g of powders to be tested, and place on the uppermost sieve which is placed on the other sieves described above and a close-fitting receiving pan and is covered with a lid. Shake the sieves in a horizontal direction for 3 minutes, and tap slightly at intervals. Weigh the amount remaining on each sieve and in the receiving pan.

6.04 Test for Acid-neutralizing Capacity of Gastrointestinal Medicines

Test for Acid-neutralizing Capacity of Gastrointestinal Medicines is a test to determine the acid-neutralizing capacity of a medicine, as a crude material or preparation, which reacts with the stomach acid and exercises an acid control action in the stomach. When performing the test according to the following procedure, the acid-neutralizing capacity of a crude material is expressed in terms of the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per g of the material, and that of a preparation is expressed by the amount (mL) of 0.1 mol/L hydrochloric acid VS consumed per dose per day (when the daily dose varies, the minimum dose is used).

Preparation of sample

A crude material and a solid preparation which conforms to Powders in the General Rules for Preparations: may be used, without any treatment, as the sample. Preparations in dose-unit packages: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, mix uniformly, and use the mixture as the sample. Granules in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: weigh accurately the content of not less than 20 packages, calculate the average mass of the content for a daily dose, powder it, and use as the sample. Granules not in dose-unit packages and other solid preparations which do not conform to Powders in the General Rules for Preparations: take not less than 20 doses, powder it, and use as the sample. Capsules and tablets: take not less than 20 doses, weigh accurately, calculate the average mass for a daily dose, powder it, and use as the sample. Liquid preparations: shake well, and use as the sample.

Procedure

Take an amount of the sample so that 'a' in the equation falls between 20 mL and 30 mL, and perform the test.

Accurately weigh the sample of the crude material or preparation, and place it in a glass-stoppered, 200-mL flask. Add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper tightly, shake at $37 \pm 2^\circ\text{C}$ for 1 hour, and filter. Take precaution against gas to be generated on the addition of 0.1 mol/L hydrochloric acid VS, and stopper tightly. After cooling, filter the solution again, if necessary. Pipet 50 mL of the filtrate, and titrate <2.50> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <2.54>, end point: pH 3.5). Perform a blank determination.

For liquid preparations, pipet the sample in a 100-mL volumetric flask, add water to make 45 mL, then add exactly 50 mL of 0.1 mol/L hydrochloric acid VS while shaking. Add water again to make the solution 100 mL. Transfer the solution to a glass-stoppered, 200-mL flask, wash the residue with 20.0 mL of water, stopper tightly, shake at $37 \pm 2^\circ\text{C}$ for 1 hour, and filter. Pipet 60 mL of the filtrate, and titrate <2.50> the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS (pH Determination <2.54>, end point: pH 3.5). Perform a blank determination.

Acid-neutralizing capacity (amount of 0.1 mol / L

hydrochloric acid VS consumed per g or daily dose) (mL)
 $= (b - a)f \times 2 \times (t/s)$

a: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

b: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank determination

f: The molarity coefficient of 0.1 mol/L sodium hydroxide VS

t: 1000 mg of crude material or daily dose of preparation (in mg of solid preparation, mL of liquid preparation)

s: Amount of the sample (in mg of crude material and solid preparation, mL of liquid preparation)

6.05 Test for Extractable Volume of Parenteral Preparations

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

♦Test for Extractable Volume of Parenteral Preparations is performed to confirm that a slightly excess volume is filled for the nominal volume to be withdrawn. Injections may be supplied in single-dose containers such as ampoules or plastic bags, or in multi-dose containers filled with a volume of injection which is sufficient to permit administration of the nominal volume declared on the label. The excess volume is determined by the characteristics of the product.♦

Suspensions and emulsions must be shaken before withdrawal of the contents and before the determination of the density. Oily and viscous preparations may be warmed according to the instructions on the label, if necessary, and thoroughly shaken immediately before removing the contents. The contents are then cooled to 20 – 25°C before measuring the volume.

(1) Single-dose containers

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. Take up individually the total contents of each container selected into a dry syringe of a capacity not exceeding three times the volume to be measured, and fitted with a 21-gauge needle not less than 2.5 cm in length. Expel any air bubbles from the syringe and needle, then discharge the contents of the syringe without emptying the needle into a standardised dry cylinder (graduated to contain rather than to deliver the designated volumes) of such size that the volume to be measured occupies at least 40% of its graduated volume. Alternatively, the volume of the contents in milliliters may be calculated as the mass in grams divided by the density.

For containers with a nominal volume of 2 mL or less the contents of a sufficient number of containers may be pooled to obtain the volume required for the measurement provided that a separate, dry syringe assembly is used for each container. The contents of containers holding 10 mL or more may be determined by opening them and emptying the contents directly into the graduated cylinder or tared beaker.

The volume is not less than the nominal volume in case of containers examined individually, or, in case of containers with a nominal volume of 2 mL or less, is not less than the sum of the nominal volumes of the containers taken collec-

tively.

(2) Multi-dose containers

For injections in multidose containers labeled to yield a specific number of doses of a stated volume, select one container and proceed as directed for single-dose containers using the same number of separate syringe assemblies as the number of doses specified. The volume is such that each syringe delivers not less than the stated dose.

(3) Cartridges and prefilled syringes

Select one container if the volume is 10 mL or more, 3 containers if the nominal volume is more than 3 mL and less than 10 mL, or 5 containers if the nominal volume is 3 mL or less. If necessary, fit the containers with the accessories required for their use (needle, piston, syringe) and transfer the entire contents of each container without emptying the needle into a dry tared beaker by slowly and constantly depressing the piston. Determine the volume in milliliters calculated as the mass in grams divided by the density.

The volume measured for each of the containers is not less than the nominal volume.

(4) Parenteral infusions

Select one container. Transfer the contents into a dry measuring cylinder of such a capacity that the volume to be determined occupies at least 40% of the nominal volume of the cylinder. Measure the volume transferred.

The volume is not less than the nominal volume.

6.06 Foreign Insoluble Matter Test for Injections

Foreign Insoluble Matter Test for Injections is a test method to examine foreign insoluble matters in injections.

Method 1. This method is applied to injections either in solutions, or in solution constituted from sterile drug solids.

Clean the exterior of containers, and inspect with the unaided eyes at a position of light intensity of approximately 1000 lx under an incandescent lamp: Injections must be clear and free from readily detectable foreign insoluble matters. As to Injections in plastic containers for aqueous injections, the inspection should be performed with the unaided eyes at a position of light intensity of approximately 8000 to 10,000 lx, with an incandescent lamp at appropriate distances above and below the container.

Method 2. This method is applied to injections with constituted solution.

Clean the exterior of containers, and dissolve the contents with constituted solution or with water for injection carefully, avoiding any contamination with extraneous foreign substances. The solution thus constituted must be clear and free from foreign insoluble matters that is clearly detectable when inspected with the unaided eyes at a position of light intensity of approximately 1000 lx, right under an incandescent lamp.

6.07 Insoluble Particulate Matter Test for Injections

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

The insoluble particulate matter test for injections or infusions is the method to test for insoluble particulates, to confirm that they are not present in excess of specified levels in the solutions. For the determination of particulate contamination 2 procedures, Method 1 (Light Obscuration Particle Count Test) and Method 2 (Microscopic Particle Count Test), are specified hereinafter. When examining injections and parenteral infusions for sub-visible particles Method 1 is preferably applied. However, it may be necessary to test some preparations by Method 2 followed by Method 1 to reach a conclusion on conformance to the requirements.

Not all parenteral preparations can be examined for sub-visible particles by one or both of these methods. When Method 1 is not applicable, e.g. in case of preparations having reduced clarity or increased viscosity, the test should be carried out according to Method 2. Emulsions, colloids, and liposomal preparations are examples. Similarly, products that produce air or gas bubbles when drawn into the sensor may also require microscopic particle count testing. If the viscosity of the preparation to be tested is sufficiently high so as to preclude its examination by either test method, a quantitative dilution with an appropriate diluent may be made to decrease viscosity, as necessary, to allow the analysis to be performed.

The results obtained in examining a discrete unit or group of units for particulate contamination cannot be extrapolated with certainty to other units that remain untested. Thus, statistically sound sampling plans must be developed if valid inferences are to be drawn from observed data to characterise the level of particulate contamination in a large group of units.

Method 1. Light Obscuration Particle Count Test

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size. ♦It is necessary to perform calibration, as well as to demonstrate the sample volume accuracy, sample flow rate, particle size response curve, sensor resolution, and counting accuracy, at least once a year. ♦

♦Calibration

Particles to be used for calibration should be subject to particle size sensitivity measurement, using spherical polystyrene particles having at least 5, 10 and 25 μm in diameter (PSL particles) in mono-dispersed suspension. The PSL particles should have either a domestic or international traceability in terms of length, with a level of uncertainty at not greater than 3%. The particles to be used for calibration should be dispersed in *particle-free water*.

Manual method

The particle size response of the system to be applied should be determined using at least 3 channels for threshold-voltage setting, according to the half counting method of window moving type. The threshold-voltage window should

be $\pm 20\%$ of the measuring particle size. After measuring the sensitivity of response for the designated particle size, the size response curve is prepared by the method indicated by the manufacturer from particle-response measuring point, and threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained.

Electronic method

In the use of multichannel peak height analyzer, the particle size response is measured by half-count method of moving window system same as the manual method, and the particle size response curve is prepared by the method designated by the instrument manufacturer, then, the threshold-voltage of 5, 10 and 25 μm of the apparatus is obtained. In this case, the instrument manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Automated method

The particle size response curve of the apparatus may be obtained by using the software developed by the user or supplied by the instrument manufacturer, whereas, the manufacturer or the user should validate the obtainability of the same result as that of the manual method.

Sample volume accuracy

Sample volume accuracy should fall within 5% of the measuring value in case measuring the decrease of test solution by the mass method after measuring the test solution of 10 mL.

Sample flow rate

The flow rate of the sample indicated into the sensor should be calculated from the observed sample volume and time, and should be conformed within the range of the manufacturer's specification for sensor used.

Sensor

There is a possibility of changes of particle size resolution and counting rate of particle-detecting sensor in each sensor by assembling accuracy and parts accuracy even in the same type sensor. The threshold accuracy also needs to be confirmed. Testing should accordingly be performed for each of particle size resolution, accuracy in counting and in threshold setting, using Particle Count Reference Standard Suspension (PSL spheres having mean diameter of approximately 10 μm , of a concentration at 1000 particles/mL $\pm 10\%$, not more than 5% of CV value).

During measurement, stirring should be made for assuring the uniformity in sample concentration.

Sensor resolution (Particle size resolution of apparatus)

Measurement should be made by either one of the following methods.

1. Manual method to obtain the spread of histogram prepared from the counting value of the apparatus.
2. Electronic method to obtain the spread of histogram of the classification of system-responding signal by using the multichannel peak height analyzer.
3. Automated method to obtain the spread of histogram of responsive signal of the test-particle by using the software prepared by the manufacturer or the user.

The difference between the threshold of particle size counting 16 and 84% of the total counts and the test-particle size should be within 10%, whereas, electronic method and automated method should be both validated for obtaining the

same result as that of the manual method.

Particle counting accuracy

Data obtained by counting particles of $5\ \mu\text{m}$ and greater should be 763 to 1155 particles per 1 mL.

Threshold accuracy

Particle size calculated from a threshold corresponding to 50% counts for particles of $5\ \mu\text{m}$ and greater should fall within $\pm 5\%$ of the mean diameter of the test particles.

Reagents

Particle-free water: The purified water containing not more than 5 particles of $10\ \mu\text{m}$ or greater size, and not more than 2 particles of $25\ \mu\text{m}$ or greater size in 10 mL of the insoluble particle number measured by the light obscuration particle counter. ♦

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filtration equipment used, except for the membrane filters, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse the equipment from top to bottom, outside and then inside, with *particle-free water*.

Take care not to introduce air bubbles into the preparation to be examined, especially when fractions of the preparation are being transferred to the container in which the determination is to be carried out.

In order to check that the environment is suitable for the test, that the glassware is properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of 5 samples of *particle-free water*, each of 5 mL, according to the method described below. If the number of particles of $10\ \mu\text{m}$ or greater size exceeds 25 for the combined 25 mL, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware and water are suitable for the test.

Method

Mix the contents of the sample by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents. Eliminate gas bubbles by appropriate measures such as allowing to stand for 2 min or sonicating.

For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units are combined in a cleaned container to obtain a volume of not less than 25 mL; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

Powders for parenteral use are reconstituted with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals

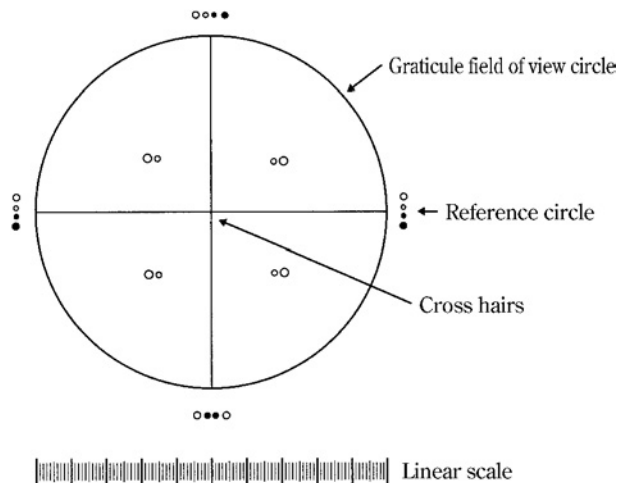


Fig. 6.07-1 Circular diameter graticule

or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Remove 4 portions, each of not less than 5 mL, and count the number of particles equal to or greater than $10\ \mu\text{m}$ and $25\ \mu\text{m}$. Disregard the result obtained for the first portion, and calculate the mean number of particles for the preparation to be examined.

Evaluation

If the average number of particles exceeds the limits, test the preparation by Method 2 (Microscopic Particle Count Test).

Test 1.A—Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of ♦ equal to or ♦ more than 100 mL

The preparation complies with the test if the average number of particles present in the units tested does not exceed 25 per milliliter equal to or greater than $10\ \mu\text{m}$ and does not exceed 3 per milliliter equal to or greater than $25\ \mu\text{m}$.

Test 1.B—Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL.

The preparation complies with the test if the average number of particles present in the units tested does not exceed 6000 per container equal to or greater than $10\ \mu\text{m}$ and does not exceed 600 per container equal to or greater than $25\ \mu\text{m}$.

Method 2. Microscopic Particle Count Test

Use a suitable binocular microscope, filter assembly for retaining particulate contamination and membrane filter for examination.

The microscope is equipped with an ocular micrometer calibrated with an objective micrometer, a mechanical stage capable of holding and traversing the entire filtration area of the membrane filter, two suitable illuminators to provide episcopic illumination in addition to oblique illumination, and is adjusted to 100 ± 10 magnifications. The ocular micrometer is a circular diameter graticule (see Fig. 6.07-1) and consists of a large circle divided by crosshairs into quadrants, transparent and black reference circles $10\ \mu\text{m}$ and $25\ \mu\text{m}$ in diameter at 100 magnifications, and a linear scale graduated in $10\ \mu\text{m}$ increments. It is calibrated using a stage micrometer that is certified by either a domestic or interna-

tional standard institution. A relative error of the linear scale of the graticule within ± 2 per cent is acceptable. The large circle is designated the graticule field of view (GFOV).

Two illuminators are required. One is an episcopic bright-field illuminator internal to the microscope, the other is an external, focussable auxiliary illuminator adjustable to give reflected oblique illumination at an angle of 10° to 20° .

The filter assembly for retaining particulate contamination consists of a filter holder made of glass or other suitable material, and is equipped with a vacuum source and a suitable membrane filter.

The membrane filter is of suitable size, black or dark gray in color, non-gridded or gridded, and $1.0\ \mu\text{m}$ or finer in nominal pore size.

General precautions

The test is carried out under conditions limiting particulate contamination, preferably in a laminar-flow cabinet.

Very carefully wash the glassware and filter assembly used, except for the membrane filter, with a warm detergent solution and rinse with abundant amounts of water to remove all traces of detergent. Immediately before use, rinse both sides of the membrane filter and the equipment from top to bottom, outside and then inside, with *particle-free water*.

In order to check that the environment is suitable for the test, that the glassware and the membrane filter are properly cleaned and that the water to be used is particle-free, the following test is carried out: determine the particulate contamination of a 50 mL volume of *particle-free water* according to the method described below. If more than 20 particles $10\ \mu\text{m}$ or larger in size or if more than 5 particles $25\ \mu\text{m}$ or larger in size are present within the filtration area, the precautions taken for the test are not sufficient. The preparatory steps must be repeated until the environment, glassware, membrane filter and water are suitable for the test.

Method

Mix the contents of the samples by slowly inverting the container 20 times successively. If necessary, cautiously remove the sealing closure. Clean the outer surfaces of the container opening using a jet of *particle-free water* and remove the closure, avoiding any contamination of the contents.

For large-volume parenterals, single units are tested. For small-volume parenterals less than 25 mL in volume, the contents of 10 or more units is combined in a cleaned container; where justified and authorised, the test solution may be prepared by mixing the contents of a suitable number of vials and diluting to 25 mL with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable. Small-volume parenterals having a volume of 25 mL or more may be tested individually.

Powders for parenteral use are constituted with *particle-free water* or with an appropriate solvent without contamination of particles when *particle-free water* is not suitable.

The number of test specimens must be adequate to provide a statistically sound assessment. For large-volume parenterals or for small-volume parenterals having a volume of 25 mL or more, fewer than 10 units may be tested, based on an appropriate sampling plan.

Wet the inside of the filter holder fitted with the membrane filter with several milliliter of *particle-free water*. Transfer to the filtration funnel the total volume of a solution pool or of a single unit, and apply vacuum. If needed add stepwise a

portion of the solution until the entire volume is filtered. After the last addition of solution, begin rinsing the inner walls of the filter holder by using a jet of *particle-free water*. Maintain the vacuum until the surface of the membrane filter is free from liquid. Place the filter in a petri dish and allow the filter to air-dry with the cover slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles that are equal to or greater than $10\ \mu\text{m}$ and the number of particles that are equal to or greater than $25\ \mu\text{m}$. Alternatively, partial filter count and determination of the total filter count by calculation is allowed. Calculate the mean number of particles for the preparation to be examined.

The particle sizing process with the use of the circular diameter graticule is carried out by transforming mentally the image of each particle into a circle and then comparing it to the $10\ \mu\text{m}$ and $25\ \mu\text{m}$ graticule reference circles. Thereby the particles are not moved from their initial locations within the graticule field of view and are not superimposed on the reference circles for comparison. The inner diameter of the transparent graticule reference circles is used to size white and transparent particles, while dark particles are sized by using the outer diameter of the black opaque graticule reference circles.

In performing the microscopic particle count test (Method 2) do not attempt to size or enumerate amorphous, semi-liquid, or otherwise morphologically indistinct materials that have the appearance of a stain or discoloration on the membrane filter. These materials show little or no surface relief and present a gelatinous or film-like appearance. In such cases the interpretation of enumeration may be aided by testing a sample of the solution by Method 1.

Evaluation

Test 2.A—Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of ♦equal to or ♦more than 100 mL

The preparation complies with the test if the average number of particles present in the units tested does not exceed 12 per milliliter equal to or greater than $10\ \mu\text{m}$ and does not exceed 2 per milliliter equal to or greater than $25\ \mu\text{m}$.

Test 2.B—Solutions for parenteral infusion or solutions for injection supplied in containers with a nominal content of less than 100 mL

The preparation complies with the test if the average number of particles present in the units tested does not exceed 3000 per container equal to or greater than $10\ \mu\text{m}$ and does not exceed 300 per container equal to or greater than $25\ \mu\text{m}$.

6.08 Insoluble Particulate Matter Test for Ophthalmic Solutions

Insoluble Particulate Matter Test for Ophthalmic Solutions is to examine for the size and the number of insoluble particulate matter in Ophthalmic Solutions.

Apparatus

Use a microscope, filter assembly for retaining insoluble particulate matter and membrane filter for determination.

Microscope: The microscope is equipped with a micrometer system, a mobile stage and an illuminator, and is adjusted

to 100 magnifications.

Filter assembly for retaining insoluble particulate matter: The filter assembly for retaining insoluble particulate matter consists of a filter holder made of glass or a proper material incapable of causing any trouble in testing, and a clip. The unit is capable of fitting with a membrane filter 25 mm or 13 mm in diameter and can be used under reduced pressure.

Membrane filter for testing: The membrane filter is white in color, 25 mm or 13 mm in diameter, not more than 10 μm in nominal pore size and is imprinted with about 3 mm grid marks. Upon preliminary testing, the insoluble particulate matter equal to or greater than 25 μm in size should not be found on the filter. When necessary, wash the filter with purified water for particulate matter test.

Reagents

Purified water for particulate matter test: Purified water which contains not more than 10 particles of 10 μm or greater size in 100 mL. Prepare before use by filtering through a membrane filter with a nominal pore size of 0.5 μm or less.

Procedure

Aqueous ophthalmic solutions

Carry out all operations carefully in clean equipment and facilities which are low in dust. Fit the membrane filter onto the membrane filter holder, and fix them with the clip. Thoroughly rinse the holder inside with the purified water for particulate matter test, and filter under reduced pressure with 200 mL of the purified water for particulate matter test at a rate of 20 to 30 mL per minute. Apply the vacuum until the surface of the membrane filter is free from water, and remove the membrane filter. Place the filter in a flat-bottomed petri dish with the cover slightly ajar, and dry the filter fully at a temperature not exceeding 50°C. After the filter has been dried, place the petri dish on the stage of the microscope. Under a down-light from an illuminating device, adjust the grid of the membrane filter to the coordinate axes of the microscope, adjust the microscope so as to get the best view of the insoluble particulate matter, then count the number of particles that are equal to or greater than 150 μm within the effective filtering area of the filter, moving the mobile stage, and ascertain that the number is not more than 1. In this case the particle is sized on the longest axis.

Fit another membrane filter to the filtration device, and fix them with the clip, then wet the inside of the filter holder with several mL of purified water for particulate matter test. Clean the outer surface of the container, and mix the sample solution gently by inverting the container several times. Remove the cap, clean the outer surface of the nozzle, and pour the sample solution into a measuring cylinder which has been rinsed well with purified water for particulate matter test. Repeat the process to prepare 25 mL of the test solution. Pour the test solution into the filter holder along the inner wall of the holder. Apply the vacuum and filter mildly so as to keep the solution always on the filter. As for viscous sample solution, dilute suitably with purified water for particulate matter test or suitable diluent and then filter as described above. When the amount of the solution on the filter becomes small, add 30 mL of purified water for particulate matter test or suitable diluent in such manner as to wash the inner wall of the filter holder. Repeat the process 3 times with 30 mL of the water. Apply the vacuum gently until the surface of the membrane filter is free from water. Place the filter in a petri dish, and dry the filter at a temperature below 50°C with the cover

slightly ajar. After the filter has been dried, place the petri dish on the stage of the microscope, and count the number of particles which are equal to or larger than 300 μm within the effective filtering area of the filter according to the same procedure of the microscope as described above. In this case the particle is sized on the longest axis.

Ophthalmic solutions which are dissolved before use

Proceed as directed in Aqueous Ophthalmic Solutions after dissolving the sample with the constituted solution.

Suspension type ophthalmic solutions

Proceed as directed in Aqueous Ophthalmic Solutions. Take 25 mL of the sample in a vessel, which has been rinsed well with purified water for particulate matter test, add a suitable amount of a suspension-solubilizing solvent or an adequate solvent, shake to dissolve the suspending particles, and use this solution as the sample solution. Use a membrane filter which is not affected by the solvent to be used.

Ophthalmic solutions contained in a single-dose container

Proceed as directed in Aqueous Ophthalmic Solutions, using 10 samples for the test. A 13-mm diameter membrane filter and a 4-mm diameter filter holder for retaining insoluble particulate matter are used.

6.09 Disintegration Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Disintegration Test is provided to determine whether tablets, capsules, ♦granules or pills◆ disintegrate within the prescribed time when placed in a liquid medium at the experimental conditions presented below.

For the purposes of this test, disintegration does not imply complete solution of the unit or even of its active constituent.

Apparatus

The apparatus consists of a basket-rack assembly, a 1000-mL, low-form beaker, 138 to 160 mm in height and having an inside diameter of 97 to 115 mm for the immersion fluid, a thermostatic arrangement for heating the fluid between 35° and 39°, and a device for raising and lowering the basket in the immersion fluid at a constant frequency rate between 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The volume of the fluid in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below the surface of the fluid and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of the basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. There is no appreciable horizontal motion or movement of the axis from the vertical.

Basket-rack assembly—The basket-rack assembly consists of six open-ended transparent tubes, each 77.5 ± 2.5 mm long and having an inside diameter of 20.7 to 23 mm and a wall 1.0 to 2.8 mm thick; the tubes are held in a vertical position by two plates, each 88 to 92 mm in diameter and 5 to 8.5 mm

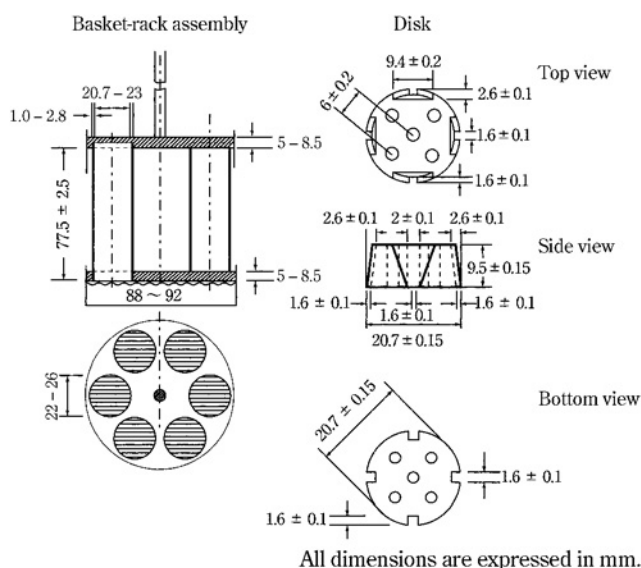
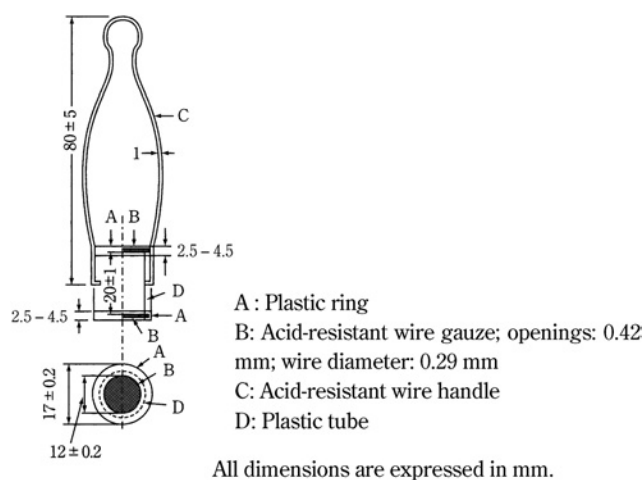


Fig. 6.09-1 Disintegration apparatus

in thickness, with six holes, each 22 to 26 mm in diameter, equidistant from the center of the plate and equally spaced from one another. Attached to the under surface of the lower plate is a woven stainless steel wire cloth, which has a plain square weave with 1.8- to 2.2-mm apertures and with a wire diameter of 0.57 to 0.66 mm. The parts of the apparatus are assembled and rigidly held by means of three bolts passing through the two plates. A suitable means is provided to suspend the basket-rack assembly from the raising and lowering device using a point on its axis. The basket-rack assembly conforms to the dimensions found in Fig. 6.09-1. The design of the basket-rack assembly may be varied somewhat provided the specifications for the glass tubes and the screen mesh size are maintained: ♦For example, in order to secure the glass tubes and the upper and the lower plastic plates in position at the top or the bottom, an acid-resistant metal plate, 88 - 92 mm in diameter and 0.5 - 1 mm in thickness, having 6 perforations, each about 22 to 26 mm in diameter, may be used which coincide with those of the upper plastic plate and upper open ends of the glass tubes. ♦

Disks—The use of disks is permitted only where specified or allowed. Each tube is provided with a cylindrical disk 9.5 ± 0.15 mm thick and 20.7 ± 0.15 mm in diameter. The disk is made of a suitable, transparent plastic material having a specific gravity of between 1.18 and 1.20. Five parallel 2 ± 0.1 mm holes extend between the ends of the cylinder. One of the holes is centered on the cylindrical axis. The other holes are centered 6 ± 0.2 mm from the axis on imaginary lines perpendicular to the axis and parallel to each other. Four identical trapezoidal-shaped planes are cut into the wall of the cylinder, nearly perpendicular to the ends of the cylinder. The trapezoidal shape is symmetrical; its parallel sides coincide with the ends of the cylinder and are parallel to an imaginary line connecting the centers of two adjacent holes 6 mm from the cylindrical axis. The parallel side of the trapezoid on the bottom of the cylinder has a length of 1.6 ± 0.1 mm, and its bottom edges lie at a depth of 1.6 ± 0.1 mm from the cylinder's circumference. The parallel side of the trapezoid on the top of the cylinder has a length of 9.4 ± 0.2 mm, and its center lies at a depth of 2.6 ± 0.1 mm from the cylinder's circum-



♦Fig. 6.09-2 Auxiliary tube♦

ference. All surfaces of the disk are smooth. If the use of disks is specified, add a disk to each tube, and operate the apparatus as directed under Procedure. The disks conform to dimensions found in Fig. 6.09-1. The use of automatic detection employing modified disks is permitted where the use of disks is specified or allowed. Such disks must comply with the requirements for density and dimension given in this chapter.

♦**Auxiliary tube**—The auxiliary tube, as illustrated in Fig. 6.09-2, consists of a plastic tube D, 12 ± 0.2 mm in inside diameter, 17 ± 0.2 mm in outside diameter, 20 ± 1 mm in length, having both outside ends screw-cut, and two plastic rings A, each 12 ± 0.2 mm in inside diameter, 17 ± 0.2 mm in outside diameter, 2.5 - 4.5 mm in length, having one inside end screw-cut. Acid-resistant woven wire gauze having 0.42-mm openings and 0.29-mm wire diameter is placed in each plastic ring and the rings are attached by screws to each end of the plastic tube. The distance between two wire gauzes is 20 ± 1 mm. A handle of an acid-resistant wire, 1 mm in diameter and 80 ± 5 mm in length, is attached to the mid portion of the plastic tube. The auxiliary tube is used for the test of granules and capsules containing enteric coated granules. ♦

Procedure

1) Immediate-release preparation

In case of tablets, capsules ♦and pills (except for pills containing crude drugs), ♦ place 1 dosage unit in each of the six tubes of the basket, and if prescribed add a disk. ♦Unless otherwise specified, operate the apparatus, using water as the immersion fluid, ♦ maintained at $37 \pm 2^\circ\text{C}$ as the immersion fluid. ♦Unless otherwise specified, carry out the test for 20 minutes for capsules, 30 minutes for plain tablets, and 60 minutes for coated tablets and pills. ♦ Lift the basket from the fluid, and observe the dosage units. ♦Complete disintegration is defined as that state in which any residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the disks, if used, is a soft mass having no palpably firm core. ♦ The test is met if all of the dosage units have disintegrated completely. If 1 or 2 dosage units fail to disintegrate, repeat the test on 12 additional dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are disintegrated.

♦For pills containing crude drugs, carry out the test for 60

minutes in the same manner, using 1st fluid for disintegration test as the immersion fluid. When any residue of the unit is observed, carry out the test successively for 60 minutes, using 2nd fluid for disintegration test. ♦

♦In case of granules, shake granules on a No. 30 (500 μm) sieve as directed in (1) Granules under Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using water as the immersion fluid, maintained at $37 \pm 2^\circ\text{C}$ as the immersion fluid, unless otherwise specified. Observe the samples after 30 minutes of operation for plain granules and after 60 minutes for coated granules, unless otherwise specified. Complete disintegration is defined as that state in which any residue of the granules, except fragments of insoluble coating in the auxiliary tube, is a soft mass having no palpably firm core. The test is met if all of 6 samples in the auxiliary tubes have disintegrated completely. If 1 or 2 samples fail to disintegrate, repeat the test on 12 additional samples. The test is met if not less than 16 of the total of 18 samples tested are disintegrated. ♦

♦2) Enteric coated preparations

Unless otherwise specified, perform the following two tests, (a) the test with 1st fluid for disintegration test and (b) the test with the 2nd fluid for disintegration test, separately.

(1) Enteric coated tablet and capsule

(a) The test with 1st fluid for disintegration test

Carry out the test for 120 minutes, using 1st fluid for disintegration test according to the procedure described in immediate release preparations. Disintegration is defined as that state in which the tablet or capsule is broken or the enteric coating film is ruptured or broken. The test is met if none of six dosage units is disintegrated. If 1 or 2 dosage units are disintegrated, repeat the test on additional 12 dosage units. The test is met if not less than 16 of the total of 18 dosage units tested are not disintegrated.

(b) The test with 2nd fluid for disintegration test

According to the procedure described in immediate-release preparations, carry out the test with new dosage units for 60 minutes, using 2nd fluid for disintegration test and determine if the test is met or not.

(2) Enteric coated granules and capsules containing the enteric coated granules

Shake granules or contents taken out from capsules on a No. 30 (500 μm) sieve as directed in (1) Granules under Particle Size Distribution Test for Preparations <6.03>, transfer 0.10 g of the residue on the sieve to each of the 6 auxiliary tubes, secure the 6 tubes to the bottom of the basket tightly, and operate the apparatus, using the 1st and 2nd fluids for disintegration test.

(a) The test with 1st fluid for disintegration test

According to the procedure described in immediate-release preparations, carry out the test for 60 minutes, using 1st fluid for disintegration test. The test is met if particles fallen from the openings of the wire gauze number not more than 15.

(b) The test with 2nd fluid for disintegration test

According to the procedure described in immediate-release preparations, carry out the test with new samples for 30 minutes, using 2nd fluid for disintegration test and determine if test is met or not. ♦

6.10 Dissolution Test

This test is harmonized with the European Pharmacopoeia and the U. S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Dissolution Test is provided to determine compliance with the dissolution requirements for dosage forms administered orally. ♦This test also aims at preventing significant bioequivalence. ♦ In this test, a dosage unit is defined as 1 tablet or 1 capsule or the amount specified equivalent to minimum dose.

Apparatus

Apparatus for Basket Method (Apparatus 1)—The assembly consists of the following: a vessel, which may be covered, made of glass or other inert, transparent material^{*1}; a motor; a drive shaft; and a cylindrical basket. The vessel is partially immersed in a suitable water bath of any convenient size or heated by a suitable device such as a heating jacket. The water bath or heating device permits holding the temperature inside the vessel at $37 \pm 0.5^\circ\text{C}$ during the test and keeping the bath fluid in constant, smooth motion. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smoothly rotating stirring element. Make the apparatus to permit observation of the specimen and stirring element during the test. The vessel is cylindrical, with a hemispherical bottom and a capacity of 1 liter. Its height is 160 mm to 210 mm and its inside diameter is 98 mm to 106 mm. Its sides are flanged at the top. Use a fitted cover to retard evaporation.^{*2} The shaft is positioned so that its axis is not more than 2 mm at any point from the vertical axis of the vessel and rotates smoothly and without significant wobble that could affect the results. Adjust a speed-regulating device to maintain the shaft rotation speed at a specified rate, within $\pm 4\%$.

Shaft and basket components of the stirring element shown in Fig. 6.10-1 are fabricated of stainless steel (SUS316) or other inert material. A basket having a gold coating of about 0.0001 inch (2.5 μm) thick may be used. The dosage unit is placed in a dry basket at the beginning of each test. The distance between the inside bottom of the vessel and the bottom of the basket is maintained at 25 ± 2 mm during the test.

Apparatus for Paddle Method (Apparatus 2)—Use the assembly from Apparatus 1, except that a paddle formed from a blade and a shaft is used as the stirring element. The shaft is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel, at any point, and rotates smoothly without significant wobble that could affect the results. The vertical center line of the blade passes through the axis of the shaft so that the bottom of the blade is flush with the bottom of the shaft. The paddle conforms to the specifications shown in Fig. 6.10-2. The distance of 25 ± 2 mm between the bottom of the blade and the inside bottom of the vessel is maintained during the test. The metallic or suitably inert, rigid blade and shaft comprise a single entity. A suitable two-part detachable design may be used provided the assembly remains firmly engaged during the test. The paddle blade and shaft may be coated with a suitable coating so as to make them inert. The dosage unit is allowed to sink to the bottom of the vessel before rotation of the blade is started. ♦When specified in the individual monograph, ♦ a small, loose piece of nonreactive

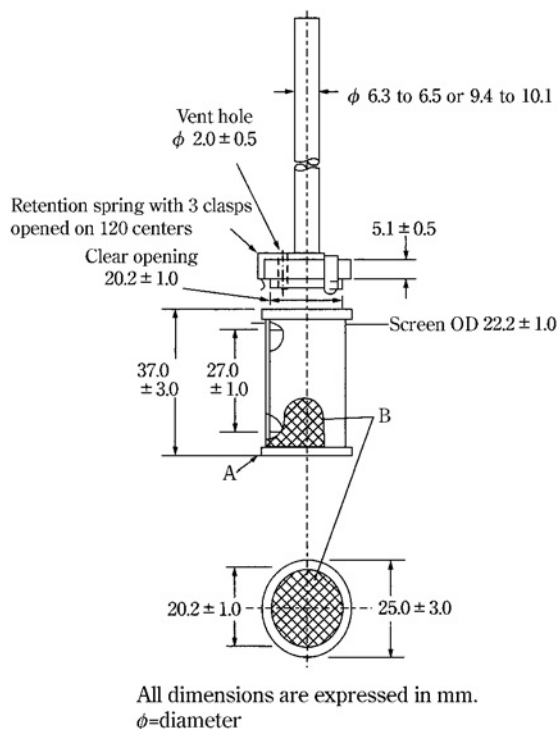


Fig. 6.10-1 Apparatus 1, Basket stirring element

A: Note—Maximum allowable runout at “A” is ± 1.0 mm when the part is rotated on center line axis with basket mounted.
 B: Screen with welded seam, 0.25–0.31 mm wire diameter with wire openings of 0.36–0.44 mm [Note—After welding, the screen may be slightly altered.]

material, such as not more than a few turns of wire helix, may be attached to dosage units that would otherwise float. An alternative sinker device is shown in Fig. 6.10-2a. Other validated sinker devices may be used.

Apparatus for Flow-Through Cell Method (Apparatus 3)—The assembly consists of a reservoir and a pump for the dissolution medium; a flow-through cell; a water bath that maintains the dissolution medium at $37 \pm 0.5^\circ\text{C}$. Use the cell size specified in the individual monograph.

The pump forces the dissolution medium upwards through the flow-through cell. The pump has a delivery range between 4 and 16 mL per minute, with standard flow rates of 4, 8, and 16 mL per minute. It must deliver a constant flow (± 5 per cent of the nominal flow rate); the flow profile should be sinusoidal with a pulsation of 120 ± 10 pulses per minute. ♦A pump without the pulsation can also be used. ♦

The flow-through cell (see Figures 6.10-3 and 6.10-4), of transparent and inert material, is mounted vertically with a filter system (specified in the individual monograph) that prevents escape of undissolved particles from the top of the cell; standard cell diameters are 12 and 22.6 mm; the bottom cone is usually filled with small glass beads of about 1-mm diameter with one bead of about 5 mm positioned at the apex to protect the fluid entry tube; a tablet holder (see Figures 6.10-3 and 6.10-4) is available for positioning of special dosage forms. The cell is immersed in a water bath, and the temperature is maintained at $37 \pm 0.5^\circ\text{C}$.

The apparatus uses a clamp mechanism of two O-rings to assemble the cell. The pump is separated from the dissolution

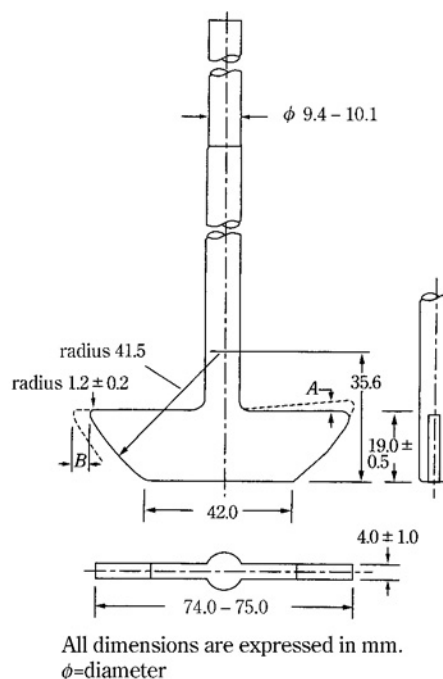


Fig. 6.10-2 Apparatus 2, Paddle stirring element

Notes:
 (1) A and B dimensions are not to vary more than 0.5 mm when part is rotated on centering axis.
 (2) Tolerances are ± 1.0 mm unless otherwise stated.

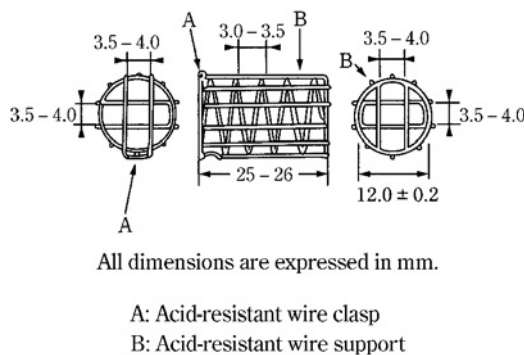


Fig. 6.10-2a Alternative sinker

unit in order to shield the latter against any vibrations originating from the pump. The position of the pump should not be on a level higher than the reservoir flasks. Tube connections are as short as possible. Use suitably inert tubing, such as polytef, with about 1.6-mm inner diameter and inert flanged-end connections.

Apparatus Suitability—The determination of suitability of a test assembly to perform dissolution testing must include conformance to the dimensions and tolerances of the apparatus as given above. In addition, critical test parameters that have to be monitored periodically during use include volume and temperature of the dissolution medium, rotation speed (Basket Method and Paddle Method), and flow rate of medium (Flow-Through Cell Method).

Determine the acceptable performance of the dissolution

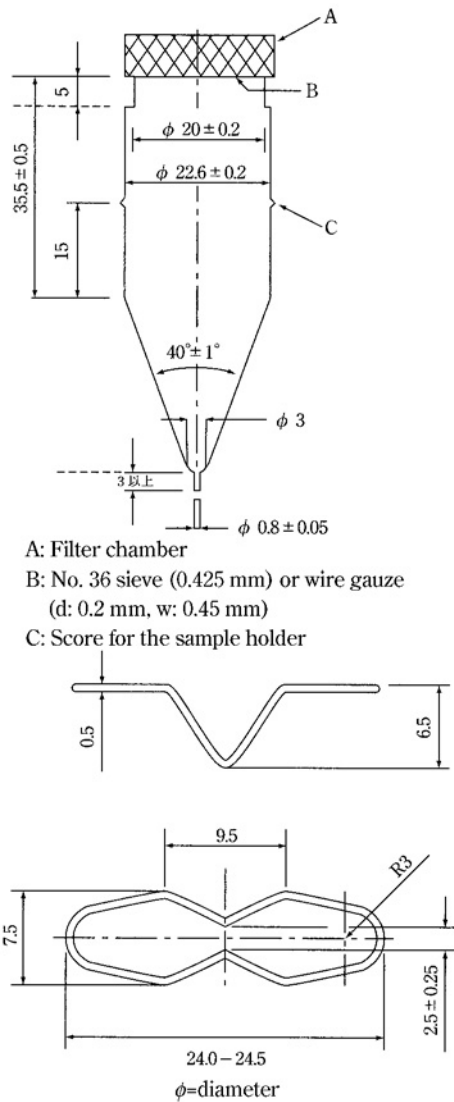


Fig. 6.10-3 Apparatus 3
Large cell for tablets and capsules (top); tablet holder for the large cell (bottom)
(All dimensions are expressed in mm unless otherwise noted.)

test assembly periodically.

Procedure

Basket Method or Paddle Method IMMEDIATE-RELEASE DOSAGE FORMS

Procedure—Place the stated volume of the dissolution medium ($\pm 1\%$) in the vessel of the specified apparatus, assemble the apparatus, equilibrate the dissolution medium to $37 \pm 0.5^\circ\text{C}$, and remove the thermometer. Place 1 dosage unit in the apparatus, taking care to exclude air bubbles from the surface of the dosage unit, and immediately operate the apparatus at the specified rate. Within the time interval specified, or at each of the times stated, withdraw a specimen from a zone midway between the surface of the Dissolution Medium and the top of the rotating basket or blade, not less than 10 mm from the vessel wall. [NOTE—Where multiple sampling times are specified, replace the aliquots withdrawn for analysis with equal volumes of fresh Dissolution Medium at 37°C or, where it can be shown that replacement of the medi-

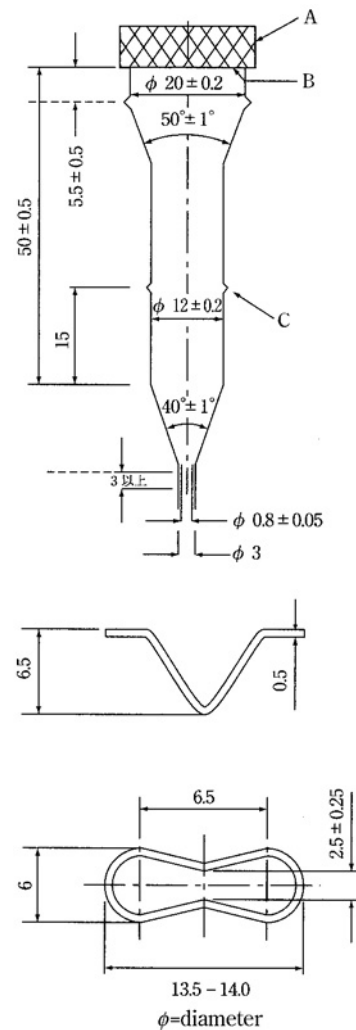


Fig. 6.10-4 Apparatus 3
Small cell for tablets and capsules (top); tablet holder for the small cell (bottom)
(All dimensions are expressed in mm unless otherwise noted.)

um is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.] Perform the analysis using an indicated assay method.*³ Repeat the test with additional dosage units.

If automated equipment is used for sampling or the apparatus is otherwise modified, verification that the modified apparatus will produce results equivalent to those obtained with the standard apparatus described in this chapter, is necessary.

Dissolution Medium—A specified dissolution medium is used. If the dissolution medium is a buffered solution, adjust the solution so that its pH is within 0.05 unit of the specified pH. [NOTE—Dissolved gases can cause bubbles to form, which may change the results of the test. If dissolved gases influence the dissolution results, remove dissolved gases prior testing.*⁴]

Time—Where a single time specification is given, the test may be concluded in a shorter period if the requirement for minimum amount dissolved is met. Specimens are to be withdrawn only at the stated times, within a tolerance of ± 2

Acceptance Table 6.10-1

Stage	Number Tested	Acceptance Criteria
S1	6	Each value is not less than $Q + 5\%$.
S2	6	Average value of the 12 dosage units (S1 + S2) is equal to or greater than Q , and no value is less than $Q - 15\%$.
S3	12	Average value of the 24 dosage units (S1 + S2 + S3) is equal to or greater than Q , not more than 2 values are less than $Q - 15\%$, and no value is less than $Q - 25\%$.

Acceptance Table 6.10-2

Level	Number Tested	Criteria
L1	6	No individual value lies outside each of the stated ranges and no individual value is less than the stated amount at the final test time.
L2	6	The average value of the 12 dosage units (L1 + L2) lies within each of the stated ranges and is not less than the stated amount at the final test time; no value is more than 10% of labeled content outside each of the stated ranges; and no value is more than 10% of labeled content below the stated amount at the final test time.
L3	12	The average value of the 24 dosage units (L1 + L2 + L3) lies within each of the stated ranges, and is not less than the stated amount at the final test time; not more than 2 of the 24 values are more than 10% of labeled content outside each of the stated ranges; not more than 2 of the 24 values are more than 10% of labeled content below the stated amount at the final test time; and no value is more than 20% of labeled content outside each of the stated ranges or more than 20% of labeled content below the stated amount at the final test time.

%.
 EXTENDED-RELEASE DOSAGE FORMS

Procedure—Proceed as described for Immediate-Release Dosage Forms.

Dissolution Medium—Proceed as directed under Immediate-Release Dosage Forms.

Time—The test-time points, generally three, are expressed in hours.

DELAYED-RELEASE DOSAGE FORMS

♦Procedure—Unless otherwise specified, proceed the acid stage test and buffer stage test separately as described for Immediate-Release Dosage Forms. ♦

♦Dissolution Medium—Acid stage: Unless 1st fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms. Buffer stage: Unless 2nd fluid for dissolution test is used, proceed as directed under Immediate-Release Dosage Forms. ♦

♦Time—Acid stage: Generally, test time is 2 hours for tablets and capsules, and 1 hour for granules. Buffer stage: The same as directed under Immediate-Release Dosage Forms. ♦ All test times stated are to be observed within a tolerance of $\pm 2\%$, unless otherwise specified.

Acceptance Table 6.10-3

Level	Number Tested	Criteria
A1	6	No individual value exceeds 10% dissolved.
A2	6	The average value of the 12 dosage units (A1 + A2) is not more than 10% dissolved, and no value is greater than 25% dissolved.
A3	12	The average value of the 24 dosage units (A1 + A2 + A3) is not more than 10% dissolved, and no value is greater than 25% dissolved.

Acceptance Table 6.10-4

Level	Number Tested	Criteria
B1	6	No value is less than $Q + 5\%$.
B2	6	The average value of the 12 dosage units (B1 + B2) is equal to or greater than Q , and no value is less than $Q - 15\%$.
B3	12	The average value of the 24 dosage units (B1 + B2 + B3) is equal to or greater than Q , not more than 2 values are less than $Q - 15\%$, and no value is less than $Q - 25\%$.

Flow-Through Cell Method

IMMEDIATE-RELEASE DOSAGE FORMS

Procedure—Place the glass beads into the cell specified in the individual monograph. Place 1 dosage unit on top of the beads or, if specified, on a wire carrier. Assemble the filter head and fix the parts together by means of a suitable clamping device. Introduce by the pump the dissolution medium warmed to $37 \pm 0.5^\circ\text{C}$ through the bottom of the cell to obtain the flow rate specified and measured with an accuracy of 5%. Collect the eluate by fractions at each of the times stated. Perform the analysis as directed. Repeat the test with additional dosage units.

Dissolution Medium—Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

Time—Proceed as directed under Immediate-Release Dosage Forms under Basket Method and Paddle Method.

EXTENDED-RELEASE DOSAGE FORMS

Procedure—Proceed as described for Immediate-Release Dosage Forms under Flow-Through Cell Method.

Dissolution Medium—Proceed as described for Immediate-Release under Flow-Through Cell Method.

Time—The test-time points, generally three, are expressed in hours.

Interpretation

IMMEDIATE-RELEASE DOSAGE FORMS

♦Follow Interpretation 1 when the value Q is specified in the individual monograph, otherwise follow Interpretation 2. ♦
 Interpretation 1:

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-1. Continue testing through the three stages unless the results conform at either S1 or S2. The quantity, Q , ♦ is the specified amount of dissolved active ingredient, ♦ expressed as a percentage of the

labeled content of the dosage unit; the 5%, 15%, and 25% values in the Acceptance Table are percentage of the labeled content so that three values and Q are in the same terms.

♦Interpretation 2:

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. ♦

EXTENDED-RELEASE DOSAGE FORMS

♦Interpretation 1: ♦

Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the dosage units tested conform to Acceptance Table 6.10-2. Continue testing through the three levels unless the results conform at either L1 or L2. Limits on the amounts of active ingredient dissolved are expressed in terms of the percentage of labeled content. The limits embrace each value of Q_i , the amount dissolved at each specified fractional dosing interval. Where more than one range is specified, the acceptance criteria apply individually to each range.

♦Interpretation 2:

Unless otherwise specified, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. Where more than one range is specified, the acceptance criteria apply individually to each range. ♦

DELAYED-RELEASE DOSAGE FORMS

♦Follow Interpretation 1 when the value Q is specified in the test using 2nd fluid for dissolution test in the individual monograph, otherwise follow Interpretation 2.

Interpretation 1:

Test using 1st fluid for dissolution test—Unless otherwise specified, the requirements of this portion of the test are met if the quantities, based on the percentage of the labeled content, of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-3. Continue testing through the three levels unless the result conforms at A2. ♦

♦Test using 2nd fluid for dissolution test. ♦—Unless otherwise specified, the requirements are met if the quantities of active ingredient dissolved from the units tested conform to Acceptance Table 6.10-4. Continue testing through the three levels unless the results of both stages conform at an earlier level. The value of Q in Acceptance Table 6.10-4 is the amount ♦ specified in monograph. ♦ of active ingredient dissolved, expressed as a percentage of the labeled content. The 5% and 15% and 25% values in Acceptance Table 6.10-4 are percentages of the labeled content so that these values and Q are in the same terms.

♦Interpretation 2:

Unless otherwise specified, both the tests using 1st fluid for dissolution test and 2nd fluid for dissolution test in acid and buffer stages, perform the test on 6 dosage forms: if the individual dissolution rate meet the requirements specified in the individual monograph, the dosage forms conform to the

test. When individual dissolution rates of 1 or 2 dosage forms fail to meet the requirements, repeat the test on another 6 dosage forms: if individual dissolution rates of not less than 10 dosage forms out of 12 meet the requirements, the dosage forms conform to the test. ♦

*1The materials should not sorb, react, or interfere with the specimen being tested.

*2If a cover is used, it provides sufficient openings to allow ready insertion of the thermometer and withdrawal of specimens.

*3Test specimens are filtered immediately upon sampling unless filtration is demonstrated to be unnecessary. Use an inert filter that does not cause adsorption of the ingredient or contain extractable substances that would interfere with the analysis.

*4One method of deaeration is as follows: Heat the medium, while stirring gently, to about 41°C, immediately filter under vacuum using a filter having a porosity of 0.45 μm or less, with vigorous stirring, and continue stirring under vacuum for about 5 minutes. Other validated deaeration techniques for removal of dissolved gases may be used.

7. Tests for Containers and Packing Materials

7.01 Test for Glass Containers for Injections

The glass containers for injections do not interact physically or chemically with the contained medicament to alter any property or quality, can protect the contained medicament from the invasion of microbes by means of perfect sealing or other suitable process, and meet the following requirements. The surface-treated container for aqueous infusion is made from glass which meets the requirements for the soluble alkali test for a container not to be fused under method 1.

(1) The containers are colorless or light brown and transparent, and have no bubbles which interfere the test of the Foreign Insoluble Matter Test for Injections <6.06>.

(2) Multiple-dose containers are closed by rubber stoppers or any other suitable stoppers. The stoppers permit penetration of an injection needle without detachment of fragments, and upon withdrawal of the needle, they reclose the containers immediately to prevent external contamination, and also do not interact physically or chemically with the contained medicaments.

Containers intended for aqueous infusions are closed by rubber stoppers meeting the requirements of the test for Rubber Closure for Aqueous Infusions <7.03>.

(3) Soluble alkali test—The testing methods may be divided into the following two methods according to the type of container or the dosage form of the medicament.

(i) Method 1: This method is applied to containers to be fused, or containers not to be fused except containers for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, dry, and roughly crush, if necessary. Transfer 30 to 40 g of the glass to a steel mortar, and crush. Sieve the crushed glass through a No. 12 (1400 μm) sieve. Transfer the portion retained on the sieve again to the steel

mortar, and repeat this crushing procedure until 2/3 of the amount of powdered glass has passed through a No. 12 (1400 μm) sieve. Combine all portions of the glass powder passed through a No. 12 (1400 μm) sieve, shake the sieve in a horizontal direction for 5 minutes with slight tapping at intervals using No. 18 (850 μm) and No. 50 (300 μm) sieves. Transfer 7 g of the powder, which has passed through a No. 18 (850 μm) sieve but not through a No. 50 (300 μm) sieve to a No. 50 (300 μm) sieve, immerse it in a suitable container filled with water, and wash the contents with gentle shaking for 1 minute. Rinse again with ethanol (95) for 1 minute, dry the washed glass powder at 100°C for 30 minutes, and allow to cool in a desiccator (silica gel). Transfer exactly 5.0 g of the powder thus prepared to a 200-mL conical flask of hard glass, add 50 mL of water, and gently shake the flask so that the powder disperses on the bottom of the flask evenly. Cover the flask with a small beaker of hard glass or a watch glass of hard glass, then heat it in boiling water for 2 hours, and immediately cool to room temperature. Decant the water from the flask into a 250-mL conical flask of hard glass, wash well the residual powdered glass with three 20-mL portions of water, and add the washings to the decanted water. Add 5 drops of bromocresol green-methyl red TS and titrate <2.50> with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed the following quantity, according to the type of containers.

Containers to be fused	0.30 mL
Containers not to be fused (including injection syringes used as containers)	2.00 mL

(ii) Method 2: This method is applied to containers not to be fused for aqueous infusions with a capacity exceeding 100 mL.

Rinse thoroughly the inside and outside of the containers to be tested with water, and dry. Add a volume of water equivalent to 90% of the overflow capacity of the container, cover it with a small beaker of hard glass or close tightly with a suitable stopper, heat in an autoclave at 121°C for 1 hour, and allow to stand until the temperature falls to room temperature, measure exactly 100 mL of the this solution, and transfer to a 250-mL conical flask of hard glass. Add 5 drops of bromocresol green-methyl red TS, and titrate <2.50> with 0.01 mol/L sulfuric acid VS until the color of the solution changes from green through slightly grayish blue to slightly grayish red-purple. Measure accurately 100 mL of water, transfer to a 250-mL conical flask of hard glass, perform a blank determination in the same manner, and make any necessary correction. The quantity of 0.01 mol/L sulfuric acid VS consumed does not exceed 0.10 mL.

(4) Soluble iron test for light-resistant containers—Rinse thoroughly five or more light-resistant containers to be tested with water, and dry at 105°C for 30 minutes. Pour a volume of 0.01 mol/L hydrochloric acid VS corresponding to the labeled volume of the container into individual containers, and fuse them. In the case of containers not to be fused, cover them with small beakers of hard glass or watch glasses of hard glass. Heat them at 105°C for 1 hour. After cooling, prepare the test solution with 40 mL of this solution according to Method 1 of the Iron Limit Test <1.10>, and perform

the test according to Method B. Prepare the control solution with 2.0 mL of the Standard Iron Solution.

(5) Light transmission test for light-resistant containers—Cut five light-resistant containers to be tested, prepare test pieces with surfaces as flat as possible, and clean the surfaces. Fix a test piece in a cell-holder of a spectrophotometer to allow the light pass through the center of the test piece perpendicularly to its surface. Measure the light transmittance of the test piece with reference to air between 290 nm and 450 nm and also between 590 nm and 610 nm at intervals of 20 nm each. The percent transmissions obtained between 290 nm and 450 nm are not more than 50% and that between 590 nm and 610 nm are not less than 60%. In the case of containers not to be fused having a wall thickness over 1.0 mm, the percent transmissions between 590 nm and 610 nm are not less than 45%.

7.02 Test Methods for Plastic Containers

Test methods for plastic containers may be used for designing and quality assurance of plastic containers. Not all tests described here will be necessary in any phases for any containers. On the other hand, the set does not include sufficient number and kinds of tests needed for any design verification and quality assurance of any containers. Additional tests may be considered if necessary.

1. Combustion Tests

1.1 Residue on ignition

Weigh accurately about 5 g of cut pieces of the container and perform the test according to the Residue on Ignition <2.44>.

1.2 Heavy metals

Place an appropriate amount of cut pieces of the container in a porcelain crucible, and perform the test according to Method 2 of the Heavy Metals Limit Test <1.07>. Prepare the control solution with 2.0 mL of Standard Lead Solution.

1.3 Lead

Method 1: Place 2.0 g of cut pieces of a container in a crucible of platinum or quartz, moisten with 2 mL of sulfuric acid, heat slowly to dryness, then heat to combustion at between 450°C and 500°C. Repeat this procedure, if necessary. After cooling, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, evaporate to dryness on a water bath, then add 1 to 5 mL of hydrochloric acid, and warm to dissolve. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter if insoluble matter remains. To the obtained filtrate add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonia TS until the color of the solution changes from yellow to green. Then add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter if necessary, and use the layer as the sample solution. Separately, to 2.0

mL of Standard Lead Solution add water to make exactly 10 mL. To 1.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of lead in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air

Lamp: Lead hollow-cathode lamp
Wavelength: 283.3 nm

Method 2: Cut a container into pieces smaller than 5-mm square, take 2.0 g of the pieces into a glass beaker, add 50 mL of 2-butanone and 0.1 mL of nitric acid, and warm to dissolve. To this solution add 96 mL of methanol gradually to precipitate a resinous substance, and filter by suction. Wash the beaker and the resinous substance with 12 mL of methanol followed by 12 mL of water, combine the washings and the filtrate, and concentrate to about 10 mL under reduced pressure. Transfer into a separator, add 10 mL of ethyl acetate and 10 mL of water, shake vigorously, and allow to stand to separate the water layer. Evaporate the water layer to dryness, add 5 mL of hydrochloric acid to the residue, and warm to dissolve. Then add 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1), and add 1 mL of a warmed solution of ammonium acetate (2 in 5). Filter through a glass filter (G3) if insoluble matter remains. To the solution so obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and then add ammonia TS until the color of the solution changes from yellow to green. Further add 10 mL of a solution of ammonium sulfate (2 in 5) and water to make 100 mL. Add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20) to this solution, mix, allow to stand for a few minutes, then add 20.0 mL of 4-methyl-2-pentanone, and shake vigorously. Allow to stand to separate the 4-methyl-2-pentanone layer, filter the layer if necessary, and use the layer as the sample solution. Separately, pipet 5 mL of Standard Lead Solution, add water to make exactly 50 mL, and to 2.0 mL of this solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, then proceed in the same manner as for the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in Method 1, and determine the concentration of lead in the sample solution.

1.4 Cadmium

Method 1: To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in Method 1 under 1.3, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in Method 1 under 1.3 and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the concentration of cadmium in the sample solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air

Lamp: Cadmium hollow-cathode lamp
Wavelength: 228.8 nm

Method 2: To 2.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, and proceed in the same manner as for the sample solution in Method 2 under 1.3, and use the solution so obtained as the standard solution. Perform the test with the sample solution obtained in Method 2 under 1.3 and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the conditions described in Method 1, and determine the concentration of cadmium in the sample solution.

1.5 Tin

Cut a container into pieces smaller than 5-mm square, place 5.0 g of the pieces in a Kjeldahl flask, add 30 mL of a mixture of sulfuric acid and nitric acid (1:1), and decompose by gentle heating in a muffle furnace, occasionally adding dropwise a mixture of sulfuric acid and nitric acid (1:1) until the content changes to a clear, light brown solution. Then heat until the color of the solution changes to a clear, light yellow, and heat to slowly concentrate to practical dryness. After cooling, dissolve the residue in 5 mL of hydrochloric acid by warming, and after cooling, add water to make exactly 10 mL. Pipet 5 mL of this solution into a 25-mL volumetric flask (A). Transfer the remaining solution to a 25-mL beaker (B) by washing out with 10 mL of water, add 2 drops of bromocresol green TS, neutralize with diluted ammonia solution (28) (1 in 2), and measure the volume consumed for neutralization as *a* mL. To the volumetric flask, A, add potassium permanganate TS dropwise until a slight pale red color develops, and add a small amount of L-ascorbic acid to decolorize. Add 1.5 mL of 1 mol/L hydrochloric acid TS, 5 mL of a solution of citric acid monohydrate (1 in 10), *a* mL of diluted ammonia solution (28) (1 in 2), 2.5 mL of polyvinyl alcohol TS, 5.0 mL of phenylfluorone-ethanol TS and water to make 25 mL. Shake well, then allow to stand for about 20 minutes, and use this solution as the sample solution. Separately, pipet 1.0 mL of Standard Tin Solution, add 5 mL of water, add potassium permanganate TS dropwise until a slight pale red color develops, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and the standard solution according to Ultraviolet-visible Spectrophotometry <2.24> at 510 nm, using water as the blank.

2. Extractable substances

Cut the container at homogeneous regions of low curvature and preferably the same thickness, gather pieces to make a total surface area of about 1200 cm² when the thickness is 0.5 mm or less, or about 600 cm² when the thickness is greater than 0.5 mm, and subdivide in general into strips approximately 0.5 cm in width and 5 cm in length. Wash them with water, and dry at room temperature. Place these strips in a 300-mL hard glass vessel, add exactly 200 mL of water, and seal the opening with a suitable stopper. After heating the vessel in an autoclave at 121 °C for 1 hour, take out the vessel, allow to stand until the temperature falls to room temperature, and use the content as the test solution.

For containers made of composite plastics, the extraction may be performed by filling a labeled volume of water in the

container. In this case, it is necessary to record the volume of water used and the inside area of the container.

When containers are deformed at 121°C, the extraction may be performed at the highest temperature which does not cause deformation among the following conditions: at 100 ± 2°C for 2 ± 0.2 hours, at 70 ± 2°C for 24 ± 2 hours, at 50 ± 2°C for 72 ± 2 hours or at 37 ± 1°C for 72 ± 2 hours.

Prepare the blank solution with water in the same manner. For containers made of composite plastics, water is used as the blank solution.

Perform the following tests with the test solution and the blank solution:

(i) Foaming test: Place 5 mL of the test solution in a glass-stoppered test tube about 15 mm in inside diameter and about 200 mm in length, shake vigorously for 3 minutes, and measure the time needed for almost complete disappearance of the foam thus generated.

(ii) pH <2.54>: To 20 mL each of the test solution and the blank solution add 1.0 mL of a solution of potassium chloride (1 in 1000), and obtain the difference in the reading of pH between these solutions.

(iii) Potassium permanganate-reducing substances: Place 20 mL of the test solution in a glass-stoppered, conical flask, add 20.0 mL of 0.002 mol/L potassium permanganate VS and 1 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper tightly, shake, then allow to stand for 10 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the test in the same manner, using 20.0 mL of the blank solution, and obtain the difference of the consumption of 0.002 mol/L potassium permanganate VS between these solutions.

(iv) UV spectrum: Read the maximum absorbances between 220 nm and 240 nm and between 241 nm and 350 nm of the test solution against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

(v) Residue on evaporation: Evaporate 20 mL of the test solution on a water bath to dryness, and weigh the residue after drying at 105°C for 1 hour.

3. Test for fine particles

Rinse thoroughly with water the inside and outside of containers to be used for the tests, fill the container with the labeled volume of 0.9 w/v% sodium chloride solution, adjust so that the amount of air in the container is about 50 mL per 500 mL of the labeled volume, stopper tightly, and heat at 121°C for 25 minutes in an autoclave. After allowing to cool for 2 hours, take out the container, and then allow to stand at ordinary temperature for about 24 hours. If the containers are deformed at 121°C, employ a suitable temperature-time combination as directed under Extractable substances. Clean the outside of the container, mix by turning upside-down 5 or 6 times, insert immediately a clean needle for filterless infusion into the container through the rubber closure of the container, take the effluent while mixing gently in a clean container for measurement, and use as the test solution. Perform the test with the solution according to the following fine particle test, and count the numbers of fine particles with diameters of 5 – 10 μm, 10 – 25 μm and larger than 25 μm in 1.0 mL of the test solution.

Fine particle test—Counting of the fine particles must be performed in dustless, clean facilities or apparatus, using a light-shielded automatic fine particle counter. The sensor of

the counter to be used must be able to count fine particles of 1.5 μm or more in diameter. The volume to be measured is 10 mL. Adjust the counter before measurement. For calibration of the diameter and number of particles, the standard particles for calibration of the light-shielded automatic fine particle counter should be used in suspension in water or 0.9 w/v % sodium chloride solution.

Count five times the numbers of particles with diameters of 5 – 10 μm, 10 – 25 μm and more than 25 μm while stirring the test solution, and calculate the average particle numbers of four counts, excluding the first, as the number of particles in 1.0 mL of the test solution.

Note: Water and 0.9 w/v% sodium chloride solution to be used for the tests should not contain more than 0.5 particles of 5 – 10 μm in size per 1.0 mL.

4. Transparency test

Method 1: This can be applied to containers which have a smooth and not embossed surface and rather low curvature. Cut the container at homogeneous regions of low curvature and preferably the same thickness to make 5 pieces of about 0.9 × 4 cm in size, immerse each piece in water filled in a cell for determination of the ultraviolet spectrum, and determine the transmittance at 450 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a cell filled with water as a blank.

Method 2: Sensory test—This can be applied to containers which have a rough or embossed surface. It can also be applied to testing of the transparency of containers in case where the turbidity of their pharmaceutical contents must be checked.

Test solutions

Hexamethylenetetramine TS Dissolve 2.5 g of hexamethylenetetramine in 25 mL of water.

Hydrazinium sulfate TS Dissolve 1.0 g of hydrazinium sulfate in water to make 100 mL.

Formadin stock suspension To 25 mL of hexamethylenetetramine TS add 25 mL of hydrazinium sulfate TS, mix, and use after standing at 25 ± 3°C for 24 hours. This suspension is stable for about 2 months after preparation, provided it is stored in a glass bottle free from inside surface defects. Mix well before use.

Standard suspension: Dilute 15 mL of the formadin stock suspension with water to make 1000 mL. Prepare before use and use within 24 hours.

Reference suspension: Dilute 50 mL of the standard suspension with water to make 100 mL.

Tests

(i) Method 2A (with control) Take 2 containers to be tested, and place in one of them the labeled volume of the reference suspension and in the other, the same volume of water. Show the two containers to five subjects, separately, who do not know which one is which, ask which one seems to be more turbid, and calculate the rate of correct answers.

(ii) Method 2B (without control) Take 6 numbered containers to be tested, and place in three of them the labeled volume of the reference suspension and in the others, the same volume of water. Show each one of the containers at random order to five subjects, separately, who do not know which one is which, ask if it is turbid or not, and calculate the percentage of the answer that it is turbid (100 X/15, X: number of containers judged as “being turbid”) in each group.

5. Water vapor permeability test

Method 1 : This test method is applicable to containers for aqueous injection. Fill the container with the labeled volume of water. After closing it hermetically, accurately weigh the container and record the value. Store the container at $65 \pm 5\%$ relative humidity and a temperature of $20 \pm 2^\circ\text{C}$ for 14 days, and then accurately weigh the container again and record the value. Calculate the mass loss during storage.

Method 2 : This test method is provided for evaluating moisture permeability of containers for hygroscopic drugs. Unless otherwise specified, perform the test according to the following procedure.

Desiccant—Place a quantity of calcium chloride for water determination in a shallow container, taking care to exclude any fine powder, then dry at 110°C for 1 hour, and cool in a desiccator.

Procedure—Select 12 containers, clean their surfaces with a dry cloth, and close and open each container 30 times in the same manner. Ten among the 12 containers are used as “test containers” and the remaining two, as “control containers”. A torque for closing screw-capped containers is specified in Table 7.02-1. Add desiccant to 10 of the containers, designated test containers, filling each to within 13 mm of the closure if the container volume is 20 mL or more, or filling each to two-thirds of capacity if the container volume is less than 20 mL. If the interior of the container is more than 63 mm in depth, an inert filler or spacer may be placed in the bottom to minimize the total mass of the container and desiccant; the layer of desiccant in such a container shall be not less than 5 cm in depth. Close each container immediately after adding desiccant, applying the torque designated in the table. To each of the control containers, add a sufficient number of glass beads to attain a mass approximately equal to that of each of the test containers, and close, applying the torque designated in the table. Record the mass of the individual containers so prepared to the nearest 0.1 mg if the container volume is less than 20 mL, to the nearest 1 mg if the container volume is 20 mL or more but less than 200 mL, or to the nearest 10 mg if the container volume is 200 mL or more, and store the containers at $75 \pm 3\%$ relative humidity and a temperature of $20 \pm 2^\circ\text{C}$. After 14 days, record the mass of the individual containers in the same manner. Completely fill 5 empty containers with water or a non-compressible, free-flowing solid such as fine glass beads, to the level indicated by the closure surface when in place. Transfer the contents of each to a graduated cylinder, and determine the average container volume, in mL. Calculate the rate of moisture permeability, in mg per day per liter, by use of the formula:

$$(1000/14 V) [(T_f - T_i) - (C_f - C_i)]$$

V : average volume (mL)

$T_f - T_i$: difference between the final and initial masses of each test container (mg)

$C_f - C_i$: average of the differences between the final and initial masses of the two controls (mg)

6. Leakage test

Fill a container with a solution of fluorescein sodium (1 in 1000), stopper tightly, place filter papers on and under the container, and apply a pressure of 6.9 N (0.7 kg)/ cm^2 at 20°C for 10 minutes. Judge the leakiness by observing the color of the paper.

Table 7.02-1 Torque Applicable to Screw-Type Container

Closure Diameter (mm)	Torque (N·cm)
8	59
10	60
13	88
15	59-98
18	78-118
20	88-137
22	98-157
24	118-206
28	137-235
30	147-265
33	167-284
38	196-294
43	196-304
48	216-343
53	235-402
58	265-451
63	284-490
66	294-510
70	314-569
83	363-735
86	451-735
89	451-794
100	510-794
110	510-794
120	618-1069
132	677-1069

7. Cytotoxicity test

The following test methods are designed to detect cytotoxic substances in plastic materials by evaluating the cytotoxicity of the culture medium extracts from plastic containers for pharmaceutical products. Other appropriate standard methods of cytotoxicity testing may be used for the evaluation, if appropriate. However, the final decision shall be made based upon the test methods given here, if the test results obtained according to the other methods are questionable.

Cell lines

The recommended cell lines are L929 (American Type Culture Collection-ATCC CCL1) and V79 (Health Science Research Resources Bank-JCRB0603). In addition, other established cell lines may be used when it is confirmed that they form well-defined colonies reproducibly, with characteristics comparable to those of L929 and V79 cells.

Culture medium

Eagle's minimum essential medium prepared as follows shall be used. Dissolve the chemicals listed below in 1000 mL of water. Sterilize the solution by autoclaving at 121°C for 20 minutes. Cool the solution to room temperature, and add 22 mL of sterilized sodium hydrogen carbonate TS and 10 mL of sterilized glutamine TS. To the resultant solution add fetal calf serum (FCS) to make 10 vol% FCS in the medium.

sodium chloride	6.80 g
potassium chloride	400 mg
sodium dihydrogen phosphate (anhydrous)	115 mg
magnesium sulfate (anhydrous)	93.5 mg
calcium chloride (anhydrous)	200 mg
glucose	1.00 g
L-arginine hydrochloride	126 mg
L-cysteine hydrochloride monohydrate	31.4 mg

L-tyrosine	36.0 mg
L-histidine hydrochloride monohydrate	42.0 mg
L-isoleucine	52.0 mg
L-leucine	52.0 mg
L-lysine hydrochloride	73.0 mg
L-methionine	15.0 mg
L-phenylalanine	32.0 mg
L-threonine	48.0 mg
L-tryptophan	10.0 mg
L-valine	46.0 mg
succinic acid	75.0 mg
succinic acid hexahydrate	100 mg
choline bitartrate	1.8 mg
folic acid	1.0 mg
myo-inositol	2.0 mg
nicotinamide	1.0 mg
calcium D-pantothenate	1.0 mg
pyridoxal hydrochloride	1.0 mg
riboflavin	0.1 mg
thiamine hydrochloride	1.0 mg
biotin	0.02 mg
phenol red	6.0 mg

Reagents

(i) Sodium hydrogen carbonate TS: Dissolve 10 g of sodium hydrogen carbonate in water to make 100 mL. Sterilize the solution either by autoclaving in a well-sealed container at 121°C for 20 minutes or by filtration through a membrane filter with a nominal pore diameter of 0.22 µm or less.

(ii) Glutamine TS: Dissolve 2.92 g of L-glutamine in water to make 100 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 µm.

(iii) Phosphate buffer solution (PBS): Dissolve 0.20 g of potassium chloride, 0.20 g of potassium dihydrogenphosphate, 8.00 g of sodium chloride, and 1.15 g of disodium hydrogenphosphate (anhydrous) in water to make 1000 mL. Sterilize the solution by autoclaving at 121°C for 20 minutes.

(iv) Trypsin TS: Dissolve 0.5 g of trypsin and 0.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in phosphate buffer solution to make 1000 mL. Sterilize the solution by passing it through a membrane filter of pore size equal to or less than 0.22 µm.

(v) Formaldehyde solution: Dilute formaldehyde solution with water by a factor of ten.

(vi) Giemsa's stain solution: Dilute a commercially available Giemsa's test solution with the diluent by a factor of fifty. Prepare before use.

(vii) Diluent: Dissolve 4.54 g of potassium dihydrogenphosphate and 4.75 g of disodium hydrogen phosphate (anhydrous) in water to make 1000 mL.

Devices and instruments

It is recommended to use the following devices and instruments for testing.

(i) Pipets : Pasteur pipet, pipet for partial delivery, measuring pipet for partial delivery, and dispenser with microtip.

(ii) Screw-capped glass bottles : 50 – 1000 mL volume.

(iii) Sterile disposable centrifuge tubes: 15 and 50 mL volume.

(iv) Sterile disposable tissue culture flasks with a flat growth area of approximately 25 or 75 cm².

(v) Sterile disposable multiple well plates (24 wells)

(vi) Inverted microscope and stereomicroscope

(vii) Humidified incubator—maintain the conditions as follows: temperature, 37°C; CO₂ gas concentration, 5%.

Control materials and substances

(i) Negative control material: polyethylene film

(ii) Positive control material (A): polyurethane film containing 0.1% zinc diethyldithiocarbamate

(iii) Positive control material (B): polyurethane film containing 0.25% zinc dibutyldithiocarbamate

(iv) Control substances: zinc diethyldithiocarbamate (reagent grade) and zinc dibutyldithiocarbamate (reagent grade)

Test procedure

(i) Sample preparation: When the material of the container consists of a single homogeneous layer, subdivide the cut pieces of a container into pieces of the size of approximately 2 × 15 mm and subject the pieces to the test. When the material of the container has multiple layers, such as laminated and coated materials, prepare cut pieces with a surface area of one side of 2.5 cm² and subject the pieces to the test without subdividing them into smaller pieces.

(ii) Preparation of test solutions: Transfer an appropriate amount of the sample to a screw-capped glass bottle or a sterile disposable centrifuge tube. Cap the bottle or tube loosely and cover the cap with clean aluminum foil. Sterilize the bottle or tube by autoclaving at 121°C for 20 minutes. When the material of the sample is not resistant to heat during autoclaving, gas sterilization with ethylene oxide (EO) may be used. In the case of EO sterilization, sufficient aeration should be achieved to avoid an additional toxic effect of residual EO in the test results. To the bottle or tube add the culture medium in a proportion of 10 mL to one gram or 1 mL to 2.5 cm² (one side) of the sample, loosely cap the bottle or tube, and allow to stand in a humidified incubator for 24 hours. Transfer the culture medium extract, which is designated 100% test solution, to a sterilized screw-capped glass bottle or a sterile disposable centrifuge tube. Dilute the 100% test solution with culture medium using a dilution factor of two to prepare serial dilutions having extract concentrations of 50%, 25%, 12.5%, 6.25%, 3.13% and so on.

(iii) Preparation of cell suspension: Remove the culture medium from the maintained cell culture flask and rinse the cells with an appropriate volume of PBS by gentle rotation of the flask two or three times, and discard the PBS. Add a sufficient volume of trypsin solution to cover the cell layer. Cap the flask and place the flask in a humidified incubator for one or two minutes. After confirming detachment of the cell layer from the bottom of the flask by using a microscope, add an appropriate volume of the fresh culture medium and gently pipet the cells completely out of the flask by using a Pasteur pipet. Transfer the pipetted cell suspension into a sterile disposable centrifuge tube and centrifuge the tube at 800 – 1000 revolutions per minute for 2 – 5 minutes. Discard the supernatant, resuspend the cells in an appropriate volume of PBS by pipetting, using a Pasteur pipet, and centrifuge the tube again. Discard the PBS, and add an appropriate volume of fresh culture medium to the flask. Resuspend the cells by pipetting and make a single cell suspension. Determine the cell concentration using a hemocytometer.

(iv) Cytotoxicity testing: Dilute the cell suspension prepared according to procedure (iii) with culture medium to adjust the cell concentration to 100 cells/mL. Place a 0.5 mL aliquot of the diluted cell suspension on each well of a sterile

disposable multiple well plate. Incubate the plate in the humidified incubator for 4 – 6 hours to attach the cells to the bottom surface of the well. Discard the medium from each well, and add a 0.5 mL aliquot of the test solution or fresh medium to quadruplicate wells. Place the plate immediately in the humidified incubator and incubate the plate for the appropriate period: 7 – 9 days for L929 cells; 6 – 7 days for V79 cells. After the incubation, discard the medium from the plate, add an appropriate volume of dilute formaldehyde TS to each well and allow the plate to stand for 30 minutes to fix the cells. Discard the dilute formaldehyde TS from each well and add an appropriate volume of dilute Giemsa's TS to each well. After ensuring good staining of the colonies, discard the stain solution from the wells and count the number of colonies in each well. Calculate a mean number of colonies for each concentration of the test solution, and divide the mean by the mean number of colonies for the fresh medium to obtain the colony formation rate (%) for each extract concentration of the test solution. Plot the extract concentration (%) of the test solution on a logarithmic scale and the colony formation rate on an ordinary scale on semi-logarithmic graph paper to obtain a colony formation inhibition curve of the container. Read the % extract concentration which inhibits colony formation to 50%, IC_{50} (%), from the inhibition curve. It is recommended to check the sensitivity and the reproducibility of the test system by the use of suitable control materials or substances in the test system, if necessary.

Plastic Containers for Aqueous Injections

Plastic containers for the aqueous injections do not interact with pharmaceuticals contained therein to alter the efficacy, safety or stability, and do not permit the contamination with microorganisms. The containers meet the following requirements.

1. Polyethylene or polypropylene containers for aqueous injections

The containers are made of polyethylene or polypropylene and free from any adhesive.

(1) Transparency—The containers have a transmittance of not less than 55%, when tested as directed in Method 1 under the Transparency test. When Method 1 can not be applied, test according to the Method 2B of the Transparency test. In this case, the rate that the water-containing container is judged as “being turbid” is not more than 20%, and the rate that the reference suspension-containing container is judged as “being turbid” is not less than 80%.

(2) Appearance—The containers do not have strips, cracks, bubbles, or other faults which cause difficulties in practical use.

(3) Water vapor permeability—Proceed as directed in Method 1 of the Water vapor permeability test. The loss of mass is not more than 0.20%.

(4) Heavy metals <1.07>—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(5) Lead—Perform the test as directed in Method 1. The absorbance of the sample solution is not more than that of the standard solution.

(6) Cadmium—Perform the test as directed in Method 1. The absorbance of the sample solution is not more than that of the standard solution.

(7) Residue on ignition—The residue is not more than 0.1 %.

(8) Extractable substances—

(i) Foaming test: the foam formed almost disappears within 3 minutes.

(ii) pH: the difference in the reading of pH between the test solution and the blank solution is not more than 1.5.

(iii) Potassium permanganate-reducing substances: The difference in the consumption of 0.002 mol/L potassium permanganate VS between the test solution and the blank solution is not more than 1.0 mL.

(iv) UV spectrum: The maximum absorbance between 220 nm and 240 nm is not more than 0.08, and that between 241 nm and 350 nm is not more than 0.05.

(v) Residue on evaporation: Not more than 1.0 mg.

(9) Cytotoxicity— IC_{50} (%) is not less than 90%. The result obtained by the other standard methods is negative.

2. Polyvinyl chloride containers for aqueous injections

The containers are composed of homopolymer of vinyl chloride, free from any adhesive, and the plasticizer added to the material should be di(2-ethylhexyl)phthalate. The containers may be covered with easily removable material to prevent the permeation of water vapor. In this case, perform the water vapor permeability test on the covered containers.

(1) Thickness—Measure the thickness of a container at five different locations. The difference between the maximum and minimum values of thickness is 0.05 mm or less.

(2) Transparency—Proceed as directed in (1) under Polyethylene or polypropylene containers for aqueous injections.

(3) Appearance—Proceed as directed in (2) under Polyethylene or polypropylene containers for aqueous injections.

(4) Leakage—Proceed with the test according to Leakage test. The solution contained does not leak.

(5) Flexibility—Insert the spike needle for infusion through a rubber closure of the container used in (4) Leakage. The contained solution is almost completely discharged without displacement by air.

(6) Water vapor permeability—Proceed as directed in (3) under Polyethylene or polypropylene containers for aqueous injections.

(7) Heavy metals <1.07>—The turbidity of the test solution is not greater than that of the control solution when the amount of the sample taken is 1.0 g.

(8) Lead—Perform the test as directed in Method 2. The absorbance of the sample solution is not more than that of the standard solution.

(9) Cadmium—Perform the test as directed in Method 2. The absorbance of the sample solution is not more than that of the standard solution.

(10) Tin—The absorbance of the sample solution is not more than that of the standard solution.

(11) Vinyl chloride—Wash a cut piece of a container with water, wipe thoroughly with a filter paper, subdivide into pieces smaller than 5-mm square, and place 1.0 g of them in a 20-mL volumetric flask. Add about 10 mL of tetrahydrofuran for gas chromatography, dissolve by occasional shaking in a cold place, add tetrahydrofuran for gas chromatography, previously cooled in a methanol-dry ice bath, to make 20 mL while cooling in a methanol-dry ice bath, and use this solution as the sample solution. Perform the tests as directed under Gas Chromatography <2.02> according to the operating conditions 1 and 2, using 10 μ L each of the sample solution and Standard Vinyl Chloride Solution. Under either operating condition, the peak height of vinyl chloride from

the sample solution is not more than that from the Standard Vinyl Chloride Solution.

Operating conditions 1—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and 2 to 3 m in length, packed with 150 to 180 μm siliceous earth for gas chromatography coated with 15% to 20% polyalkylene glycol monoether for gas chromatography.

Column temperature: A constant temperature of between 60°C and 70°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 1.5 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

Operating conditions 2—

Detector: A hydrogen flame-ionization detector. Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with 150 to 180 μm porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore size: 0.06 – 0.08 μm ; 100 – 200 m^2/g).

Column temperature: A constant temperature of about 120°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of vinyl chloride is about 3 minutes.

Selection of column: Proceed with 10 μL of Standard Vinyl Chloride Solution under the above operating conditions. Use a column from which vinyl chloride and ethanol are eluted in that order, with a good resolution between their peaks.

Detection sensitivity: Adjust the detection sensitivity so that the peak height from 10 μL of the Standard Vinyl Chloride Solution is 5 to 7 mm.

(12) Fine particles—The number of fine particles in 1.0 mL of the test solution is counted as not more than 100 of 5 to 10 μm , not more than 10 of 10 to 25 μm and not more than 1 of 25 μm or more.

(13) Residue on ignition—The residue is not more than 0.1%.

(14) Extractable substances—Proceed as directed in (8) under Polyethylene or polypropylene containers for aqueous injections.

(15) Cytotoxicity—Proceed as directed in (9) under Polyethylene or polypropylene containers for aqueous injections.

3. Plastic containers for aqueous injections being not described above

The containers meet the following specifications and other necessary specifications for their materials with regard to heavy metals, residue on ignition and extractable substances, etc.

(1) Transparency—Proceed as directed in (1) under Polyethylene or polypropylene containers for aqueous injections.

(2) Appearance—Proceed as directed in (2) under Polyethylene or polypropylene containers for aqueous injections.

(3) Vapor permeability—Proceed as directed in (3) under Polyethylene or polypropylene containers for aqueous injections.

tions.

(4) Cytotoxicity—Proceed as directed in (9) under Polyethylene or polypropylene containers for aqueous injections.

7.03 Test for Rubber Closure for Aqueous Infusions

The rubber closure for aqueous infusions means a rubber closure (containing material coated or laminated with the stuff like plastics) used for a container for aqueous infusion having a capacity of 100 mL or more, and is in direct contact with the contained aqueous infusion. The rubber closure when in use does not interact physically or chemically with the contained medicament to alter any property or quality, does not permit the invasion of microbes, does not disturb the use of the contained infusion, and meets the following requirements.

(1) Cadmium—Wash the rubber closures with water, dry at room temperature, cut into minute pieces, mix well, place 2.0 g of them in a crucible of platinum or quartz, moisten them with 2 mL of sulfuric acid, heat gradually to dryness, and ignite between 450°C and 500°C until the residue is incinerated. When incineration was insufficient, moisten the residue with 1 mL of sulfuric acid, heat to dryness, and ignite again. Repeat the above-mentioned procedure if necessary. Cool the crucible, moisten the residue with water, add 2 to 4 mL of hydrochloric acid, heat on a water bath to dryness, add 1 to 5 mL of hydrochloric acid, and dissolve by heating. Then add 0.5 to 1 mL of a mixture of a solution of citric acid monohydrate (1 in 2) and hydrochloric acid (1:1) and 0.5 to 1 mL of a warmed solution of ammonium acetate (2 in 5). When any insoluble residue remains, filter through a glass filter. To the solution thus obtained add 10 mL of a solution of diammonium hydrogen citrate (1 in 4), 2 drops of bromothymol blue TS and ammonium TS until the color of the solution changes from yellow to green. Then add 10 mL of ammonium sulfate solution (2 in 5) and water to make 100 mL. Next, add 20 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 20), mix, allow to stand for a few minutes, add 20.0 mL of 4-methyl-2-pentanone, and mix by vigorous shaking. Allow to stand to separate the 4-methyl-2-pentanone layer from the solution, filter if necessary, and use as the sample solution. On the other hand, to 10.0 mL of Standard Cadmium Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen

Supporting gas—Air

Lamp: Cadmium hollow-cathode lamp

Wavelength: 228.8 nm

(2) Lead—To 1.0 mL of the Standard Lead Solution add 10 mL of a solution of diammonium hydrogen citrate (1 in 4) and 2 drops of bromothymol blue TS, proceed as directed for the sample solution under (1), and use this solution as the

standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, using the sample solution obtained in (1) and the standard solution. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air

Lamp: Lead hollow-cathode lamp

Wavelength: 283.3 nm

(3) Extractable substances—Wash the rubber closures with water, and dry at room temperature. Place them in a glass container, add water exactly 10 times the mass of the test material, close with a suitable stopper, heat at 121°C for 1 hour in an autoclave, take out the glass container, allow to cool to room temperature, then take out immediately the rubber closures, and use the remaining solution as the test solution. Prepare the blank solution with water in the same manner. Perform the following tests with the test solution and the blank solution.

(i) Description: The test solution is clear and colorless. Read the transparency of the test solution at 430 nm and 650 nm (10 mm), using the blank solution as the blank. Both of them are not less than 99.0%.

(ii) Foam test: Place 5 mL of the test solution in a glass-stoppered test tube of about 15 mm in inner diameter and about 200 mm in length, and shake vigorously for 3 minutes. The foam arisen disappears almost completely within 3 minutes.

(iii) pH <2.54>: To 20 mL each of the test solution and the blank solution add 1.0 mL each of potassium chloride solution, prepared by dissolving 1.0 g of potassium chloride in water to make 1000 mL. The difference of pH between the two solutions is not more than 1.0.

(iv) Zinc: To 10.0 mL of the test solution add diluted dilute nitric acid (1 in 3) to make 20 mL, and use this solution as the sample solution. Further, to 1.0 mL of Standard Zinc Solution for atomic absorption spectrophotometry add diluted nitric acid (1 in 3) to make exactly 20 mL, and use this solution as the standard solution. Perform the tests according to the Atomic Absorption Spectrophotometry <2.23>, using these solutions, under the following conditions. The absorbance of the sample solution is not more than that of the standard solution.

Gas: Combustible gas—Acetylene
Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

Standard Zinc Solution for atomic absorption spectrophotometry: Measure exactly 10 mL of the Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. One mL of this solution contains 0.01 mg of zinc (Zn).

(v) Potassium Permanganate-reducing substances: Measure 100 mL of the test solution in a glass-stoppered, Erlenmeyer flask, add 10.0 mL of 0.002 mol/L potassium permanganate VS and 5 mL of dilute sulfuric acid, and boil for 3 minutes. After cooling, add 0.10 g of potassium iodide, stopper, mix by shaking, then allow to stand for 10 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 5 drops of starch TS). Perform the blank test in the same manner, using 100 mL of the blank solution. The difference in mL of 0.002 mol/L potassium permanganate VS required between the tests is not more than 2.0 mL.

(vi) Residue on evaporation: Measure 100 mL of the test solution, evaporate on a water bath to dryness, and dry the residue at 105°C for 1 hour. The mass of the residue is not more than 2.0 mg.

(vii) UV spectrum: Read the absorbance of the test solution between 220 nm and 350 nm against the blank solution as directed under Ultraviolet-visible Spectrophotometry <2.54>: it is not more than 0.20.

(4) Acute systemic toxicity—The test solution meets the requirements, when examined under the following conditions against the blank solution.

Preparation of the test solution and the blank solution: Wash the rubber closures with water and Water for Injection successively, and dry under clean conditions at room temperature. Transfer the rubber closures to a glass container. Add isotonic sodium chloride solution 10 times the mass of the test material, stopper adequately, heat in an autoclave at 121°C for 1 hour, take out the glass container, and allow to cool to room temperature. The solution thus obtained is used as the test solution. The blank solution is prepared in the same manner.

(i) Test procedures

Test animals: Use healthy male mice of inbred strain or from a closed colony, weighing 17 to 23 g.

Procedure: Separate the animals into two groups of 10 mice, and inject intravenously 50 mL each of the solutions per kg body mass.

(ii) Interpretation

Observe the animals for 5 days after injection: During the observation period, none of the animals treated with the test solution show any abnormality or death.

(5) Pyrogen test—The test solution specified in (4) meets the requirements of the Pyrogen Test <4.04> as does the blank solution.

(6) Hemolysis test—When 0.1 mL of defibrinated blood of rabbit is added to 10 mL of the test solution specified in (4) and the mixture is allowed to stand at 37°C for 24 hours, hemolysis is not observed. Perform the blank test in the same manner, using 10 mL of the blank solution.

8. Other Methods

8.01 Sterilization and Aseptic Manipulation, and Reverse Osmosis-Ultrafiltration

(1) Sterilization and Aseptic Manipulation

1. Sterilization

Sterilization means a process whereby the killing or removal of all living microorganisms is accomplished. Generally, the sterilization process requires the choice of appropriate procedure and accurately controlled operation and conditions depending on the kind of microorganism, the conditions of contamination and the quality and nature of the sub-

stance to be sterilized.

The adequacy of sterilization is decided by means of the Sterility Test <4.06>.

The procedure for sterilization should be carried out after confirming that the temperature, pressure, etc. are adequate for the desired sterilization.

For the choice of the conditions for sterilization or verification of the integrity of sterilization, biological indicators suitable for individual conditions of sterilization may be used.

2. Aseptic manipulation

Aseptic manipulation is a technique used for processing the sterile drug products which are not terminally sterilized in their final containers, and applied to a series of aseptic processing of the sterile products which are prepared by the filtration sterilization and/or with sterile raw materials.

Generally, aseptic manipulation requires the presterilization of all equipments and materials used for processing the sterile products, and then the products are processed in a way to give a defined sterility assurance level in the aseptic processing facilities where microbial and particulate levels are adequately maintained.

(2) Reverse Osmosis-Ultrafiltration

Reverse Osmosis-Ultrafiltration is a water filtration method by means of crucial flow filtration utilizing either a reverse osmotic membrane or an ultrafilter, or an apparatus combining both.

When Water for Injection is prepared by the reverse osmosis-ultrafiltration, pretreatment facilities, facilities for preparation of water for injection, and facilities for supplying water for injection are usually used. The pretreatment facilities, placed before the preparation facilities, are used to remove solid particles, dissolved salts and colloids in original water, so as to reduce load on the preparation facilities. They are assemblies having a cohesion apparatus, precipitation-separation apparatus, filtration apparatus, chlorine sterilization apparatus, oxidation-reduction apparatus, residual chlorine removing apparatus, precise filtration apparatus, reverse osmosis apparatus, ultrafiltration apparatus, ion exchange apparatus, etc., which are combined properly depending upon the quality of original water. The facilities for preparing water for injection consist of a pretreatment water supplying apparatus, ultraviolet sterilization apparatus, heat exchange apparatus, membrane module, cleansing-sterilization apparatus, etc. The facilities for supplying water for injection consist of a reservoir with a capacity to meet changing demand, tubes for distributing Water for Injection, heat exchange apparatus, circulation pump, pressure control apparatus, etc. Usually, Water for Injection prepared by the reverse osmosis-ultrafiltration circulates in the facilities at a temperature not lower than 80°C for prevention of microbial proliferation.

For preparing water for Injection by means of the reverse osmosis-ultrafiltration, use a membrane module which removes microorganisms and substances of molecular masses approximately not less than 6000.

9. Reference Standards; Standard Solutions; Reagents, Test Solutions; Measuring Instruments, Appliances, etc.

Reference Standards

9.01 Reference Standards

Reference Standards are the reference substances prepared to a specified quality necessary with regard to their intended use as prescribed in monographs of the Pharmacopoeia.

The Japanese Pharmacopoeia Reference Standards are as follows:

- * A: Assay
- AF: Anti-factor IIa activity
- AH: Potency test for anti-heparin
- B: Bacterial Endotoxins Test <4.01>
- C: Content ratio of active principle
- D: Dissolution
- DG: Digestion Test <4.03>
- I: Identification
- IS: Isomer ratio
- M: Melting Point Determination <2.60>
- P: Purity
- T: Thermal Analysis <2.52>
- U: Uniformity of dosage units
- V: Vitamin A Assay <2.55>

Reference Standard	Intended Use*
Aceglutamide	I, P, A
Acetaminophen	I, A
Aclarubicin	A
Actinomycin D	I, A
Adrenaline Bitartrate	P
Alprostadiol	I, P, A
Amikacin Sulfate	I, A
<i>p</i> -Aminobenzoyl Glutamic Acid	P
Amitriptyline Hydrochloride	I, D, A
Amoxicillin	I, A
Amphotericin B	I, P, A
Ampicillin	I, P, A
Anhydrous Lactose	I
Arbekacin Sulfate	I, A
Ascorbic Acid	A
Aspirin	A
Aspoxicillin	I, P, A
Astromicin Sulfate	I, A
Atropine Sulfate	I, A
Azathioprine	I, A
Azithromycin	I, A
Aztreonam	I, P, A

Bacampicillin Hydrochloride	I, A	Cholecalciferol	I, A
Bacitracin	I, A	Ciclacillin	I, A
Baclofen	I, D, A	Ciclosporin	I, P, A
Baicalin	I, A	Cilostazol	I, U, D, A
Beclometasone Dipropionate	I, A	Cisplatin	I, A
Bekanamycin Sulfate	I, A	Clarithromycin	I, P, U, D, A
Benzyloxyphenacillin Potassium	I, A	Clindamycin Hydrochloride	I, U, D, A
Berberine Chloride	I, A	Clindamycin Phosphate	I, P, A
Betamethasone	I, P, U, D, A	Clofibrate	I, A
Betamethasone Sodium Phosphate	I, A	Clomifene Citrate	I, A
Betamethasone Valerate	I, A	Cloxacillin Sodium	I, P, A
Bisacodyl	I, A	Colistin Sodium Methanesulfonate	I, A
Bleomycin A ₂ Hydrochloride	A	Colistin Sulfate	A
Caffeine	A	Cortisone Acetate	I, A
Calcium Folate	I, A	Cyanocobalamin	I, P, A
Calcium Oxalate Monohydrate	T	Cycloserine	I, A
Camostat Mesilate	I, A	Daunorubicin Hydrochloride	I, A
<i>d</i> -Camphor	A	Deferoxamine Mesilate	I, A
<i>dl</i> -Camphor	A	Demethylchlortetracycline Hydrochloride	I, P, A
Carbidopa	I, P, A	Deslanoside	I, P, A
Carumonam Sodium	I, P, A	Dexamethasone	I, A
Cefaclor	I, P, U, D, A	Dibekacin Sulfate	I, P, A
Cefadroxil	I, A	Diclofenamide	I, P, D, A
Cefalexin	A	Dicloxacillin Sodium	I, A
Cefalotin Sodium	I, P, A	Diethanolammonium Fusidate	A
Cefapirin Sodium	I, A	Diethylcarbamazine Citrate	A
Cefatrizine Propylene Glycolate	I, A	Digitoxin	I, U, D, A
Cefazolin	P, A	Digoxin	I, U, D, A
Cefbuperazone	A	Dihydroergotoxine Mesilate	A
Cefcapene Pivoxil Hydrochloride	I, U, A	Dobutamine Hydrochloride	I, A
Cefdinir	I, P, D, A	Doxorubicin Hydrochloride	I, A
Cefditoren Pivoxil	I, U, D, A	Doxycycline Hydrochloride	I, A
Cefepime Dihydrochloride	I, A	Edrophonium Chloride	I, A
Cefixime	I, P, A	Elcatonin	A
Cefmenoxime Hydrochloride	I, P, A	Endotoxin 100	B
Cefmetazole	A	Endotoxin 10000	B
Cefminox Sodium	I, A	Enviomycin Sulfate	A
Cefodizime Sodium	I, P, A	Epirubicin Hydrochloride	I, P, A
Cefoperazone	A	Epitiostanol	P, A
Cefotaxime	P, A	Ergocalciferol	I, A
Cefotetan	I, P, A	Ergometrine Maleate	P, U, A
Cefotiam Hexetil Hydrochloride	I, P, IS, A	Erythromycin	I, P, A
Cefotiam Hydrochloride	I, P, A	Estradiol Benzoate	I, P, A
Cefozopran Hydrochloride	I, A	Estriol	I, U, D, A
Cefpiramide	P, A	Ethenzamide	A
Cefpirome Sulfate	I, A	Ethinylestradiol	I, U, D, A
Cefpodoxime Proxetil	I, IS, A	Ethyl Aminobenzoate	A
Cefroxadine	I, A	Ethyl Icosapentate	I, P, A
Cefsulodin Sodium	I, P, A	Etoposide	I, A
Ceftazidime	I, A	Faropenem Sodium	I, U, A
Cefteram Pivoxil Mesitylene Sulfonate	A	Flomoxef Triethylammonium	P, A
Ceftibuten Hydrochloride	A	Fluocinolone Acetonide	I, A
Ceftizoxime	P, A	Fluocinonide	I, A
Ceftriaxone Sodium	I, A	Fluorometholone	I, A
Cefuroxime Axetil	I, P, IS, A	Fluoxymesterone	I, A
Cefuroxime Sodium	I, A	Folic Acid	I, A
Cellulose	I	Fosfestrol	I, D, A
Chloramphenicol	I, A	Fosfomycin Phenethylammonium	A
Chloramphenicol Palmitate	I, A	Fradiomycin Sulfate	I, A
Chloramphenicol Succinate	A	Furosemide	I, U, D, A
Chlordiazepoxide	I, P, A	Fursultiamine Hydrochloride	I, A
Chlormadinone Acetate	I, A	Gabexate Mesilate	I, P, A
Chlorpheniramine Maleate	I, U, A		

Gentamicin Sulfate	I, A	Metildigoxin	I, A
Ginsenoside Rb ₁	I, A	Mexiletine Hydrochloride	I, P, A
Ginsenoside Rg ₁	I, A	Micronomicin Sulfate	I, A
Gitoxin	P	Midecamycin	I, A
Glycyrrhizic Acid	I, A	Midecamycin Acetate	I, A
Gonadorelin Acetate	I, A	Minocycline Hydrochloride	I, P, A
Gramicidin	I, A	Mitomycin C	I, A
Griseofulvin	I, P, A	Mupirocin Lithium	P, A
Guaifenesin	I, A	Neostigmine Methylsulfate	I, A
Heparin Sodium	AH, A	Netilmicin Sulfate	I, A
High-molecular Mass Urokinase	A	Nicotinamide	I, A
Human Chorionic Gonadotrophin	A	Nicotinic Acid	I, A
Human Insulin	I, A	Nilvadipine	I, U, D, A
Human Menopausal Gonadotrophin	P, A	Noradrenaline Bitartrate	P, A
Hydrochlorothiazide	I, A	Norgestrel	I, U, D, A
Hydrocortisone	I, P, A	Nystatin	I, P, A
Hydrocortisone Acetate	I, A	Oxytetracycline Hydrochloride	I, A
Hydrocortisone Sodium Phosphate	I, A	Oxytocin	P, A
Hydrocortisone Succinate	I, A	Paeoniflorin	I, A
Hypromellose Phthalate	I	Panipenem	A
Idarubicin Hydrochloride	I, U, A	Pentobarbital	P, A
Idoxuridine	I, A	Peplomycin Sulfate	I, A
Imipenem	I, U, A	Perphenazine	I, U, D, A
Imipramine Hydrochloride	I, D, A	Phenethicillin Potassium	A
Indomethacin	I, P, D, A	Phytonadione	A
Insulin	P, A	Pimaricin	I, A
Interleukin-2	A	Piperacillin	A
Isepamicin Sulfate	I, P, A	Pirarubicin	I, P, A
Isoflurane	I, P, A	Pivmecillinam Hydrochloride	I, P, A
Josamycin	I, C, A	Polymixin B Sulfate	I, A
Josamycin Propionate	I, A	Potassium Sucrose Octasulfate	P, A
Kallidinogenase	A	Povidone	I
Kanamycin Monosulfate	I, P, A	Pravastatin 1,1,3,3-tetramethylbutylam- monium	I, A
Lactose	I	Prednisolone	I, U, D, A
Lactulose	P, A	Prednisolone Acetate	I, A
Lanatoside C	I, P, U, D, A	Prednisolone Succinate	I, A
Latamoxef Ammonium	P, A	Primidone	A
Lenampicillin Hydrochloride	I, A	Probenecid	I, D, A
Leucomycin A ₅	C, A	Prochlorperazine Maleate	I, A
Limaprost	P, A	Progesterone	I, A
Lincomycin Hydrochloride	I, P, A	Protamine Sulfate	P
Lithium Clavulanate	A	Puerarin	I, A
Low-molecular Mass Heparin	AF, A	Pyridoxine Hydrochloride	I, A
Loxoprofen	A	Pyrrrolnitrin	I, A
Lysozyme	A	Ranitidine Hydrochloride	I, A
Maltose	A	Reserpine	I, U, D, A
Mecobalamin	I, A	Retinol Acetate	I, V
Melting Point Standard-Acetanilide	M	Retinol Palmitate	I, V
Melting Point Standard-Acetophenetidine	M	Riboflavin	I, A
Melting Point Standard-Caffeine	M	Ribostamycin Sulfate	I, A
Melting Point Standard-Sulfanilamide	M	Rifampicin	I, P, A
Melting Point Standard-Sulfapyridine	M	Ritodrine Hydrochloride	I, U, D, A
Melting Point Standard-Vanillin	M	Rokitamycin	I, A
Menatetrenone	I, P, A	Roxatidine Acetate Hydrochloride	I, U, D, A
Meropenem	I, A	Roxithromycin	I, P, A
Mestranol	I, A	Saccharated Pepsin	A
Methotrexate	I, A	Scopolamine Hydrobromide	I, A
Methoxsalen	I, A	Sennoside A	I, A
Methyldopa	I, A	Sennoside B	A
Methylergometrine Maleate	I, U, D, A	Serum Gonadotrophin	A
Methylprednisolone Succinate	I, P, A	Siccanin	I, A
Methyltestosterone	I, U, A	Sisomicin Sulfate	I, A

Spectinomycin Hydrochloride	I, A
Spiramycin Acetate II	C, A
Spironolactone	I, A
Streptomycin Sulfate	I, A
Sulbactam	P, A
Sulbenicillin Sodium	I, A
Sulfadiazine Silver	I, A
Sulfapyrazone	I, A
Sultamicillin Tosilate	I, A
Swertiamarin	I, A
Talampicillin Hydrochloride	I, A
Teicoplanin	I, A
Testosterone Propionate	I, A
Tetracycline Hydrochloride	I, P, A
Thiamine Chloride Hydrochloride	I, P, A
Thiamylal	A
Thrombin	A
Tobramycin	I, A
Tocopherol	I, P, A
Tocopherol Acetate	I, A
Tocopherol Nicotinate	I, A
Tocopherol Succinate	A
Tolazamide	I, A
Tolbutamide	D
Tolnaftate	I, A
Tranexamic Acid	I, P, D, A
Triamcinolone	I, A
Triamcinolone Acetonide	I, A
Trichlormethiazide	I, U, D, A
Trichomyacin	A
Trihexyphenidyl Hydrochloride	I, U, D, A
Tubocurarine Chloride Hydrochloride	I, A
Tyrosine	A, DG
Ubidecarenone	I, A
Ulinastatin	A
Vancomycin Hydrochloride	I, A
Vasopressin	A
Vinblastine Sulfate	I, U, A
Warfarin Potassium	I, U, A
Zinostatin Stimalamer.	I, A

Standard Solutions

9.21 Standard Solutions for Volumetric Analysis

Standard Solutions for Volumetric Analysis are the solutions of reagent with an accurately known concentration, mainly used for the volumetric analysis. They are prepared to a specified molar concentration. A 1 molar solution is a solution which contains exactly 1 mole of a specified substance in each 1000 mL of the solution and is designated as 1 mol/L. If necessary, these solutions are diluted to other specified molar concentrations and the diluted solutions are also used as standard solutions. For example, 0.1 mol/L solution is obtained by diluting 1 mol/L solution 10 times by volume.

Unless otherwise directed, standard solutions for volumetric analysis should be stored in colorless or light-resistant,

glass-stoppered bottles.

Preparation and Standardization

A volumetric standard solution is prepared according to one of the following methods. The degree of difference from a specified concentration n (mol/L) is expressed as a factor (molar concentration coefficient) f . Usually, standard solutions are prepared so that the factor is in the range of 0.970 – 1.030. The determination procedure of the factor is called standardization of the standard solution.

(1) Weigh accurately a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the pure substance, and dissolve it in the specified solvent to make exactly 1000 mL to prepare a standard solution having a concentration close to the specified molarity n (mol/L). In this case, the factor f of the standard solution is obtained by dividing the mass of the pure substance taken (g) by the molecular mass of the substance (g) and the specified molarity number n .

When a pure substance is not obtainable, it is permissible to use a highly purified substance whose purity has been exactly determined and certified.

(2) In the case where a pure substance or a highly purified substance is not obtainable, weigh a quantity equivalent to about 1 mole or its multiple or a fractional mole number of the substance specified for each standard solution and dissolve it in the specified solvent to make about 1000 mL to prepare a standard solution having a concentration close to the specified molarity n (mol/L). The factor f of this solution is determined by applying the standardization procedure described for the respective standard solution. The procedure is classified into direct and indirect methods, as follows:

a) Direct method

Weigh accurately a standard reagent or an indicated substance specified for each standard solution, dissolve it in the specified solvent, then titrate with the prepared standard solution to be standardized, and determine the factor f by applying the following equation.

$$f = \frac{1000m}{VMn}$$

M : Molecular mass equivalent to 1 mole of the standard reagent or the specified substance (g)

m : Mass of the standard reagent or the specified substance taken (g)

V : Volume of the prepared standard solution consumed for the titration (mL)

n : Arithmetical mole number of the specified molar concentration of the standard solution to be standardized (e.g. $n=0.02$ for 0.02 mol/L standard solution)

b) Indirect method

When an appropriate standard reagent is not available, titrate a defined volume V_2 (mL) of a standard solution to be standardized with the specified standard solution having a known factor (f_1), and calculate the factor (f_2) by applying the following equation.

$$f_2 = \frac{V_1 \times f_1}{V_2}$$

f_1 : Factor of the titrating standard solution having a known factor

f_2 : Factor of the prepared standard solution to be standardized

V_1 : Volume of the titrating standard solution consumed (mL)

V_2 : Volume of the prepared standard solution taken (mL)

(3) Standard solutions may be prepared by diluting exactly an accurately measured volume of a standard solution having a known factor, according to the specified dilution procedure. During this dilution procedure, the original factor of the standard solution is assumed to remain constant.

Ammonium Thiocyanate, 0.1 mol/L

1000 mL of this solution contains 7.612 g of ammonium thiocyanate (NH_4SCN : 76.12).

Preparation—Dissolve 8 g of ammonium thiocyanate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the 0.1 mol/L silver nitrate VS, and add 50 mL of water, 2 mL of nitric acid and 2 mL of ammonium iron (III) sulfate TS. Titrate <2.50> the solution with the prepared ammonium thiocyanate solution to the first appearance of a persistent red-brown color with shaking. Calculate the molarity factor.

Note: Store protected from light.

Ammonium Thiocyanate, 0.02 mol/L

1000 mL of this solution contains 1.5224 g of ammonium thiocyanate (NH_4SCN : 76.12).

Preparation—Before use, dilute 0.1 mol/L ammonium thiocyanate VS with water to make exactly 5 times the initial volume.

Ammonium Iron (III) Sulfate, 0.1 mol/L

1000 mL of this solution contains 48.22 g of ammonium iron (III) sulfate dodecahydrate [$\text{Fe}(\text{NH}_4)(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$: 482.19].

Preparation—Dissolve 49 g of ammonium iron (III) sulfate dodecahydrate in a cooled mixture of 6 mL of sulfuric acid and 300 mL of water, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared ammonium iron (III) sulfate solution into an iodine flask, add 5 mL of hydrochloric acid, and shake the mixture. Dissolve 2 g of potassium iodide, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 50 mL of water, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period of time, should be restandardized.

Ammonium Iron (II) Sulfate, 0.1 mol/L

1000 mL of this solution contains 39.214 g of ammonium iron (II) sulfate hexahydrate [$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$: 392.14].

Preparation—Dissolve 40 g of ammonium iron (II) sulfate hexahydrate in a cooled mixture of 30 mL of sulfuric acid and 300 mL of water, dilute with water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared ammonium iron (II) sulfate solution, and add 25 mL of water

and 5 mL of phosphoric acid. Titrate <2.50> the solution with 0.02 mol/L potassium permanganate VS. Calculate the molarity factor.

Note: Prepare before use.

Ammonium Iron (II) Sulfate, 0.02 mol/L

1000 mL of this solution contains 7.843 g of ammonium iron (II) sulfate hexahydrate [$\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$: 392.14].

Preparation—Before use, dilute 0.1 mol/L ammonium iron (II) sulfate VS with diluted sulfuric acid (3 in 100) to make exactly 5 times the initial volume.

Barium chloride, 0.1 mol/L

1000 mL of this solution contains 24.426 g of barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$: 244.26).

Preparation—Dissolve 24.5 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of the prepared solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), previously warmed, heat the mixture on a water bath for 30 minutes, and allow it to stand overnight. Filter the mixture, wash the precipitate on the filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat again at about 700°C for 2 hours. After cooling, weigh accurately the mass of the residue, and calculate the molarity factor as barium sulfate (BaSO_4).

Each mL of 0.1 mol/L barium chloride VS
= 23.34 mg of BaSO_4

Barium Chloride, 0.02 mol/L

1000 mL of this solution contains 4.885 g of barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$: 244.26).

Preparation—Dissolve 4.9 g of barium chloride dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 100 mL of the prepared barium chloride solution, add 3 mL of hydrochloric acid, and warm the mixture. Add 40 mL of diluted sulfuric acid (1 in 130), warmed previously, heat the mixture on a water bath for 30 minutes, and allow to stand overnight. Filter the mixture, wash the collected precipitate of filter paper with water until the last washing shows no turbidity with silver nitrate TS, transfer the precipitate together with the filter paper to a tared crucible, and then heat strongly to ashes. After cooling, add 2 drops of sulfuric acid, and heat strongly again at about 700°C for 2 hours. After cooling, weigh accurately the residue as barium sulfate (BaSO_4), and calculate the molarity factor.

Each mL of 0.02 mol/L barium chloride VS
= 4.668 mg of BaSO_4

Barium Chloride, 0.01 mol/L

1000 mL of this solution contains 2.4426 g of barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$: 244.26).

Preparation—Before use, dilute 0.02 mol/L barium chloride VS with water to make exactly twice the initial volume.

Barium Perchlorate, 0.005 mol/L

1000 mL of this solution contains 1.6812 g of barium perchlorate $[\text{Ba}(\text{ClO}_4)_2]$: 336.23].

Preparation—Dissolve 1.7 g of barium perchlorate in 200 mL of water, dilute with 2-propanol to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of the prepared barium perchlorate solution, add 55 mL of methanol and 0.15 mL of arsenazo III TS. Titrate <2.50> the solution with 0.005 mol/L sulfuric acid VS until its purple color changes through red-purple to red. Calculate the molarity factor.

Bismuth Nitrate, 0.01 mol/L

1000 mL of this solution contains 4.851 g of bismuth nitrate pentahydrate $[\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}]$: 485.07].

Preparation—Dissolve 4.86 g of bismuth nitrate pentahydrate in 60 mL of dilute nitric acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared bismuth nitrate solution, add 50 mL of water and 1 drop of xylenol orange TS, and titrate <2.50> the solution with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red color changes to yellow. Calculate the molarity factor.

Bromine, 0.05 mol/L

1000 mL of this solution contains 7.990 g of bromine (Br: 79.90).

Preparation—Dissolve 2.8 g of potassium bromate and 15 g of potassium bromide in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared solution into an iodine flask. Add 120 mL of water, quickly add 5 mL of hydrochloric acid, stopper the flask immediately, and shake it gently. Then add 5 mL of potassium iodide TS, re-stopper immediately, shake the mixture gently, and allow to stand for 5 minutes. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Cerium (IV) Tetraammonium Sulfate, 0.1 mol/L

1000 mL of this solution contains 63.26 g of cerium (IV) tetraammonium sulfate dihydrate $[\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}]$: 632.55].

Preparation—Dissolve 64 g of cerium (VI) tetraammonium sulfate dihydrate in 0.5 mol/L sulfuric acid VS to make 1000 mL, allow to stand for 24 hours, filter the solution through a glass filter (G3 or G4), if necessary, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared cerium (IV) tetraammonium sulfate solution into an iodine flask. Add 20 mL of water and 20 mL of dilute sulfuric acid, then dissolve 1 g of potassium iodide in the mixture. Immediately titrate <2.50> the solution with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored

for a long period of time, should be restandardized.

Cerium (IV) Tetraammonium Sulfate, 0.01 mol/L

1000 mL of this solution contains 6.326 g of cerium (IV) tetraammonium sulfate dihydrate $[\text{Ce}(\text{NH}_4)_4(\text{SO}_4)_4 \cdot 2\text{H}_2\text{O}]$: 632.55].

Preparation—Before use, dilute 0.1 mol/L cerium (IV) tetraammonium sulfate VS with 0.5 mol/L sulfuric acid VS to make exactly 10 times the initial volume.

Ceric Ammonium Sulfate, 0.1 mol/L

See cerium (IV) tetraammonium sulfate, 0.1 mol/L.

Ceric Ammonium Sulfate, 0.01 mol/L

See cerium (IV) tetraammonium sulfate, 0.01 mol/L.

Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.1 mol/L

1000 mL of this solution contains 37.224 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate $(\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O})$: 372.24).

Preparation—Dissolve 38 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Wash zinc (standard reagent) with dilute hydrochloric acid, water and then acetone, dry at 110°C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 1.3 g of this zinc, add 20 mL of dilute hydrochloric acid and 8 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Pipet 25 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetraacetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

Each mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 6.541 mg of Zn

Note: Store in polyethylene bottles.

Disodium Dihydrogen Ethylenediamine Tetraacetate, 0.05 mol/L

1000 mL of this solution contains 18.612 g of disodium dihydrogen ethylenediamine tetraacetate dehydrate $(\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O})$: 372.24).

Preparation—Dissolve 19 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Wash zinc (standard reagent) with dilute hydrochloric acid, water and then acetone, dry at 110°C for 5 minutes, and allow to cool in a desiccator (silica gel). Weigh accurately about 0.8 g of this zinc, add 12 mL of dilute hydrochloric acid and 5 drops of bromine TS, and dissolve it by gentle warming. Expel any excess of bromine by boiling, and add water to make exactly 200 mL. Measure exactly 20 mL of this solution, and neutralize with sodium hydroxide solution (1 in 50). Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator. Titrate <2.50> the solution with the prepared disodium dihydrogen ethylenediamine tetra-

cetate solution until the red-purple color changes to blue-purple. Calculate the molarity factor.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 3.271 mg of Zn

Note: Store in polyethylene bottles.

**Disodium Dihydrogen Ethylenediamine Tetraacetate,
0.02 mol/L**

1000 mL of this solution contains 7.445 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation—Dissolve 7.5 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, but weigh accurately 0.3 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and with acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, and add 5 mL of dilute hydrochloric acid and 5 drops of bromine TS.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.308 mg of Zn

Note: Store in polyethylene bottles.

**Disodium Dihydrogen Ethylenediamine Tetraacetate,
0.01 mol/L**

1000 mL of this solution contains 3.7224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation—Before use, dilute 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly twice the initial volume.

**Disodium Dihydrogen Ethylenediamine Tetraacetate,
0.001 mol/L**

1000 mL of this solution contains 0.37224 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24).

Preparation—Before use, dilute 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS with water to make exactly 10 times the initial volume.

Ferric Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (III) Sulfate, 0.1 mol/L.

Ferrous Ammonium Sulfate, 0.1 mol/L

See Ammonium Iron (II) Sulfate, 0.1 mol/L.

Ferrous Ammonium Sulfate, 0.02 mol/L

See Ammonium Iron (II) Sulfate, 0.02 mol/L.

Hydrochloric Acid, 2 mol/L

1000 mL of this solution contains 72.92 g of hydrochloric acid (HCl: 36.46).

Preparation—Dilute 180 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh about 1.5 g

of sodium carbonate (standard reagent) accurately, and dissolve in 100 mL of water.

Each mL of 2 mol/L hydrochloric acid VS
= 106.0 mg of Na_2CO_3

Hydrochloric Acid, 1 mol/L

1000 mL of this solution contains 36.461 g of hydrochloric acid (HCl: 36.46).

Preparation—Dilute 90 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.8 g of sodium carbonate (standard reagent), previously heated between 500 °C and 650 °C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of water, and titrate <2.50> with the prepared hydrochloric acid to calculate the molarity factor (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end-point is approached, boil the content carefully, stopper the flask loosely, allow to cool, and continue the titration until the color of the solution changes to persistent orange to orange-red. In the potentiometric titration, titrate with vigorous stirring, without boiling.

Each mL of 1 mol/L hydrochloric acid VS
= 52.99 mg of Na_2CO_3

Hydrochloric Acid, 0.5 mol/L

1000 mL of this solution contains 18.230 g of hydrochloric acid (HCl: 36.46).

Preparation—Dilute 45 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.5 mol/L hydrochloric acid VS
= 26.50 mg of Na_2CO_3

Hydrochloric Acid, 0.2 mol/L

1000 mL of this solution contains 7.292 g of hydrochloric acid (HCl: 36.46).

Preparation—Dilute 18 mL of hydrochloric acid with water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L hydrochloric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.2 mol/L hydrochloric acid VS
= 10.60 mg of Na_2CO_3

Hydrochloric Acid, 0.1 mol/L

1000 mL of this solution contains 3.6461 g of hydrochloric acid (HCl: 36.46).

Preparation—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly twice the initial volume.

Hydrochloric Acid, 0.05 mol/L

1000 mL of this solution contains 1.8230 g of hydrochloric acid (HCl: 36.46).

Preparation—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 4 times the initial volume.

Hydrochloric Acid, 0.02 mol/L

1000 mL of this solution contains 0.7292 g of hydrochloric acid (HCl: 36.46).

Preparation—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 10 times the initial volume.

Hydrochloric Acid, 0.01 mol/L

1000 mL of this solution contains 0.36461 g of hydrochloric acid (HCl: 36.46).

Preparation—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 20 times the initial volume.

Hydrochloric Acid, 0.001 mol/L

1000 mL of this solution contains 0.036461 g of hydrochloric acid (HCl: 36.46).

Preparation—Before use, dilute 0.2 mol/L hydrochloric acid VS with water to make exactly 200 times the initial volume.

Iodine, 0.05 mol/L

1000 mL of this solution contains 12.690 g of iodine (I: 126.90).

Preparation—Dissolve 13 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid and water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 15 mL of the iodine solution, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (Indicator method: starch TS; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS, and continue the titration until the blue color disappears. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized before use.

Iodine, 0.01 mol/L

1000 mL of this solution contains 2.5381 g of iodine (I: 126.90).

Preparation—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 5 times the initial volume.

Iodine, 0.005 mol/L

1000 mL of this solution contains 1.2690 g of iodine (I: 126.90).

Preparation—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 10 times the initial volume.

Iodine, 0.002 mol/L

1000 mL of this solution contains 0.5076 g of iodine (I: 126.90).

Preparation—Before use, dilute 0.05 mol/L iodine VS with water to make exactly 25 times the initial volume.

Magnesium Chloride, 0.05 mol/L

1000 mL of this solution contains 10.165 g of magnesium chloride hexahydrate (MgCl₂·6H₂O: 203.30).

Preparation—Dissolve 10.2 g of magnesium chloride hexa-

hydrate in freshly boiled and cooled water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared magnesium chloride solution. Add 50 mL of water, 3 mL of pH 10.7 ammonia-ammonium chloride buffer solution and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue-purple. Calculate the molarity factor.

Magnesium Chloride, 0.01 mol/L

1000 mL of this solution contains 2.0330 g of magnesium chloride hexahydrate (MgCl₂·6H₂O: 203.30).

Preparation—Before use, dilute 0.05 mol/L magnesium chloride VS with water to make exactly 5 times the initial volume.

Oxalic Acid, 0.05 mol/L

1000 mL of this solution contains 6.303 g of oxalic acid (C₂H₂O₄·2H₂O: 126.07).

Preparation—Dissolve 6.3 g of oxalic acid dihydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared oxalic acid solution in a 500-mL conical flask, and add 200 mL of diluted sulfuric acid (1 in 20), previously boiled for 10 to 15 minutes and then cooled to 27 ± 3°C. Transfer freshly standardized 0.02 mol/L potassium permanganate VS to a burette. Add quickly 22 mL of the 0.02 mol/L potassium permanganate VS to the oxalic acid solution from the burette under gentle stirring, and allow to stand until the red color of the mixture disappears. Heat the solution up to between 55°C and 60°C, and complete the titration <2.50> by adding 0.02 mol/L potassium permanganate VS until a faint red color persists for 30 seconds. Add the last 0.5 to 1 mL dropwise, being particularly careful to allow the solution to become decolorized before the next drop is added. Calculate the molarity factor.

Note: Store protected from light.

Oxalic Acid, 0.005 mol/L

1000 mL of this solution contains 0.6303 g of oxalic acid dihydrate (C₂H₂O₄·2H₂O: 126.07).

Preparation—Before use, dilute 0.05 mol/L oxalic acid VS with water to make exactly 10 times the initial volume.

Perchloric Acid, 0.1 mol/L

1000 mL of this solution contains 10.046 g of perchloric acid (HClO₄: 100.46).

Preparation—Add slowly 8.7 mL of perchloric acid to 1000 mL of acetic acid (100) while keeping the temperature at about 20°C. Allow the mixture to stand for about 1 hour. Perform quickly the test as directed under Water Determination with 3.0 mL of the mixture, and designate the water content as A (g/dL). To the rest mixture add slowly [(A - 0.03) × 52.2] mL of acetic anhydride with shaking at about 20°C. Allow the solution to stand for 24 hours, and standardize it as follows:

Standardization—Weigh accurately about 0.3 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of acetic acid (100), and titrate <2.50> the solution with the prepared perchloric acid solution

(Indicator method: 3 drops of crystal violet TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a blue color. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L perchloric acid VS
= 20.42 mg of $\text{KHC}_6\text{H}_4(\text{COO})_2$

Note: Store protected from moisture.

Perchloric Acid, 0.05 mol/L

1000 mL of this solution contains 5.023 g of perchloric acid (HClO_4 : 100.46).

Preparation—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly twice the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add $[(A - 0.03) \times 52.2]$ mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

Perchloric Acid, 0.02 mol/L

1000 mL of this solution contains 2.0092 g of perchloric acid (HClO_4 : 100.46).

Preparation—Before use, dilute 0.1 mol/L perchloric acid VS with acetic acid for nonaqueous titration to make exactly 5 times the initial volume. Perform quickly the test as directed under Water Determination with 8.0 mL of acetic acid for nonaqueous titration, and designate the water content as A (g/dL). If A is not less than 0.03, add $[(A - 0.03) \times 52.2]$ mL of acetic anhydride to 1000 mL of acetic acid for nonaqueous titration, and use it for the preparation.

Perchloric Acid-1,4-Dioxane, 0.1 mol/L

1000 mL of this solution contains 10.046 g of perchloric acid (HClO_4 : 100.46).

Preparation—Dilute 8.5 mL of perchloric acid with 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.5 g of potassium hydrogen phthalate (standard reagent), previously dried at 105°C for 4 hours and allowed to cool in a desiccator (silica gel). Dissolve it in 80 mL of acetic acid for nonaqueous titration, and add 3 drops of crystal violet TS. Titrate <2.50> the solution with the prepared perchloric acid-1,4-dioxane solution until it acquires a blue color. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS
= 20.42 mg of $\text{KHC}_6\text{H}_4(\text{COO})_2$

Note: Store in a cold place, protected from moisture.

Perchloric Acid-1,4-Dioxane, 0.05 mol/L

1000 mL of this solution contains 5.023 g of perchloric acid (HClO_4 : 100.46).

Preparation—Before use, dilute 0.1 mol/L perchloric acid-1,4-dioxane VS with 1,4-dioxane to make exactly twice the initial volume.

Perchloric Acid-1,4-Dioxane, 0.004 mol/L

1000 mL of this solution contains 0.4018 g of perchloric acid (HClO_4 : 100.46).

Preparation—Before use, dilute 0.1 mol/L perchloric acid-

1,4-dioxane VS with 1,4-dioxane to make exactly 25 times the initial volume.

Potassium Bichromate, 1/60 mol/L

See Potassium Dichromate, 1/60 mol/L

Potassium Bromate, 1/60 mol/L

1000 mL of this solution contains 2.7833 g of potassium bromate (KBrO_3 : 167.00).

Preparation—Dissolve 2.8 g of potassium bromate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared potassium bromate solution into an iodine flask. Add 2 g of potassium iodide and 5 mL of dilute sulfuric acid, stopper the flask, and allow the solution to stand for 5 minutes. Add 100 mL of water, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Potassium Dichromate, 1/60 mol/L

1000 mL of this solution contains 4.903 g of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$: 294.18).

Preparation—Weigh accurately about 4.903 g of potassium dichromate (standard reagent), previously powdered, dried between 100°C and 110°C for 3 to 4 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and calculate the molarity factor.

Potassium Ferricyanide, 0.1 mol/L

See Potassium Hexacyanoferrate (III), 0.1 mol/L

Potassium Ferricyanide, 0.05 mol/L

See Potassium Hexacyanoferrate (III), 0.05 mol/L.

Potassium Hexacyanoferrate (III), 0.1 mol/L

1000 mL of this solution contains 32.924 g of potassium hexacyanoferrate (III) [$\text{K}_3\text{Fe}(\text{CN})_6$: 329.24].

Preparation—Dissolve 33 g of potassium hexacyanoferrate (III) in water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 25 mL of the prepared potassium hexacyanoferrate (III) solution into an iodine flask. Add 2 g of potassium iodide and 10 mL of dilute hydrochloric acid, stopper the flask, and allow to stand for 15 minutes. Add 15 mL of zinc sulfate TS, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

Potassium Hexacyanoferrate (III), 0.05 mol/L

1000 mL of this solution contains 16.462 g of potassium hexacyanoferrate (III) [$\text{K}_3\text{Fe}(\text{CN})_6$: 329.24].

Preparation—Before use, dilute 0.1 mol/L potassium hexacyanoferrate (III) VS with water to make exactly twice the initial volume.

Potassium Hydroxide, 1 mol/L

1000 mL of this solution contains 56.11 g of potassium hydroxide (KOH: 56.11).

Preparation—Dissolve 65 g of potassium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Shake the mixture thoroughly, and allow it to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

Standardization—Weigh accurately about 2.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and add 2 drops of bromothymol blue TS. Titrate <2.50> the solution with the prepared potassium hydroxide solution until it acquires a green color. Calculate the molarity factor.

Each mL of 1 mol/L potassium hydroxide VS
= 97.09 mg of HOSO₂NH₂

Note: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda-lime). This solution, if stored for a long period, should be restandardized.

Potassium Hydroxide, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation—Weigh 32 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 1.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L potassium hydroxide VS
= 48.55 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Potassium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation—Weigh 6.5 g of potassium hydroxide, proceed as directed for preparation under 1 mol/L potassium hydroxide VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L potassium hydroxide VS, but weigh accurately about 0.25 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L potassium hydroxide VS
= 9.709 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L potassium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Potassium Hydroxide-Ethanol, 0.5 mol/L

1000 mL of this solution contains 28.053 g of potassium hydroxide (KOH: 56.11).

Preparation—Dissolve 35 g of potassium hydroxide in 20 mL of water, and add aldehyde-free ethanol to make 1000

mL. Allow the solution to stand for 24 hours in a tightly stoppered bottle. Then quickly decant the supernatant liquid, and standardize the solution as follows:

Standardization—Measure exactly 15 mL of 0.25 mol/L sulfuric acid VS, add 50 mL of water, and titrate with the prepared potassium hydroxide-ethanol solution to calculate the molarity factor (Indicator method: 2 drops of phenolphthalein TS; or potentiometric titration). In the indicator method, titrate <2.50> until the solution acquires a pale red color.

Note: Store in tightly stoppered bottles, protected from light. Standardize before use.

Potassium Hydroxide-Ethanol, 0.1 mol/L

1000 mL of this solution contains 5.611 g of potassium hydroxide (KOH: 56.11).

Preparation—Weigh 7 g of potassium hydroxide, proceed as directed for preparation under 0.5 mol/L potassium hydroxide-ethanol VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.5 mol/L potassium hydroxide-ethanol VS, but measure exactly 15 mL of 0.05 mol/L sulfuric acid VS.

Note: Store as directed under 0.5 mol/L potassium hydroxide-ethanol VS. Standardize before use.

Potassium Iodate, 0.05 mol/L

1000 mL of this solution contains 10.700 g of potassium iodate (KIO₃: 214.00).

Preparation—Weigh accurately about 10.700 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

Potassium Iodate, 1/60 mol/L

1000 mL of this solution contains 3.567 g of potassium iodate (KIO₃: 214.00).

Preparation—Weigh accurately about 3.567 g of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

Potassium Iodate, 1/1200 mol/L

1000 mL of this solution contains 0.17833 g of potassium iodate (KIO₃: 214.00).

Preparation—Weigh accurately about 0.17833 g of potassium iodate, previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and dissolve it in water to make exactly 1000 mL. Calculate the molarity factor.

Potassium Permanganate, 0.02 mol/L

1000 mL of this solution contains 3.1607 g of potassium permanganate (KMnO₄: 158.03).

Preparation—Dissolve 3.2 g of potassium permanganate in water to make 1000 mL, and boil the solution for 15 minutes. Allow the solution to stand for at least 48 hours in a tightly stoppered flask, and filter it through a glass filter (G3 or G4). Standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 1 to 1.5 hours and allowed to cool in a desicca-

tor (silica gel), transfer it to a 500 mL conical flask, dissolve in 30 mL of water, add 250 mL of diluted sulfuric acid (1 in 20), and warm the mixture between 30°C and 35°C. Transfer the prepared potassium permanganate solution to a buret, add quickly 40 mL of the solution under gentle stirring from the buret, and allow to stand until the red color of the mixture disappears. Warm the solution between 55°C and 60°C, and complete the titration <2.50> with the potassium permanganate solution until a faint red color persists for 30 seconds. Add the last 0.5 to 1 mL dropwise before the end point, being particularly careful to allow the solution to be decolorized before the next drop is added. Calculate the molarity factor.

Each mL of 0.02 mol/L potassium permanganate VS
= 6.700 mg of $\text{Na}_2\text{C}_2\text{O}_4$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

Potassium Permanganate, 0.002 mol/L

1000 mL of this solution contains 0.31607 g of potassium permanganate (KMnO_4 : 158.03).

Preparation—Before use, dilute 0.02 mol/L potassium permanganate VS with water to make exactly 10 times the initial volume.

Silver Nitrate, 0.1 mol/L

1000 mL of this solution contains 16.987 g of silver nitrate (AgNO_3 : 169.87).

Preparation—Dissolve 17.0 g of silver nitrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 80 mg of sodium chloride (standard reagent), previously dried between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel), dissolve it in 50 mL of water, and titrate <2.50> under vigorous stirring with the prepared silver nitrate solution to calculate the molarity factor (Indicator method: 3 drops of fluorescein sodium TS; or potentiometric titration: silver electrode). In the indicator method, titrate until the color of the solution changes from yellow-green to orange through yellow.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Note: Store protected from light.

Silver Nitrate, 0.02 mol/L

1000 mL of this solution contains 3.3974 g of silver nitrate (AgNO_3 : 169.87).

Preparation—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 5 times the initial volume.

Silver Nitrate, 0.01 mol/L

1000 mL of this solution contains 1.6987 g of silver nitrate (AgNO_3 : 169.87).

Preparation—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 10 times the initial volume.

Silver Nitrate, 0.005 mol/L

1000 mL of this solution contains 0.8494 g of silver nitrate (AgNO_3 : 169.87).

Preparation—Before use, dilute 0.1 mol/L silver nitrate VS with water to make exactly 20 times the initial volume.

Silver Nitrate, 0.001 mol/L

1000 mL of this solution contains 0.16987 g of silver nitrate (AgNO_3 : 169.87).

Preparation—Dilute 0.1 mol/L silver nitrate VS with water to make exactly 100 times of the initial volume before use.

Sodium Acetate, 0.1 mol/L

1000 mL of this solution contains 8.203 g of sodium acetate (CH_3COONa : 82.03).

Preparation—Dissolve 8.20 g of anhydrous sodium acetate in acetic acid (100) to make 1000 mL, and standardize the solution as follows:

Standardization—Pipet 25 mL of the prepared sodium acetate solution, add 50 mL of acetic acid (100) and 1 mL of *p*-naphtholbenzene TS, and titrate <2.50> with 0.1 mol/L perchloric acid VS until a yellow-brown color changes through yellow to green. Perform a blank determination. Calculate the molarity factor.

Sodium Hydroxide, 1 mol/L

1000 mL of this solution contains 39.997 g of sodium hydroxide (NaOH : 40.00).

Preparation—Dissolve 42 g of sodium hydroxide in 950 mL of water. Add a freshly prepared, saturated solution of barium hydroxide octahydrate until no more precipitate is produced. Mix well the mixture, and allow to stand for 24 hours in a tightly stoppered bottle. Decant the supernatant liquid or filter the solution through a glass filter (G3 or G4), and standardize the solution as follows:

Standardization—Weigh accurately about 1.5 g of amidosulfuric acid (standard reagent), previously dried in a desiccator (in vacuum, silica gel) for about 48 hours. Dissolve it in 25 mL of freshly boiled and cooled water, and titrate <2.50> the solution with the prepared sodium hydroxide solution to calculate the molarity factor (Indicator method: 2 drops of bromothymol blue TS; or potentiometric titration). In the indicator method, titrate until the solution acquires a green color.

Each mL of 1 mol/L sodium hydroxide VS
= 97.09 mg of HOSO_2NH_2

Note: Store in tightly stoppered bottles or in containers provided with a carbon dioxide-absorbing tube (soda lime). This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.5 mol/L

1000 mL of this solution contains 19.999 g of sodium hydroxide (NaOH : 40.00).

Preparation—Weigh 22 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.7 g of amidosulfuric acid (standard reagent).

Each mL of 0.5 mol/L sodium hydroxide VS
= 48.55 mg of HOSO_2NH_2

Note: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.2 mol/L

1000 mL of this solution contains 7.999 g of sodium hydroxide (NaOH: 40.00).

Preparation—Weigh 9 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.3 g of amidosulfuric acid (standard reagent).

Each mL of 0.2 mol/L sodium hydroxide VS
= 19.42 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 3.9997 g of sodium hydroxide (NaOH: 40.00).

Preparation—Weigh 4.5 g of sodium hydroxide, proceed as directed for preparation under 1 mol/L sodium hydroxide VS, and standardize the solution as follows.

Standardization—Proceed as directed for standardization under 1 mol/L sodium hydroxide VS, but weigh accurately about 0.15 g of amidosulfuric acid (standard reagent).

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.709 mg of HOSO₂NH₂

Note: Store as directed under 1 mol/L sodium hydroxide VS. This solution, if stored for a long period, should be restandardized.

Sodium Hydroxide, 0.05 mol/L

1000 mL of this solution contains 1.9999 g of sodium hydroxide (NaOH: 40.00).

Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly twice the initial volume.

Sodium Hydroxide, 0.02 mol/L

1000 mL of this solution contains 0.7999 g of sodium hydroxide (NaOH: 40.00).

Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Sodium Hydroxide, 0.01 mol/L

1000 mL of this solution contains 0.39997 g of sodium hydroxide (NaOH: 40.00).

Preparation—Before use, dilute 0.1 mol/L sodium hydroxide VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

Sodium Lauryl Sulfate, 0.01 mol/L

1000 mL of this solution contains 2.8838 g of sodium lauryl sulfate (C₁₂H₂₅NaO₄S: 288.38).

Preparation—Dissolve 2.9 g of sodium lauryl sulfate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of papaverine hydrochloride for assay, previously dried, and dissolve

in water to make exactly 100 mL. Pipet 10 mL of this solution into a glass-stoppered conical flask, add 5 mL each of water and dilute sulfuric acid and 60 mL of dichloromethane, then add 5 to 6 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.50>, while vigorous shaking, with the sodium lauryl sulfate solution prepared above, using a buret with a minimum graduation of 0.02 mL. End point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of the sodium lauryl sulfate solution, vigorous shaking and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS
= 3.759 mg of C₂₀H₂₁NO₄·HCl

Sodium Methoxide, 0.1 mol/L

1000 mL of this solution contains 5.402 g of sodium methoxide (CH₃ONa: 54.02).

Preparation—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add benzene to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of benzoic acid, previously dried for 24 hours in a desiccator (silica gel), dissolve it in 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide VS
= 12.21 mg of C₆H₅COOH

Note: Store in a cold place, protected from moisture. Standardize before use.

Sodium Methoxide-Dioxane, 0.1 mol/L

See Sodium Methoxide-1,4-Dioxane, 0.1 mol/L.

Sodium Methoxide-1,4-Dioxane, 0.1 mol/L

1000 mL of this solution contains 5.402 g of sodium methoxide (CH₃ONa: 54.02).

Preparation—Add little by little 2.5 g of freshly cut sodium pieces to 150 mL of methanol cooled in ice-water. After the sodium has dissolved, add 1,4-dioxane to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 80 mL of *N,N*-dimethylformamide, and add 3 drops of thymol blue-*N,N*-dimethylformamide TS. Titrate <2.50> the solution with the prepared sodium methoxide-1,4-dioxane solution until a blue color appears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium methoxide-1,4-dioxane VS
= 12.21 mg of C₆H₅COOH

Note: Store in a cold place, protected from moisture. Standardize before use.

Sodium Nitrite, 0.1 mol/L

1000 mL of this solution contains 6.900 g of sodium nitrite (NaNO₂: 69.00).

Preparation—Dissolve 7.2 g of sodium nitrite in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.44 g of sulfanilamide for titration of diazotization, previously dried at 105°C for 3 hours and allowed to cool in a desiccator (silica gel), dissolve in 10 mL of hydrochloric acid, 40 mL of water and 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate with the prepared sodium nitrite solution as directed in the potentiometric titration or amperometric titration under Endpoint Detection Methods in Titrimetry <2.50>. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium nitrite VS
= 17.22 mg of $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$

Note: Store protected from light. This solution, if stored for a long period, should be restandardized.

Sodium Oxalate, 0.005 mol/L

1000 mL of this solution contains 0.6700 g of sodium oxalate ($\text{Na}_2\text{C}_2\text{O}_4$: 134.00).

Preparation—Weigh accurately about 0.6700 g of sodium oxalate (standard reagent), previously dried between 150°C and 200°C for 2 hours and allowed to cool in a desiccator (silica gel), dissolve it in water to make exactly 1000 mL, and calculate the molarity factor.

Sodium Tetraphenylborate, 0.02 mol/L

1000 mL of this solution contains 6.844 g of sodium tetraphenylborate [$\text{NaB}(\text{C}_6\text{H}_5)_4$: 342.22].

Preparation—Dissolve 7.0 g of sodium tetraphenylborate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh 0.5 g of potassium hydrogen phthalate (standard reagent), dissolve it in 100 mL of water, add 2 mL of acetic acid (31), and warm to 50°C in a water bath. Add slowly 50 mL of the prepared sodium tetraphenylborate solution under stirring from a buret, then cool the mixture quickly, and allow to stand for 1 hour at room temperature. Transfer the precipitate to a tared glass filter (G4), wash with three 5 mL portions of potassium tetraphenylborate TS, dry at 105°C for 1 hour, and weigh accurately the glass filter. Calculate the molarity factor from the mass of potassium tetraphenylborate [$\text{KB}(\text{C}_6\text{H}_5)_4$: 358.32].

Each mL of 0.02 mol/L sodium tetraphenylborate VS
= 7.167 mg of $\text{KB}(\text{C}_6\text{H}_5)_4$

Note: Prepare before use.

Sodium Tetraphenylboron, 0.02 mol/L

See Sodium Tetraphenylborate, 0.02 mol/L.

Sodium Thiosulfate, 0.1 mol/L

1000 mL of this solution contains 24.818 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Dissolve 25 g of sodium thiosulfate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL, allow to stand for 24 hours, and standardize the solution as follows:

Standardization—Weigh accurately about 50 mg of potassium iodate (standard reagent), previously dried between 120°C and 140°C for 1.5 to 2 hours and allowed to cool in a desiccator (silica gel), and transfer to an iodine flask. Dissolve it in 25 mL of water, add 2 g of potassium iodide and 10

mL of dilute sulfuric acid, and stopper the flask. After allowing the mixture to stand for 10 minutes, add 100 mL of water, and titrate <2.50> the liberated iodine with the prepared sodium thiosulfate solution (Indicator method; or potentiometric titration: platinum electrode). In the indicator method, when the solution assumes a pale yellow color as the end point is approached, add 3 mL of starch TS. Continue the titration, until the blue color disappears. Perform a blank determination. Calculate the molarity factor.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.567 mg of KIO_3

Note: This solution, if stored for a long period, should be restandardized.

Sodium Thiosulfate, 0.05 mol/L

1000 mL of this solution contains 12.409 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 2 times the initial volume.

Sodium Thiosulfate, 0.02 mol/L

1000 mL of this solution contains 4.964 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Sodium Thiosulfate, 0.01 mol/L

1000 mL of this solution contains 2.4818 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 10 times the initial volume.

Sodium Thiosulfate, 0.005 mol/L

1000 mL of this solution contains 1.2409 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 20 times the initial volume.

Sodium Thiosulfate, 0.002 mol/L

1000 mL of this solution contains 0.4964 g of sodium thiosulfate pentahydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Preparation—Before use, dilute 0.1 mol/L sodium thiosulfate VS with freshly boiled and cooled water to make exactly 50 times the initial volume.

Sulfuric Acid, 0.5 mol/L

1000 mL of this solution contains 49.04 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Add slowly, under stirring, 30 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

Standardization—Weigh accurately about 0.8 g of sodium carbonate (standard reagent), previously heated between 500°C and 650°C for 40 to 50 minutes and allowed to cool in a desiccator (silica gel). Dissolve it in 50 mL of water, and titrate <2.50> the solution with the prepared sulfuric acid (Indicator method: 3 drops of methyl red TS; or potentiometric titration). In the indicator method, when the end point is ap-

proached, boil the solution carefully, stopper the flask loosely, allow to cool, and continue the titration, until the color of the solution changes to persistent orange to orange-red. Calculate the molarity factor. In the potentiometric titration, titrate with vigorous stirring without boiling.

Each mL of 0.5 mol/L sulfuric acid VS
= 52.99 mg of Na_2CO_3

Sulfuric Acid, 0.25 mol/L

1000 mL of this solution contains 24.520 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Add slowly, under stirring, 15 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.4 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.25 mol/L sulfuric acid VS
= 26.50 mg of Na_2CO_3

Sulfuric Acid, 0.1 mol/L

1000 mL of this solution contains 9.808 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Add slowly, under stirring, 6 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 0.15 g of sodium carbonate (standard reagent), and dissolve in 50 mL of water.

Each mL of 0.1 mol/L sulfuric acid VS
= 10.60 mg of Na_2CO_3

Sulfuric Acid, 0.05 mol/L

1000 mL of this solution contains 4.904 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Add slowly, under stirring, 3 mL of sulfuric acid to 1000 mL of water, allow to cool, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.5 mol/L sulfuric acid VS, but weigh accurately about 80 mg of sodium carbonate (standard reagent), and dissolve in 30 mL of water.

Each mL of 0.05 mol/L sulfuric acid VS
= 5.299 mg of Na_2CO_3

Sulfuric Acid, 0.025 mol/L

1000 mL of this solution contains 2.4520 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly twice the initial volume.

Sulfuric Acid, 0.01 mol/L

1000 mL of this solution contains 0.9808 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 5 times the initial volume.

Sulfuric Acid, 0.005 mol/L

1000 mL of this solution contains 0.4904 g of sulfuric acid

(H_2SO_4 : 98.08).

Preparation—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 10 times the initial volume.

Surfuric Acid, 0.0005 mol/L

1000 mL of this solution contains 0.04904 g of sulfuric acid (H_2SO_4 : 98.08).

Preparation—Before use, dilute 0.05 mol/L sulfuric acid VS with water to make exactly 100 times the initial volume.

Tetrabutylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 25.947 g of tetrabutylammonium hydroxide $[(\text{C}_4\text{H}_9)_4\text{NOH}]$: 259.47].

Preparation—Before use, dilute a volume of 10% tetrabutylammonium hydroxide-methanol TS, equivalent to 26.0 g of tetrabutylammonium hydroxide, with 2-propanol to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.3 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 50 mL of acetone, and titrate $\langle 2.50 \rangle$ the solution with the prepared tetrabutylammonium hydroxide solution (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
= 12.21 mg of $\text{C}_6\text{H}_5\text{COOH}$

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethylammonium Hydroxide, 0.2 mol/L

1000 mL of this solution contains 18.231 g of tetramethylammonium hydroxide $[(\text{CH}_3)_4\text{NOH}]$: 91.15].

Preparation—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 18.4 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

Standardization—Weigh accurately about 0.4 g of benzoic acid, previously dried in a desiccator (silica gel) for 24 hours, dissolve it in 60 mL of *N,N*-dimethylformamide, and titrate $\langle 2.50 \rangle$ the solution with the prepared 0.2 mol/L tetramethylammonium hydroxide solution (Indicator method: 3 drops of thymol blue-*N,N*-dimethylformamide TS; or potentiometric titration). In the indicator method, titrate until a blue color is produced. Perform a blank determination in the same manner. Calculate the molarity factor.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS
= 24.42 mg of $\text{C}_6\text{H}_5\text{COOH}$

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethylammonium Hydroxide, 0.1 mol/L

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide $[(\text{CH}_3)_4\text{NOH}]$: 91.15].

Preparation—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.2 mol/L tetramethylammonium hydroxide VS. Weigh accurately about 0.2 g of benzoic acid and titrate $\langle 2.50 \rangle$.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 12.21 mg of C_6H_5COOH

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Tetramethylammonium Hydroxide, 0.02 mol/L

1000 mL of this solution contains 1.8231 g of tetramethylammonium hydroxide $[(CH_3)_4NOH]$: 91.15].

Preparation—Before use, dilute 0.1 mol/L tetramethylammonium hydroxide VS with freshly boiled and cooled water to make exactly 5 times the initial volume.

Tetramethylammonium Hydroxide-Methanol, 0.1 mol/L

1000 mL of this solution contains 9.115 g of tetramethylammonium hydroxide $[(CH_3)_4NOH]$: 91.15].

Preparation—Before use, dilute a volume of tetramethylammonium hydroxide-methanol TS, equivalent to 9.2 g of tetramethylammonium hydroxide, with methanol to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.1 mol/L tetramethylammonium hydroxide VS.

Note: Store in tightly stoppered bottles. This solution, if stored for a long period, should be restandardized.

Titanium (III) Chloride, 0.1 mol/L

1000 mL of this solution contains 15.423 g of titanium (III) chloride $(TiCl_3)$: 154.23].

Preparation—Add 75 mL of hydrochloric acid to 75 mL of titanium (III) chloride, and dilute with freshly boiled and cooled water to make 1000 mL. Transfer the solution into a buret provided with a reservoir protected from light, replace the air with hydrogen, and allow to stand for 48 hours. Before use, standardize the solution as follows:

Standardization—Weigh 3 g of ammonium iron (II) sulfate hexahydrate in a wide-mouthed, 500 mL conical flask. Passing carbon dioxide through the flask, dissolve it in 50 mL of freshly boiled and cooled water, and add 25 mL of diluted sulfuric acid (27 in 100). Rapidly add exactly 40 mL of 0.02 mol/L potassium permanganate VS to the mixture, while passing carbon dioxide through the flask. Titrate <2.50> with the prepared titanium (III) chloride solution until the calculated end point is approached, then add 5 g of ammonium thiocyanate immediately, and continue the titration with the prepared titanium (III) chloride solution until the color of the solution disappears. Perform a blank determination. Calculate the molarity factor.

Note: Store after the air has been displaced with hydrogen.

Titanium Trichloride, 0.1 mol/L

See Titanium (III) Chloride, 0.1 mol/L.

Zinc, 0.1 mol/L

1000 mL of this solution contains 6.541 g of zinc (Zn: 65.41).

Preparation—To 6.541 g of zinc (standard reagent), previously washed with dilute hydrochloric acid, with water and then acetone, and cooled in a desiccator (silica gel) after drying at 110°C for 5 minutes, add 80 mL of dilute hydrochloric acid and 2.5 mL of bromine TS, dissolve by gentle warming, evaporate excess bromine by boiling, and add water to make exactly 1000 mL.

Zinc Acetate, 0.05 mol/L

1000 mL of this solution contains 10.977 g of zinc acetate dihydrate $[Zn(CH_3COO)_2 \cdot 2H_2O]$: 219.53].

Preparation—Dissolve 11.1 g of zinc acetate dihydrate in 40 mL of water and 4 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Measure exactly 20 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and add 50 mL of water, 3 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride reagent. Titrate <2.50> the solution with the prepared zinc acetate solution, until the blue color changes to blue-purple. Calculate the molarity factor.

Zinc Acetate, 0.02 mol/L

1000 mL of this solution contains 4.391 g of zinc acetate dihydrate $[Zn(CH_3COO)_2 \cdot 2H_2O]$: 219.53].

Preparation—Dissolve 4.43 g of zinc acetate dihydrate in 20 mL of water and 2 mL of dilute acetic acid, add water to make 1000 mL, and standardize the solution as follows:

Standardization—Proceed as directed for standardization under 0.05 mol/L zinc acetate VS, but measure exactly 20 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS.

Zinc Sulfate, 0.1 mol/L

1000 mL of this solution contains 28.758 g of zinc sulfate heptahydrate $(ZnSO_4 \cdot 7H_2O)$: 287.58].

Preparation—Dissolve 28.8 g of zinc sulfate heptahydrate in water to make 1000 mL, and standardize the solution as follows:

Standardization—Pipet 25 mL of the prepared zinc sulfate solution, add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue-purple. Calculate the molarity factor.

9.22 Standard Solutions

Standard Solutions are used as the standard for the comparison in a text of the Pharmacopoeia.

Borate pH Standard Solution See pH Determination <2.54>.

Calcium Hydroxide pH Standard Solution See pH Determination <2.54>.

Carbonate pH Standard Solution See pH Determination <2.54>.

Oxalate pH Standard Solution See pH Determination <2.54>.

pH Standard Solution, Borate See pH Determination <2.54>.

pH Standard Solution, Calcium Hydroxide See pH Determination <2.54>.

pH Standard Solution, Carbonate See pH Determination <2.54>.

pH Standard Solution, Oxalate See pH Determination <2.54>.

pH Standard Solution, Phosphate See pH Determination <2.54>.

pH Standard Solution, Phthalate See pH Determination <2.54>.

Phosphate pH Standard Solution See pH Determination <2.54>.

Phthalate pH Standard Solution See pH Determination <2.54>.

Standard Aluminum Stock Solution Weigh 1.0 g of aluminum, add 60 mL of diluted hydrochloric acid (1 in 2), dissolve by heating, cool, add water to make 1000 mL. Pipet 10 mL of this solution, add 30 mL of water and 5 mL of acetic acid-ammonium acetate buffer solution, pH 3.0, and adjust the pH to about 3 with ammonia TS added dropwise. Then, add 0.5 mL of Cu-PAN TS, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS while boiling until the color of the solution changes from red to yellow lasting for more than 1 minute. Perform a blank determination.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.2698 mg of Al

Standard Ammonium Solution Dissolve 2.97 g of ammonium chloride, exactly weighed, in purified water for ammonium limit test to make exactly 1000 mL. Measure exactly 10 mL of this solution, and add purified water for ammonium limit test to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of ammonium (NH_4^+).

Standard Arsenic Stock Solution See Arsenic Limit Test <1.11>.

Standard Arsenic Solution See Arsenic Limit Test <1.11>.

Standard Boron Solution Weigh exactly 0.286 g of boric acid, previously dried in a desiccator (silica gel) to constant mass, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.5 μg of boron (B).

Standard Cadmium Stock Solution Dissolve 1.000 g of cadmium ground metal, exactly weighed, in 100 mL of dilute nitric acid by gentle heating, cool, and add dilute nitric acid to make exactly 1000 mL.

Standard Cadmium Solution Measure exactly 10 mL of Standard Cadmium Stock Solution, and add diluted nitric acid (1 in 3) to make exactly 1000 mL. Pipet 10 mL of this solution, and add diluted nitric acid (1 in 3) to make 100 mL. Each mL of this solution contains 0.001 mg of cadmium (Cd). Prepare before use.

Standard Calcium Solution Weigh exactly 0.250 g of calcium carbonate, add 5 mL of dilute hydrochloric acid and 25 mL of water, and dissolve by heating. After cooling, add water to make exactly 1000 mL. Each mL of this solution contains 0.1 mg of calcium (Ca).

Standard Calcium Solution for Atomic Absorption Spectrophotometry Weigh accurately 0.250 g of calcium carbonate, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of calcium (Ca).

Standard Copper Solution Pipet 10 mL of Standard Copper Stock Solution, and dilute with water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of copper (Cu). Prepare before use.

Standard Copper Stock Solution Weigh exactly 1.000 g of copper (standard reagent), add 100 mL of dilute nitric acid, and dissolve by heating. After cooling, add water to make exactly 1000 mL.

Standard Cyanide Stock Solution Dissolve 2.5 g of potassium cyanide in water to make exactly 1000 mL. Measure exactly 100 mL of this solution, add 0.5 mL of 4-dimethylaminobenzylidene rhodanine TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS until the solution shows a red color.

Each mL of 0.1 mol/L silver nitrate VS = 5.204 mg of CN

Standard Cyanide Solution Measure exactly a volume of Standard Cyanide Stock Solution, equivalent to 10 mg of cyanide (CN), add 100 mL of sodium hydroxide TS and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of cyanide (CN). Prepare before use.

Standard Fluorine Solution See Oxygen Flask Combustion Method <1.06>.

Standard Gold Stock Solution Dissolve 0.209 g of hydrogen tetrachloroaurate (III) tetrahydrate, exactly weighed, in 2 mL of aqua regia, heat on a water bath for 10 minutes, and add 1 mol/L hydrochloric acid TS to make exactly 100 mL. Each mL of this solution contains 1.00 mg of gold (Au).

Standard Gold Solution for Atomic Absorption Spectrophotometry To 25 mL of Standard Gold Stock Solution, exactly measured, add water to make exactly 1000 mL. Each mL of this solution contains 0.025 mg of gold (Au).

Standard Iron Solution Weigh exactly 86.3 mg of ammonium iron (III) sulfate dodecahydrate, dissolve in 100 mL of water, and add 5 mL of dilute hydrochloric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of iron (Fe).

Standard Lead Stock Solution Weigh exactly 159.8 mg of lead (II) nitrate, dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Prepare and store this solution using glass containers, free from soluble lead salts.

Standard Lead Solution Measure exactly 10 mL of Standard Lead Stock Solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.01 mg of lead (Pb). Prepare before use.

Standard Liquids for Calibrating Viscosimeters [JIS, Standard Liquids for Calibrating Viscosimeters (Z 8809)]

Standard Mercury Solution Weigh exactly 13.5 mg of mercury (II) chloride, previously dried for 6 hours in a desiccator (silica gel), dissolve in 10 mL of dilute nitric acid, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add 10 mL of dilute nitric acid and water to make exactly 1000 mL. Each mL of this solution contains 0.1 μg of

mercury (Hg). Prepare before use.

Standard Methanol Solution See Methanol Test <1.12>.

Standard Nickel Solution Dissolve 6.73 g of ammonium nickel (II) sulfate hexahydrate, exactly weighed, in water to make exactly 1000 mL. Pipet 5 mL of this solution, add water to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of nickel (Ni).

Standard Nitric Acid Solution Weigh exactly 72.2 mg of potassium nitrate, dissolve in water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of nitrogen (N).

Standard Phosphoric Acid Solution Weigh exactly 0.358 g of potassium dihydrogen phosphate, previously dried to constant mass in a desiccator (silica gel), and add 10 mL of diluted sulfuric acid (3 in 10) and water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Each mL of this solution contains 0.025 mg of phosphoric acid (as PO₄).

Standard Potassium Stock Solution Weigh exactly 9.534 g of potassium chloride, previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 5.00 mg of potassium (K).

Standard Selenium Solution To exactly 1 mL of Standard Selenium Stock Solution add water to make exactly 1000 mL. Prepare before use. It contains 1.0 µg of selenium (Se) per mL.

Standard Selenium Stock Solution Dissolve exactly 1.405 g of selenium dioxide in 0.1 mol/L nitric acid to make exactly 1000 mL.

Standard Silver Stock Solution Dissolve 1.575 g of silver nitrate, exactly weighed, in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of silver (Ag).

Standard Silver Solution for Atomic Absorption Spectrophotometry Measure exactly 10 mL of Standard Silver Stock Solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of silver (Ag). Prepare before use.

Standard Sodium Dodecylbenzene Sulfonate Solution Weigh exactly 1.000 g of sodium dodecylbenzene sulfonate, and dissolve in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of sodium dodecylbenzene sulfonate [CH₃(CH₂)₁₁C₆H₄SO₃Na].

Standard Sodium Stock Solution Weigh exactly 2.542 g of sodium chloride (standard reagent), previously dried at 130°C for 2 hours, and dissolve in water to make exactly 1000 mL. Each mL of this solution contains 1.00 mg of sodium (Na).

Standard Tin Solution Weigh exactly 0.250 g of tin, and dissolve in 10 mL of sulfuric acid by heating. After cooling, transfer this solution with 400 mL of diluted hydrochloric acid (1 in 5) to a 500-mL volumetric flask, and add diluted hydrochloric acid (1 in 5) to make 500 mL. Pipet 10 mL of this solution, and add diluted hydrochloric acid (1 in 5) to make exactly 1000 mL. Each mL of this solution contains 0.005 mg of tin (Sn). Prepare before use.

Standard Vinyl Chloride Solution Transfer about 190

mL of ethanol for gas chromatography into a 200-mL volumetric flask, and stopper with a silicone rubber stopper. Cooling this volumetric flask in a methanol-dry ice bath, inject 0.20 g of vinyl chloride, previously liquidized, through the silicone rubber stopper, and then inject ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, through the silicone rubber stopper to make 200 mL. Then pipet 1 mL of this solution, add ethanol for gas chromatography, previously cooled in a methanol-dry ice bath, to make exactly 200 mL. Pipet 1 mL of this solution, add ethanol for gas chromatography, cooled previously in a methanol-dry ice bath to make exactly 100 mL. Preserve in a hermetic container, at a temperature not exceeding -20°C.

Standard Water-Methanol Solution See Water Determination <2.48>.

Standard Zinc Stock Solution Dissolve exactly 1.000 g of zinc (standard reagent), in 100 mL of water and 5 mL of hydrochloric acid with the aid of gentle heating, cool, and add water to make exactly 1000 mL.

Standard Zinc Solution Measure exactly 25 mL of Standard Zinc Stock Solution, and add water to make exactly 1000 mL. Prepare before use. Each mL of this solution contains 0.025 mg of zinc (Zn).

Standard Zinc Solution for Atomic Absorption Spectrophotometry See Test for Rubber Closure for Aqueous Infusions <7.03>.

9.23 Matching Fluids for Color

Matching Fluids for Color are used as the reference for the comparison of color in a text of the Pharmacopoeia.

They are prepared from the following colorimetric stock solutions. Colorimetric stock solutions are prepared by the following procedures and stored in glass-stoppered bottles. When the color of the solution is compared with Matching Fluids for Color, unless otherwise specified, transfer both solutions and fluids to Nessler tubes and view transversely against a white background.

Cobalt (II) Chloride Colorimetric Stock Solution Weigh 65 g of cobalt (II) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of the solution, add 75 mL of water and 0.05 g of murexide-sodium chloride indicator, and add dropwise diluted ammonia solution (28) (1 in 10) until the color of the solution changes from red-purple to yellow. Titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes, after the addition of 0.2 mL of diluted ammonia solution (28) (1 in 10) near the endpoint, from yellow to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.379 mg of CoCl₂·6H₂O

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 59.5 mg of cobalt

(II) chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$: 237.93) in each mL, and use this solution as the colorimetric stock solution.

Cobaltous Chloride Colorimetric Stock Solution See Cobalt (II) Chloride Colorimetric Stock Solution.

Copper (II) Sulfate Colorimetric Stock Solution Weigh 65 g of copper (II) sulfate pentahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, and add water to make exactly 250 mL. Measure exactly 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 2 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator. Titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from green to purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.497 mg of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 62.4 mg of copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 249.69) in each mL, and use this solution as the colorimetric stock solution.

Copper Sulfate Colorimetric Stock Solution See Copper (II) Sulfate Colorimetric Stock Solution.

Iron (III) Chloride Colorimetric Stock Solution Weigh 55 g of iron (III) chloride hexahydrate, and dissolve in 25 mL of hydrochloric acid and water to make 1000 mL. Measure exactly 10 mL of this solution, transfer to an iodine flask, add 15 mL of water and 3 g of potassium iodide, stopper tightly, and allow to stand in a dark place for 15 minutes. Add 100 mL of water to the mixture, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 27.03 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$

According to the titrated value, add diluted hydrochloric acid (1 in 40) to make a solution containing 45.0 mg of iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$: 270.30) in each mL, and use this solution as the colorimetric stock solution.

Matching Fluids for Color Measure exactly the volume of colorimetric stock solutions and water shown in the following table with a buret or a pipet graduated to less than 0.1 mL, and mix.

Table 9.23-1 Matching fluid for color

Matching fluid for color	Parts of cobalt (II) chloride colorimetric stock solution (mL)	Parts of iron (III) chloride colorimetric stock solution (mL)	Parts of copper (II) sulfate colorimetric stock solution (mL)	Parts of water (mL)
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	3.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	—	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	—	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	—	—
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	—	4.9	0.1	—
O	0.1	4.8	0.1	—
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	—	4.7
T	0.5	0.5	0.4	3.6

Reagents, Test Solutions, etc.

9.41 Reagents, Test Solutions

Reagents are the substances used in the tests of the Pharmacopoeia. The reagents that are described as “Standard reagent for volumetric analysis”, “Special class”, “First class”, “For water determination”, etc. in square brackets meet the corresponding requirements of the Japan Industrial Standards (JIS). The tests for them are performed according to the test methods of JIS. In the case where the reagent name in the Pharmacopoeia differs from that of JIS, the JIS name is given in the brackets. The reagents for which a monograph’s title is given in the brackets meet the requirements of the corresponding monograph. In the case of the reagents that are described merely as test items, the corresponding test method of the Pharmacopoeia is applied.

Test Solutions are the solutions prepared for use in the tests of the Pharmacopoeia.

Acenaphthene $\text{C}_{12}\text{H}_{10}$ White to pale yellowish white crystals or crystalline powder, having a characteristic aroma. Freely soluble in diethyl ether and in chloroform, soluble in acetonitrile, sparingly soluble in methanol, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of acenaphthene according to the paste method under Infrared Spectrophotometry <2.25>, with 5 mg of acenaphthene: it exhibits absorption at the wave numbers of about 1605 cm^{-1} , 840 cm^{-1} , 785 cm^{-1} and 750 cm^{-1} .

Melting point <2.60>: $93 - 96^\circ\text{C}$

Purity—Dissolve 0.1 g of acenaphthene in 5 mL of chloro-

form, and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of acenaphthene by the area percentage method: it shows a purity of not less than 98.0%.

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 150- to 180- μ m siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acenaphthene is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acenaphthene obtained from 2 μ L of the solution prepared by adding chloroform to 1.0 mL of the sample solution to make 100 mL is 5% to 15% of the full scale.

Time span of measurement: About 3 times as long as the retention time of acenaphthene beginning after the solvent peak.

Residue on ignition <2.44>—Not more than 0.1% (1 g).

Acetal C₆H₁₄O₂ A clear and colorless volatile liquid. Miscible with water and with ethanol (95).

Refractive index <2.45> n_D^{20} : about 1.382

Specific gravity <2.56> d_4^{20} : about 0.824

Boiling point <2.57>: about 103°C

Acetaldehyde CH₃CHO [K 8030, First class]

Acetaldehyde for assay Distil 100 mL of acetaldehyde under reduced pressure, discard the first 20 mL of the distillate, and use the subsequent. Prepare before use.

Acetaldehyde for gas chromatography C₂H₄O A clear and colorless, flammable liquid. Miscible with water and with ethanol (95).

Refractive index <2.45> n_D^{20} : about 1.332

Specific gravity <2.56> d_4^{20} : about 0.788

Boiling point <2.57>: about 21°C

2-Acetamidoglutarimide C₇H₁₀N₂O₃: 170.17

Identification—Determine the infrared absorption spectrum of 2-acetamidoglutarimide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3350 cm⁻¹, 1707 cm⁻¹, 1639 cm⁻¹ and 1545 cm⁻¹.

Purity Related substances—Dissolve 10 mg of 2-acetamidoglutarimide in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Proceed with exactly 20 μ L each of the sample solution and standard solution as directed in the Purity (3) under Acetylglutamide Aluminum: the total of the peak areas other than 2-acetamidoglutarimide from the sample solution is not more than the peak area from the standard solution.

Content: not less than 98.0%. Assay—Weigh accurately about 20 mg of 2-acetamidoglutarimide, and perform the test

as directed under Nitrogen Determination <1.08>.

Each mL of 0.01 mol/L sulfuric acid VS
= 0.8509 mg of C₇H₁₀N₂O₃

Acetaminophen C₈H₉NO₂ [Same as the namesake monograph]

Acetanilide C₈H₉NO₂ White, crystals or crystalline powder.

Melting point <2.60>: 114 – 117°C

p-Acetanilide C₉H₁₁NO₂ White to purplish white, crystals or crystalline powder, having a characteristic odor.

It is freely soluble in ethanol (95) and in acetonitrile, and very slightly soluble in water.

Melting point <2.60>: 126 – 132°C

Content: not less than 98.0%. Assay—Dissolve 0.1 g of p-acetanilide in 5 mL of ethanol (95). Perform the test with 2 μ L of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content} = \frac{\text{peak area of } p\text{-acetanilide}}{\text{total of all peak areas}} \times 100$$

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass tube 3 mm in inside diameter and 2 m in length, packed with acid-treated and silanized siliceous earth for gas chromatography coated with alkylene glycol phthalate ester for gas chromatography in 1% (177–250 μ m in particle diameter).

Column temperature: A constant temperature of about 210°C

Carrier gas: Nitrogen

Flow rate: Adjust to a constant flow rate of between 30 and 50 mL per minute and so that the retention time of p-acetanilide is between 11 and 14 minutes.

Time span of measurement: About 3 times as long as the retention time of p-acetanilide beginning after the solvent peak.

Acetate buffer solution, pH 3.5 Dissolve 50 g of ammonium acetate in 100 mL of 6 mol/L hydrochloric acid TS, adjust to pH 3.5 with ammonia TS or 6 mol/L hydrochloric acid TS, if necessary, and add water to make 200 mL.

Acetate buffer solution, pH 4.5 Dissolve 63 g of anhydrous sodium acetate in a suitable amount of water, add 90 mL of acetic acid (100) and water to make 1000 mL.

Acetate buffer solution, pH 5.4 To 5.78 mL of acetic acid (100) add water to make 1000 mL (solution A). Dissolve 8.2 g of anhydrous sodium acetate in water to make 1000 mL (solution B). Mix 176 mL of the solution A and 824 mL of the solution B, and adjust, if necessary, the pH to 5.4 with the solution A or the solution B.

0.01 mol/L Acetate buffer solution, pH 5.0 Dissolve 385 g of ammonium acetate in 900 mL of water, add acetic acid (31) to adjust the pH to 5.0, and then add water to make 1000 mL.

Acetate buffer solution, pH 5.5 Dissolve 2.72 g of sodium acetate trihydrate in water to make 1000 mL, and adjust the pH to 5.5 with diluted acetic acid (100) (3 in 2500).

Acetic acid See acetic acid (31).

Acetic acid-ammonium acetate buffer solution, pH 3.0
Add acetic acid (31) to ammonium acetate TS, and adjust the pH to 3.0.

Acetic acid-ammonium acetate buffer solution, pH 4.5
Dissolve 77 g of ammonium acetate in about 200 mL of water, adjust the pH to 4.5 by adding acetic acid (100), and add water to make 1000 mL.

Acetic acid-ammonium acetate buffer solution, pH 4.8
Dissolve 77 g of ammonium acetate in about 200 mL of water, and add 57 mL of acetic acid (100) and water to make 1000 mL.

Acetic acid, dilute Dilute 6 g of acetic acid (100) with water to make 100 mL (1 mol/L).

Acetic acid for nonaqueous titration [K 8355, Special class. meeting with following requirement.]

Purity Acetic anhydride—Dissolve 1.0 g of aniline in acetic acid for nonaqueous titration to make 100 mL, and use this solution as the sample solution. Pipet 25 mL of the sample solution, titrate <2.50> with 0.1 mol/L perchloric acid VS, and designate the consumed volume as A (mL). A is not less than 26 mL. Pipet 25 mL of the sample solution, add 75 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS, and designate the consumed volume as B (mL) (potentiometric titration). A – B is not more than 0.1 (mL) (not more than 0.001 g/dL).

Acetic acid, glacial See acetic acid (100).

Acetic acid-potassium acetate buffer solution, pH 4.3
Dissolve 14 g of potassium acetate in 20.5 mL of acetic acid (100), and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, 0.05 mol/L, pH 4.0 To 3.0 g of acetic acid (100) add water to make 1000 mL. Adjust to pH 4.0 with a solution prepared by dissolving 3.4 g of sodium acetate trihydrate in water to make 500 mL.

0.05 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0 To 3.0 g of acetic acid (100) add water to make 1000 mL. To this solution add a sufficient amount of a solution of sodium acetate trihydrate (3.4 in 500) to adjust to pH 4.0.

Acetic acid-sodium acetate buffer solution, pH 4.5 To 80 mL of sodium acetate TS add 120 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 4.5, for iron limit test Dissolve 75.4 mL of acetic acid (100) and 111 g of sodium acetate trihydrate in 1000 mL of water.

Acetic acid-sodium acetate buffer solution, pH 4.7 Dissolve 27.2 g of sodium acetate trihydrate in 900 mL of water, adjust the pH to 4.7 by adding acetic acid (100) dropwise, and add water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.0 To 140 mL of sodium acetate TS add 60 mL of dilute acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate buffer solution, pH 5.5
Dissolve 20 g of sodium acetate trihydrate in 80 mL of water, adjust the pH to 5.5 by adding acetic acid (100) dropwise, and add water to make 100 mL.

Acetic acid-sodium acetate buffer solution, pH 5.6

Dissolve 12 g of sodium acetate trihydrate in 0.66 mL of acetic acid (100) and water to make 100 mL.

1 mol/L Acetic acid-sodium acetate buffer solution, pH 5.0 To sodium acetate TS add dilute acetic acid, and adjust the pH to 5.0.

0.1 mol/L Acetic acid-sodium acetate buffer solution, pH 4.0 Dissolve 13.61 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL.

0.05 mol/L Acetic acid-sodium acetate buffer solution, pH 4.6 Dissolve 6.6 g of sodium acetate trihydrate in 900 mL of water, and add 3 mL of acetic acid and water to make 1000 mL.

Acetic acid-sodium acetate TS Mix 17 mL of 1 mol/L sodium hydroxide VS with 40 mL of dilute acetic acid, and add water to make 100 mL.

0.02 mol/L Acetic acid-sodium acetate TS Dissolve 2.74 g of sodium acetate trihydrate in a suitable amount of water, and add 2 mL of acetic acid (100) and water to make 1000 mL.

6 mol/L Acetic acid TS Dilute 36 g of acetic acid (100) with water to make 100 mL.

0.25 mol/L Acetic acid TS To 3 g of acetic acid (100) add water to make 200 mL.

Acetic acid (100) CH₃COOH [K 8355, Acetic Acid, Special class]

Acetic acid (100)-sulfuric acid TS To 5 mL of acetic acid (100) add cautiously 5 mL of sulfuric acid while cooling in an ice bath, and mix.

Acetic acid (31) Dilute 31.0 g of acetic acid (100) with water to make 100 mL (5 mol/L).

Acetic anhydride (CH₃CO)₂O [K 8886, Special class]

Acetic anhydride-pyridine TS Place 25 g of acetic anhydride in a 100 mL volumetric flask, add pyridine to make 100 mL, and mix well. Preserve in light-resistant containers, protected from air. This solution may be used even if it becomes colored during storage.

Acetone CH₃COCH₃ [K 8034, Special class]

Acetone for nonaqueous titration Add potassium permanganate to acetone in small portions, and shake. When the mixture keeps its purple color after standing for 2 to 3 days, distil, and dehydrate with freshly ignited anhydrous potassium carbonate. Distil by using a fractionating column under protection from moisture, and collect the fraction distilling at 56°C.

Acetone for purity of crude drug [K 8034, Special class] Use acetone meeting the following additional specification. Evaporate 300.0 mL of acetone to be tested in vacuum at a temperature not higher than 40°C, add the acetone to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of γ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this so-

lution as the standard solution (1). Perform the test with exactly 1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of γ -BHC beginning from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of γ -BHC obtained from 1 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of γ -BHC from 1 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ -BHC beginning after the solvent peak.

Acetonitrile CH_3CN [K 8032, Special class]

Acetonitrile for liquid chromatography CH_3CN Colorless and clear liquid. Mixable with water.

Purity Ultraviolet light absorbing substances—Determine the absorbances at the following wavelengths as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control: not more than 0.07 at 200 nm, not more than 0.046 at 210 nm, not more than 0.027 at 220 nm, not more than 0.014 at 230 nm and not more than 0.009 at 240 nm.

Acetrizoic acid $\text{C}_9\text{H}_6\text{I}_3\text{NO}_3$ White powder.

Purity Related substances—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL. To 10 mL of this solution add water to make 100 mL, and use this solution as the sample solution. Proceed the test with 5 μL of the sample solution as directed in the Assay under Meglumine Sodium Amidotrizoate Injection: any peaks other than the principal peak are not observed.

Acetylacetone $\text{CH}_3\text{COCH}_2\text{COCH}_3$ [K 8027, Special class]

Acetylacetone TS Dissolve 150 g of ammonium acetate in a sufficient quantity of water, and add 3 mL of acetic acid (100), 2 mL of acetylacetone and water to make 1000 mL. Prepare before use.

Acetylene See dissolved acetylene.

Acidic ferric chloride TS See iron (III) chloride TS, acidic.

Acidic potassium chloride TS See potassium chloride TS, acidic.

Acidic potassium permanganate TS See potassium permanganate TS, acidic.

Acidic stannous chloride TS See tin (II) chloride TS, acidic.

Acid-treated gelatin See gelatin, acid-treated.

Aconitine for purity $\text{C}_{34}\text{H}_{47}\text{NO}_{11}$ White, crystals or crys-

talline powder. Sparingly soluble in acetonitrile and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 185°C (with decomposition).

Identification—Determine the infrared absorption spectrum of aconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500 cm^{-1} , 1718 cm^{-1} , 1278 cm^{-1} , 1111 cm^{-1} , 1097 cm^{-1} and 717 cm^{-1} .

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (230 nm): 211 – 243 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

Purity Related substances—

(1) Dissolve 5.0 mg of aconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of aconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of aconitine and the solvent obtained with the sample solution is not larger than the peak area of aconitine with the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconitine Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of aconitine is about 26 minutes.

Time span of measurement: About 3 times as long as the retention time of aconitine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of aconitine obtained from 10 μL of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μL of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μL of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating

conditions, the relative standard deviation of the peak area of aconitine is not more than 1.5%.

Water <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Aconitum diester alkaloids standard solution for purity It is a solution containing 10 mg of aconitine for purity, 10 mg of jesaconitine for purity, 30 mg of hyaconitine for purity and 20 mg of mesaconitine for purity in 1000 mL of a mixture of phosphate buffer solution for processed aconite root and acetonitrile (1:1). When proceed the test with 20 μ L of this solution as directed in the Purity under Processed Aconite Root at the detection wavelength 231 nm, the peaks of aconitine, jesaconitine, hyaconitine and mesaconitine are observed, and the ratio of their peak heights is about 10:1:35:30. When proceed the test at the detection wavelength 254 nm, the peaks of aconitine, jesaconitine, hyaconitine and mesaconitine are observed, and the ratio of their peak heights is about 2:8:7:6.

Acrinol $C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$ [Same as the monograph Acrinol Hydrate]

Acrylamide $CH_2CHCONH_2$ Pale yellow crystalline powder.

Melting point <2.60>: 83 – 86°C

Content: not less than 97.0%.

Activated alumina Aluminum oxide with specially strong adsorptive activity.

Activated charcoal [Same as the monograph Medicinal Carbon]

Activated thromboplastin-time assay reagent It is prepared by lyophilization of phospholipid (0.4 mg/mL) which is suspended in 1 mL of a solution of 2-[4-(2-hydroxymethyl)-1-piperazinyl]propanesulfonic acid (61 in 5000), mixed with both silica-gel (4.3 mg/mL) and dextran after the extraction and purification from rabbit brain. Activated thromboplastin-time: 25 – 45 seconds (as assayed with human normal plasma).

Activated thromboplastin-time assay solution Dissolve an aliquot of activated thromboplastin-time assay reagent equivalent to 0.4 mg of phospholipid in 1 mL of water.

Adipic acid $C_4H_8(COOH)_2$ White crystals or crystalline powder. Freely soluble in ethanol (95), and sparingly soluble in water.

Melting point <2.60>: 151 – 154°C

Content: not less than 98.0%. **Assay**—Weigh accurately about 1 g of adipic acid, and 100 mL of water, dissolve by warming, cool, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 73.07 mg of $C_6H_{10}O_4$

Agar [K 8263, Special class. Same as the monograph Agar or Agar Powder. Loss on drying is not more than 15%.]

Agar medium, ordinary See ordinary agar medium.

Agar slant Dispense portions of about 10 mL of ordinary agar medium into test tubes, and sterilize by autoclaving. Before the medium congeals, allow to stand in a slanting

position, and solidify. When the coagulating water is lost, reprepare by dissolving with the aid of heat.

Ajmaline for assay $C_{20}H_{26}N_2O_2$ [Same as the monograph Ajmaline. When dried, it contains not less than 99.0% of ajmaline ($C_{20}H_{26}N_2O_2$).]

Alacepril $C_{20}H_{26}N_2O_5S$ [Same as the namesake monograph]

Alacepril for assay [Same as the monograph Alacepril. When dried, it contains not less than 99.0% of alacepril ($C_{20}H_{26}N_2O_5S$).]

L-Alanine $C_3H_7NO_2$ [K 9101, Special class]

Albiflorin $C_{23}H_{28}O_{11} \cdot xH_2O$ Colorless powder having no odor. Freely soluble in water and in methanol, and practically insoluble in diethyl ether.

Purity—Dissolve 1 mg in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed in the Assay under Peony Root; when measure the peak areas about 2 times as long as the retention time of peoniflorin, the total area of the peaks other than albiflorin and other than the solvent is not larger than 1/10 of the total area of the peaks other than the solvent peak.

Albumin TS Carefully separate the white from the yolk of a fresh hen's egg. Shake the white with 100 mL of water until the mixture is thoroughly mixed, and filter. Prepare before use.

Aldehyde dehydrogenase Each mg contains not less than 2 enzyme activity units. White powder.

Assay—Dissolve about 20 mg of aldehyde dehydrogenase, accurately weighed, in 1 mL of water, add ice-cold solution of bovine serum albumin (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. In a spectrophotometric cell, place 2.50 mL of pyrophosphate buffer solution, pH 9.0, 0.20 mL of a solution prepared by dissolving 20.0 mg of β -nicotinamide adenine dinucleotide (NAD) to make exactly 1 mL, 0.10 mL of a pyrazole solution (17 in 2500) and 0.10 mL of the sample solution, stir, stopper tightly, and allow to stand at $25 \pm 1^\circ C$ for 2 minutes. To this solution add 0.01 mL of an acetaldehyde solution (3 in 1000), stir, stopper tightly, determine every 30 seconds the absorbance at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate a change (ΔA) in absorbance per minute starting from the spot where the relation of time and absorbance is shown with a straight line. One enzyme activity unit means an amount of enzyme which oxidizes 1 μ mol of acetaldehyde per minute when the test is conducted under the conditions of the Procedure.

Enzyme activity unit (unit/mg) of aldehyde dehydrogenase

$$= \frac{2.91 \times \Delta A \times 200}{6.3 \times W \times 0.10 \times 1000}$$

W : Amount (g) of sample

Aldehyde dehydrogenase TS Dissolve an amount equivalent to 70 aldehyde dehydrogenase units in 10 mL of water. Prepare before use.

Aldehyde-free ethanol See ethanol, aldehyde-free.

Alisol A for thin-layer chromatography $C_{30}H_{50}O_5$ A

white to pale yellow powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Optical rotation <2.49> $[\alpha]_D^{20}$: +86– +106° (5 mg previously dried on silica gel for 24 hours, methanol, 1 mL, 50 mm).

Purity Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 5 μ L of this solution as directed in the Identification (6) under Saireito Extract: no spot appears other than the principal spot of around *Rf* 0.3.

Alizarin complexone $C_{19}H_{15}NO_8$ (1,2-Dihydroxyanthra-quinone-3-ylmethylamine-*N,N*-diacetate) A yellow-brown powder. Soluble in ammonia TS, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Sensitivity—Dissolve 0.1 g of alizarin complexone by adding 2 drops of ammonia solution (28), 2 drops of ammonium acetate TS and 20 mL of water. To 10 mL of this solution add acetic acid-potassium acetate buffer solution, pH 4.3, to make 100 mL. Place 1 drop of this solution on a white spot plate, add 1 drop of a solution of sodium fluoride (1 in 100,000) and 1 drop of cerium (III) nitrate hexahydrate TS, stir, and observe under scattered light after 1 minute: a blue-purple color is produced, and the color of the control solution is red-purple. Use a solution prepared in the same manner, to which 1 drop of water is added in place of a solution of sodium fluoride, as the control solution.

Alizarin complexone TS Dissolve 0.390 g of alizarin complexone in 20 mL of a freshly prepared solution of sodium hydroxide (1 in 50), then add 800 mL of water and 0.2 g of sodium acetate trihydrate, and dissolve. Adjust the pH to 4 to 5 with 1 mol/L hydrochloric acid VS, and add water to make 1000 mL.

Alizarin red S $C_{14}H_7NaO_7S \cdot H_2O$ [K 8057, Special class]

Alizarin red S TS Dissolve 0.1 g of alizarin red S in water to make 100 mL, and filter if necessary.

Alizarin S See alizarin red S.

Alizarin S TS See alizarin red S TS.

Alizarin yellow GG $C_{13}H_8N_3NaO_5$ [K 8056, Special class]

Alizarin yellow GG-thymolphthalein TS Mix 10 mL of alizarin GG TS with 20 mL of thymolphthalein TS.

Alizarin yellow GG TS Dissolve 0.1 g of alizarin yellow GG in 100 mL of ethanol (95), and filter if necessary.

Alkali copper TS Dissolve 70.6 g of disodium hydrogen phosphate dodecahydrate, 40.0 g of potassium sodium tartrate tetrahydrate and 180.0 g of anhydrous sodium sulfate in 600 mL of water, and add 20 mL of a solution of sodium hydroxide (1 in 5). To this mixture add, with stirring, 100 mL of a solution of copper (II) sulfate (2 in 25), 33.3 mL of 0.05 mol/L potassium iodate VS and water to make 1000 mL.

Alkaline blue tetrazolium TS See blue tetrazolium TS, alkaline.

Alkaline copper solution Dissolve 0.8 g of sodium hydroxide in water to make 100 mL, and dissolve 4 g of anhydrous sodium carbonate in this solution to make solution A. Combine 1 mL of a solution of copper (II) sulfate pentahydrate (1 in 50) and 1 mL of a solution of sodium tartrate de-

hydrate (1 in 25) to make solution B. Mix 50 mL of freshly prepared solution A and 1 mL of freshly prepared solution B.

Alkaline copper TS Dissolve 2 g of anhydrous sodium carbonate in 100 mL of 0.1 mol/L sodium hydroxide TS. To 50 mL of this solution add 1 mL of a mixture of a solution of copper (II) sulfate pentahydrate (1 in 100) and a solution of potassium tartrate (1 in 50) (1:1), and mix.

Alkaline copper TS for protein content determination Dissolve 0.8 g of sodium hydroxide in water to make 100 mL. Dissolve 4 g of anhydrous sodium carbonate in this solution to make solution A. Combine 1 mL of copper (II) sulfate pentahydrate solution (1 in 50) and 1 mL of sodium tartrate dihydrate solution (1 in 25) to make solution B. Mix 50 mL of solution A and 1 mL of solution B. Prepare at the time of use.

Alkaline copper (II) sulfate solution See copper (II) sulfate solution, alkaline.

Alkaline glycerin TS To 200 g of glycerin add water to make 235 g, and add 142.5 mL of sodium hydroxide TS and 47.5 mL of water.

Alkaline hydroxylamine TS See hydroxylamine TS, alkaline.

Alkaline *m*-dinitrobenzene TS See 1,3-dinitrobenzene TS, alkaline.

Alkaline picric acid TS See 2,4,6-trinitrophenol TS, alkaline.

Alkaline potassium ferricyanide TS See potassium hexacyanoferrate (III) TS, alkaline.

Alkylene glycol phthalate ester for gas chromatography Prepared for gas chromatography.

Alternative thioglycolate medium See Sterility Test <4.06> under the General Tests, Processes and Apparatus.

Aluminon $C_{22}H_{23}N_3O_9$ [K 8011, Special class]

Aluminon TS Dissolve 0.1 g of aluminon in water to make 100 mL, and allow this solution to stand for 24 hours.

Aluminum Al [K 8069, Special class]

Aluminum chloride See aluminum (III) chloride hexahydrate.

Aluminum chloride TS See Aluminum (III) chloride TS.

Aluminum (III) chloride TS Dissolve 64.7 g of aluminum (III) chloride hexahydrate in 71 mL of water, add 0.5 g of activated charcoal, then shake for 10 minutes, and filter. Adjust the pH of the filtrate to 1.5 with a solution of sodium hydroxide (1 in 100) with stirring, and filter if necessary.

Aluminum (III) chloride hexahydrate $AlCl_3 \cdot 6H_2O$ [K 8114, Special class]

Aluminum oxide Al_2O_3 White crystals, crystalline powder, or powder. Boiling point: about 3000°C. Melting point: about 2000°C.

Aluminum potassium sulfate dodecahydrate $AlK(SO_4)_2 \cdot 12H_2O$ [K 8255, Special class]

Amidosulfuric acid (standard reagent) $HOSO_2NH_2$ [K 8005, Standard substance for volumetric analysis]

Amidotrizoic acid for assay $C_{11}H_9I_3N_2O_4$ [Same as the monograph Amidotrizoic Acid] It contains not less than 99.0% of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), calculated on the dried basis.

Aminoacetic acid See glycine.

p-Aminoacetophenone See 4-aminoacetophenone.

p-Aminoacetophenone TS See 4-aminoacetophenone TS.

4-Aminoacetophenone $H_2NC_6H_4COCH_3$ Light yellow, crystals or crystalline powder, having a characteristic odor.
Melting point <2.60>: 105 – 108°C

4-Aminoacetophenone TS Dissolve 0.100 g of 4-aminoacetophenone in methanol to make exactly 100 mL.

4-Aminoantipyrine $C_{11}H_{13}N_3O$ [K 8048, Special class]

4-Aminoantipyrine hydrochloride $C_{11}H_{13}N_3O.HCl$ Light yellow crystalline powder. It dissolves in water. *Melting point*: 232 – 238°C (decomposition).

Purity Clarity of solution—Dissolve 1 g of 4-aminoantipyrine hydrochloride in 25 mL of water: the solution is almost clear.

Content: 100.6 – 108.5%. *Assay*—Weigh accurately about 0.5 g of 4-aminoantipyrine hydrochloride, dissolve in 50 mL of water, and, if necessary, neutralize with 0.1 mol/L sodium hydroxide VS (indicator: red litmus paper). Add 4 drops of dichlorofluorescein TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS.

Each mL of 0.1 mol/L silver nitrate VS
= 23.97 mg of $C_{11}H_{13}N_3O.HCl$

4-Aminoantipyrine hydrochloride TS Dissolve 1 g of 4-aminoantipyrine hydrochloride in water to make 50 mL.

4-Aminoantipyrine TS Dissolve 0.1 g of 4-aminoantipyrine in 30 mL of water, add 10 mL of a solution of sodium carbonate decahydrate (1 in 5), 2 mL of sodium hydroxide TS and water to make 100 mL. Prepare before use.

p-Aminobenzoic acid See 4-aminobenzoic acid.

4-Aminobenzoic acid $C_7H_7NO_2$ White to very pale yellow crystalline powder. A solution of 4-aminobenzoic acid in ethanol (95) (1 in 100) is clear.

2-Amino-1-butanol $CH_3CH_2CH(NH_2)CH_2OH$ Clear, colorless to light yellow liquid. Miscible with water and dissolves in methanol.

Refractive index <2.45> n_D^{20} : 1.450 – 1.455

Specific gravity <2.56> d_{20}^{20} : 0.944 – 0.950

Purity Related substances—Dissolve 50 mg of 2-amino-1-butanol in 10 mL of methanol, measured exactly, and perform the test with 2 μ L of this solution as directed in the Purity (4) under Ethambutol Hydrochloride: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

2-Amino-5-chlorobenzophenone for thin-layer chromatography $C_{13}H_{10}ClNO$ Yellow, crystalline powder.

Melting point <2.60>: 97 – 101°C

Purity Related substances—Dissolve 10 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and perform the test with this solution as directed in the purity (3) under Chlor-

diazepoxide: any spot other than the principal spot at the *Rf* value about 0.7 does not appear.

4-Amino-N,N-diethylaniline sulfate $H_2NC_6H_4N(C_2H_5)_2.H_2SO_4.H_2O$ White to slightly colored powder. It dissolves in water.

Melting point <2.60>: 173 – 176°C

Residue on ignition <2.44>: not more than 0.1% (1 g).

4-Amino-N,N-diethylaniline sulfate TS Dissolve 0.2 g of 4-amino-N,N-diethylaniline sulfate in water to make 100 mL. Prepare before use, protected from light.

2-Aminoethanethiol hydrochloride $H_2NCH_2CH_2SH.HCl$ White crystal or granule.

Melting point <2.60>: 65 – 71°C

2-Aminoethanol $NH_2CH_2CH_2OH$ [K 8109, Special class]

N-Aminohexamethyleneimine $(CH_2)_6NNH_2$ Clear, colorless to pale yellow liquid.

Refraction index <2.45> n_D^{20} : 1.482 – 1.487

Specific gravity <2.56> d_{20}^{20} : 0.936 – 0.942

2-Amino-2-hydroxymethyl-1,3-propanediol $C_4H_{11}NO_3$ [K 9704, Special class]

2-Amino-2-hydroxymethyl-1,3-propanediol hydrochloride $C_4H_{11}NO_3.HCl$ White crystals or crystalline powder

4-(Aminomethyl)benzoic acid $C_8H_9NO_2$ A white powder.

Purity—Dissolve 10 mg of 4-(aminomethyl)benzoic acid in 100 mL of water, and use this as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the operating conditions as directed in the Purity (5) under Tranexamic Acid, and determine each peak area by the automatic integration method: each area of the peak other than 4-(aminomethyl)benzoic acid obtained from the sample solution is not more than the peak area of 4-(aminomethyl)benzoic acid from the standard solution.

3-(2-Aminoethyl)indole $C_{10}H_{12}N_2$ Yellowish-brown crystals.

Melting point <2.60>: about 118°C.

1-Amino-2-naphthol-4-sulfonic acid $C_{10}H_9NO_4S$ [K 8050, Special class]

1-Amino-2-naphthol-4-sulfonic acid TS Mix thoroughly 5 g of anhydrous sodium sulfite, 94.3 g of sodium bisulfite and 0.7 g of 1-amino-2-naphthol-4-sulfonic acid. Before use, dissolve 1.5 g of this mixture in water to make 10 mL.

m-Aminophenol See 3-aminophenol.

3-Aminophenol $H_2NC_6H_4OH$ White, crystals or crystalline powder.

Melting point <2.60>: 121 – 125°C

Content: not less than 97.0%. *Assay*—Weigh accurately about 0.2 g, dissolve in 50 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.91 mg of $H_2NC_6H_4OH$

***p*-Aminophenol hydrochloride** See 4-aminophenol hydrochloride.

4-Aminophenol hydrochloride $\text{HOC}_6\text{H}_4\text{NH}_2\cdot\text{HCl}$
White to pale colored crystals. Freely soluble in water and in ethanol (95). Melting point: about 306°C (with decomposition).

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.17 g of 4-aminophenol hydrochloride, dissolve in 50 mL of acetic acid for nonaqueous titration and 5 mL of mercury (II) acetate TS for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid-1,4-dioxane VS (indicator: 1 mL of α -naphtholbenzene TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-1,4-dioxane VS = 14.56 mg of $\text{C}_6\text{H}_8\text{NOCl}$

Storage—Preserve in tight, light-resistant containers.

Aminopropylsilanized silica gel for pretreatment Prepared for pretreatment.

L-2-Aminosuberic acid $\text{C}_8\text{H}_{15}\text{NO}_4$ White, crystals or crystalline powder. Odorless.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +19.1 – +20.1° (after drying, 0.1 g, 5 mol/L hydrochloric acid TS, 100 mm).

Loss on drying <2.41>: not more than 0.3% (1 g, 105°C, 2 hours).

Assay—Weigh accurately about 0.3 g of L-2-aminosuberic acid, previously dried, add exactly 6 mL of formic acid to dissolve, then add exactly 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS = 18.92 mg of $\text{C}_8\text{H}_{15}\text{NO}_4$

Ammonia-ammonium acetate buffer solution, pH 8.0 To ammonium acetate TS add ammonia TS dropwise to adjust the pH to 8.0.

Ammonia-ammonium acetate buffer solution, pH 8.5 Dissolve 50 g of ammonium acetate in 800 mL of water and 200 mL of ethanol (95), and add ammonia solution (28) to adjust the pH to 8.5.

Ammonia-ammonium chloride buffer solution, pH 8.0 Dissolve 1.07 g of ammonium chloride in water to make 100 mL, and adjust the pH to 8.0 by adding diluted ammonia TS (1 in 30).

Ammonia-ammonium chloride buffer solution, pH 10.0 Dissolve 70 g of ammonium chloride in water, add 100 mL of ammonia solution (28), dilute with water to make 1000 mL, and add ammonia solution (28) dropwise to adjust the pH to 10.0.

Ammonia-ammonium chloride buffer solution, pH 10.7 Dissolve 67.5 g of ammonium chloride in water, add 570 mL of ammonia solution (28), and dilute with water to make 1000 mL.

Ammonia-ammonium chloride buffer solution, pH 11.0 Dissolve 53.5 g of ammonium chloride in water, add 480 mL of ammonia solution (28), and dilute with water to make 1000 mL.

Ammonia copper TS To 0.5 g of cupric carbonate monohydrate add 10 mL of water, triturate, and add 10 mL of ammonia solution (28).

Ammonia-ethanol TS To 20 mL of ammonia solution (28) add 100 mL of ethanol (99.5).

Ammonia gas NH_3 Prepare by heating ammonia solution (28).

Ammonia-saturated 1-butanol TS To 100 mL of 1-butanol add 60 mL of diluted ammonia solution (28) (1 in 100), shake vigorously for 10 minutes, and allow to stand. Use the upper layer.

Ammonia solution (28) NH_4OH [K 8085, Ammonia Water, Special class, Specific gravity: about 0.90, Density: 0.908 g/mL, Content: 28–30%]

Ammonia TS To 400 mL of ammonia solution (28) add water to make 1000 mL (10%).

Ammonia water See ammonia TS.

1 mol/L Ammonia water To 65 mL of ammonia solution (28) add water to make 1000 mL.

13.5 mol/L Ammonia water To exactly 9 mL of water add ammonia solution (28) to make exactly 50 mL.

Ammonia water, strong See ammonia solution (28).

Ammonium acetate $\text{CH}_3\text{COONH}_4$ [K 8359, Special class]

Ammonium acetate TS Dissolve 10 g of ammonium acetate in water to make 100 mL.

0.5 mol/L Ammonium acetate TS Dissolve 38.5 g of ammonium acetate in water to make 1000 mL.

Ammonium amidosulfate $\text{NH}_4\text{OSO}_2\text{NH}_2$ [K 8588, Special class]

Ammonium amidosulfate TS Dissolve 1 g of ammonium amidosulfate in water to make 40 mL.

Ammonium aminetrichloroplatinate for liquid chromatography $\text{Cl}_3\text{H}_7\text{N}_2\text{Pt}$ To 20 g of cisplatin add 600 mL of 6 mol/L hydrochloric acid TS, and heat under a reflux condenser for 4–6 hours to boil while stirring. After cooling, evaporate the solvent, and dry the orange residue at room temperature under reduced pressure. To the residue so obtained add 300 mL of methanol, and heat at about 50°C to dissolve. Filter, separate insoluble yellow solids, and wash the solids with 10 mL of methanol. Combine the filtrate and the washing, heat at about 50°C, and add slowly 100 mL of ethyl acetate while stirring. Cool the mixture to room temperature avoiding exposure to light, and allow to stand at –10°C for 1 hour. Filter the mixture to take off the formed crystals, wash the crystals with 100 mL of acetone, combine the washing to the filtrate, and evaporate to dryness to obtain orange crystals. If necessary, repeat the purification procedure described above to take off the insoluble crystals. To the orange crystals obtained add 300 to 500 mL of a mixture of acetone and methanol (5:1), and heat at about 50°C while stirring to dissolve. Filter while hot to take off the insoluble crystals, wash the crystals with the mixture, and combine the filtrate and washing. Repeat the procedure several times, and evaporate to dryness. Suspend the crystals so obtained in 50

mL of acetone, filter, wash the crystals with 20 mL of acetone, and dry the crystals at room temperature under reduced pressure. It is a yellow-brown crystalline powder.

Identification—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 80°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3480 cm⁻¹, 3220 cm⁻¹, 1622 cm⁻¹, 1408 cm⁻¹ and 1321 cm⁻¹.

Related substances—Cisplatin Conduct this procedure using light-resistant vessels. Dissolve 10 mg in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of Cisplatin in *N,N*-dimethylformamide to make exactly 50 mL. Pipet 5 mL of this solution, add *N,N*-dimethylformamide to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of cisplatin by the automatic integration method: the peak area from the sample solution is not more than that from the standard solution.

Operating conditions

Proceed as directed in the operating conditions in the Assay under Cisplatin.

System suitability

System performance: When the procedure is run with 40 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 2500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 5.0%.

Ammonium aurintricarboxylate See aluminon.

Ammonium carbonate [K 8613, Special class]

Ammonium carbonate TS Dissolve 20 g of ammonium carbonate in 20 mL of ammonia TS and water to make 100 mL.

Ammonium chloride NH₄Cl [K 8116, Special class]

Ammonium chloride-ammonia TS To ammonia solution (28) add an equal volume of water, and saturate this solution with ammonium chloride.

Ammonium chloride buffer solution, pH 10 Dissolve 5.4 g of ammonium chloride in water, and add 21 mL of ammonia solution (28) and water to make 100 mL.

Ammonium chloride TS Dissolve 10.5 g of ammonium chloride in water to make 100 mL (2 mol/L).

Ammonium citrate See diammonium hydrogen citrate.

Ammonium dihydrogenphosphate NH₄H₂PO₄ [K 9006, Special class]

0.02 mol/L Ammonium dihydrogenphosphate TS Dissolve 2.30 g of ammonium dihydrogen phosphate in water to make 1000 mL.

Ammonium formate HCOONH₄ Colorless crystals. Very soluble in water.

Melting point <2.60>: 116 – 119°C

0.05 mol/L Ammonium formate buffer solution, pH 4.0 Dissolve 3.5 g of ammonium formate in about 750 mL of water, adjust the pH to 4.0 with formic acid, and add water to make 1000 mL.

Ammonium hydrogen carbonate NH₄HCO₃ White or semi-transparency crystals, crystalline powder or masses, having an ammonia odor.

Ammonium iron (II) sulfate hexahydrate FeSO₄(NH₄)₂SO₄·6H₂O [K 8979, Special class]

Ammonium iron (III) citrate [Same as the monograph Ferric Ammonium Citrate in the Japanese Standards of Food Additives]

Ammonium iron (III) sulfate TS Dissolve 8 g of ammonium iron (III) sulfate dodecahydrate in water to make 100 mL.

Ammonium iron (III) sulfate TS, acidic Dissolve 20 g of ammonium iron (III) sulfate dodecahydrate in a suitable amount of water, add 9.4 mL of sulfuric acid, and add water to make 100 mL.

Ammonium iron (III) sulfate TS, dilute To 2 mL of ammonium iron (III) sulfate TS add 1 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

Ammonium iron (III) sulfate dodecahydrate FeNH₄(SO₄)₂·12H₂O [K 8982, Special class]

Ammonium molybdate See hexaammonium heptamolybdate tetrahydrate.

Ammonium molybdate-sulfuric acid TS See hexaammonium heptamolybdate-sulfuric acid TS

Ammonium molybdate TS See hexaammonium heptamolybdate TS.

Ammonium nickel (II) sulfate hexahydrate NiSO₄(NH₄)₂SO₄·6H₂O [K 8990, Special class]

Ammonium nitrate NH₄NO₃ [K 8545, Special class]

Ammonium oxalate See ammonium oxalate monohydrate.

Ammonium oxalate monohydrate (NH₄)₂C₂O₄·H₂O [K 8521, Special class]

Ammonium oxalate TS Dissolve 3.5 g of ammonium oxalate monohydrate in water to make 100 mL (0.25 mol/L).

Ammonium peroxodisulfate (NH₄)₂S₂O₈ [K 8252, Special class]

10% Ammonium peroxodisulfate TS Dissolve 1 g of ammonium peroxodisulfate in water to make 10 mL.

Ammonium persulfate See ammonium peroxodisulfate.

Ammonium polysulfide TS (NH₄)₂S_x [K 8943, Ammonium Sulfide Solution (yellow), First class]

Ammonium sodium hydrogenphosphate tetrahydrate NaNH₄HPO₄·4H₂O [K 9013, Special class]

Ammonium sulfamate See ammonium amidosulfate.

Ammonium sulfamate TS See ammonium amidosulfate TS.

Ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$ [K 8960, Special class]

Ammonium sulfate buffer solution Dissolve 264 g of ammonium sulfate in 1000 mL of water, add 1000 mL of 0.5 mol/L sulfuric acid TS, shake, and filter. The pH of this solution is about 1.

Ammonium sulfide TS $(\text{NH}_4)_2\text{S}$ [K 8943, Ammonium Sulfide Solution, (colorless), First class] Store in small, well-filled containers, protected from light.

Ammonium tartrate See L-ammonium tartrate.

L-Ammonium tartrate $\text{C}_4\text{H}_{12}\text{N}_2\text{O}_6$ [K 8534, (+) Ammonium tartrate, Special class]

Ammonium thiocyanate NH_4SCN [K 9000, Special class]

Ammonium thiocyanate-cobalt (II) nitrate TS Dissolve 17.4 g of ammonium thiocyanate and 2.8 g of cobalt (II) nitrate hexahydrate in water to make 100 mL.

Ammonium thiocyanate TS Dissolve 8 g of ammonium thiocyanate in water to make 100 mL (1 mol/L).

Ammonium vanadate See ammonium vanadate (V).

Ammonium vanadate (V) NH_4VO_3 [K 8747, Special class]

Amoxicillin $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}\cdot 3\text{H}_2\text{O}$ [Same as the namesake monograph]

Amphoteric electrolyte solution for pH 3 to 10 Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 3 to 10 when mixed with polyacrylamide gel and placed in an electric field.

Amphoteric electrolyte solution for pH 6 to 9 Forms a pH gradient over a pH range of 6 to 9 when mixed with polyacrylamide gel and placed in an electric field. Prepare by diluting a 0.35 mmol/pH-mL buffer capacity solution about 20-fold with water. Almost colorless.

Amphoteric electrolyte solution for pH 8 to 10.5 Extremely pale yellow liquid. Mixture consisting of multiple types of molecules, buffer capacity is 0.35 mmol/pH-mL. Forms a pH gradient over a pH range of 8 to 10.5 when mixed with polyacrylamide gel and placed in an electric field.

Amygdalin for thin-layer chromatography $\text{C}_{20}\text{H}_{27}\text{NO}_{11}$ A white, odorless powder. Freely soluble in water, sparingly soluble in methanol, and practically insoluble in diethyl ether.

Purity Related substances—Dissolve 20 mg of amygdalin for thin-layer chromatography in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Apricot Kernel: any spot other than the principal spot at the R_f value of about 0.5 obtained from the sample solution is not more intense than the spot from the standard solution.

n-Amyl alcohol $\text{CH}_3(\text{CH}_2)_4\text{OH}$ Clear, colorless liquid, having a characteristic odor. Sparingly soluble in water, and miscible with ethanol (95) and with diethyl ether.

Refractive index $\langle 2.45 \rangle$ n_D^{20} : 1.409 – 1.411

Specific gravity $\langle 2.56 \rangle$ d_4^{20} : 0.810 – 0.820

Distilling range $\langle 2.57 \rangle$: 135 – 140°C, not less than 95 vol%.

t-Amyl alcohol $(\text{CH}_3)_2\text{C}(\text{OH})\text{CH}_2\text{CH}_3$ Clear, colorless liquid, having a characteristic odor. Miscible with *tert*-butanol and with 2-butanone, and freely soluble in water.

Specific gravity $\langle 2.56 \rangle$ d_{20}^{20} : 0.808 – 0.815

Purity Acid and ester—To 20 mL of *t*-amyl alcohol add 20 mL of ethanol (95) and 5.0 mL of 0.1 mol/L sodium hydroxide VS, and heat gently under a reflux condenser in a water bath for 10 minutes. Cool, add 2 drops of phenolphthalein TS, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L hydrochloric acid VS. Perform a blank determination: not more than 1.25 mL of 0.1 mol/L sodium hydroxide VS is consumed.

Nonvolatile residue—Evaporate 50 mL of *t*-amyl alcohol, and dry at 105°C for 1 hour: the residue is not more than 1.6 mg.

Distilling range $\langle 2.57 \rangle$: 100 – 103°C, not less than 95 vol%.

tert-Amyl alcohol See *t*-amyl alcohol.

Amyl alcohol, iso See 3-methyl-1-butanol.

Anesthetic ether See ether, anesthetic.

Anhydrous caffeine See caffeine, anhydrous.

Anhydrous cupric sulfate See copper (II) sulfate (anhydrous).

Anhydrous dibasic sodium phosphate See disodium hydrogen phosphate.

Anhydrous dibasic sodium phosphate for pH determination See disodium hydrogen phosphate for pH determination.

Anhydrous hydrazine for amino acid analysis Prepared for amino acid analysis.

Anhydrous lactose $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ [Same as the monograph Anhydrous Lactose]

Anhydrous potassium carbonate See potassium carbonate.

Anhydrous sodium acetate See sodium acetate, anhydrous.

Anhydrous sodium carbonate See sodium carbonate, anhydrous.

Anhydrous sodium sulfate See sodium sulfate, anhydrous.

Anhydrous sodium sulfite See sodium sulfite, anhydrous.

Aniline $\text{C}_6\text{H}_5\text{NH}_2$ [K 8042, Special class]

Animal tissue peptone See peptone, animal tissue.

p-Anisaldehyde See 4-methoxybenzaldehyde.

p-Anisaldehyde-acetic acid TS See 4-methoxybenzaldehyde-acetic acid TS.

p-Anisaldehyde-sulfuric acid TS See 4-methoxybenzaldehyde-sulfuric acid TS.

Anisole $\text{C}_7\text{H}_8\text{O}$ [K 8041, Special class]

Anthrone $\text{C}_{14}\text{H}_{10}\text{O}$ [K 8082, Special class]

Anthrone TS Dissolve 35 mg of anthrone in 100 mL of sulfuric acid.

Anti-A type antibody for blood typing Conforms to the requirements of antibody for blood typing.

Anti-B type antibody for blood typing Conforms to the requirements of antibody for blood typing.

Antibody fragment (Fab') Purify *E. coli* protein antibody by affinity chromatography using *Staphylococcus aureus* protein A as a ligand, and fractionate IgG. Digest this fraction using pepsin, remove the pepsin and Fc fragment by gel filtration chromatography, and obtain F(ab')₂ fragment after removing undigested IgG by affinity chromatography with protein A as ligand. Reduce this with 2-mercaptoethylamine.

Anti-bradykinin antibody A colorless to light brown, clear solution prepared by dissolving rabbit origin anti-bradykinin antibody in 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL of bovine serum albumin.

Performance test—To a suitable amount of anti-bradykinin antibody to be tested add 0.04 mol/L phosphate buffer solution, pH 7.0 containing 1 mg/mL bovine serum albumin to make a 1 vol% solution. Perform the test with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the absorbances at 490–492 nm, A_1 and A_2 , of the standard solution (1) and the standard solution (7): the value, $A_2 - A_1$, is not less than 1.

Anti-bradykinin antibody TS To 0.15 mL of anti-bradykinin antibody, 15 mg of bovine serum albumin, 2.97 mg of sodium dihydrogen phosphate dihydrate, 13.5 mg of disodium hydrogen phosphate dodecahydrate and 13.5 mg of sodium chloride add water to make 15 mL, and lyophilize. Dissolve this in 15 mL of water. Prepare before use.

Anti-E. coli protein antibody stock solution Taking *E. coli* protein stock solution as the immunogen, mix with Freund's complete adjuvant, and immunize rabbits by subcutaneous injection at 3 week intervals to obtain antiserum. Treat the antiserum obtained by ammonium sulfate precipitation.

Protein concentration: Dilute anti-E. coli protein antibody stock solution with 0.05 mol/L tris hydrochloride buffer solution (pH 7.5), measure the absorbance at 280 nm using 0.05 mol/L tris hydrochloride buffer solution (pH 7.5) as a control as direct under Ultraviolet-visible Spectrophotometry <2.24>, and determine the protein concentration (absorbance 1.0 = 0.676 mg/mL).

Antimony (III) chloride SbCl₃ [K 8400, Special class]

Antimony (III) chloride TS Wash chloroform with an equal volume of water twice or three times, add freshly ignited and cooled potassium carbonate, and allow to stand overnight in a well-closed container protected from light. Separate the chloroform layer, and distil it, preferably with protection from light. With this chloroform, wash the surface of antimony (III) chloride until the rinsing solution becomes clear, add the chloroform to this antimony (III) chloride to make a saturated solution, and place in light-resistant, glass-stoppered bottles. Prepare before use.

Antimony trichlorid See antimony (III) chloride.

Antimony trichlorid TS See antimony (III) chlorid TS.

Antipyrine C₁₁H₁₂N₂O [Same as the namesake monograph]

Anti-rabbit antibody-coated wells Wells of a polystyrene microplate coated with goat origin anti-rabbit IgG antibody.

Anti-thrombin III A white powder.

Water <2.48>: not more than 5%.

Content: not less than 80% and not more than 130% of the labeled amount.

Anti-thrombin III TS Dissolve 10 unit of anti-thrombin III in 10 mL of water.

Anti-ulinastatin rabbit serum To a suitable amount of Ulinastatin having the specific activity of more than 3000 Units per mg protein add isotonic sodium chloride solution so that each mL of the solution contains about 1 mg of protein. To 1 mL of this solution add 1 mL of Freund's complete adjuvant, and emulsify completely. Intracutaneously, inject the emulsion so obtained into a rabbit weighing about 2 kg. Repeat the injection at least 4 times at one-week intervals, and draw the blood of the animal from the carotid artery after the antibody titer reaches 16 times or more. Separate the serum after the blood has coagulated. Preserve at below -20°C.

Anti-urokinase serum Take Urokinase containing not less than 140,000 Unit per mg of protein, dissolve in isotonic sodium chloride solution to make a solution containing 1 mg of protein per mL, and emulsify with an equal volume of Freund's complete adjuvant. Inject intracutaneously three 2-mL portions of the emulsion to a healthy rabbit weighed between 2.5 kg and 3.0 kg in a week interval. Collect the blood from the rabbit at 7 to 10 days after the last injection, and prepare the anti-serum.

Performance test—Dissolve 1.0 g of agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish to make a depth of about 2 mm. After cooling, bore three of a pair-well 2.5 mm in diameter with a space of 6 mm each other. In one of the wells of each pair-well, place 10 μL of anti-urokinase serum, and in each another well, place 10 μL of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution, 10 μL of human serum and 10 μL of human urine, respectively, and allow to stand for overnight: a precipitin line appears between anti-urokinase serum and urokinase, and not appears between anti-urokinase serum and human serum or human urine.

α-Apoxytetracycline C₂₂H₂₂N₂O₈ Yellow-brown to green powder.

Melting point <2.60>: 200–205°C

β-Apoxytetracycline C₂₂H₂₂N₂O₈ Yellow-brown to brown powder.

Purity Related substances—Dissolve 8 mg of β-apoxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as the sample solution. Proceed the test with 20 μL of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than β-apoxytetracycline is not more than 10%.

Aprotinin A clear and colorless liquid containing aprotinin extracted from the lung or parotid gland of a healthy cattle. The pH is between 5.0 and 7.0.

Content: not less than 15,000 KIE Units and not more than 25,000 KIE Units of aprotinin per mL. **Assay—**(i) Trypsin solution: Weigh an amount of crystallized trypsin equivalent to about 250 FIP Units of trypsin according to the labeled FIP Units, and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 10 mL. Prepare before use, and preserve in ice. (ii) Sample solution: Dilute a suitable quantity of aprotinin with sodium tetraborate-calcium chloride buffer solution, pH 8.0 so that each mL of the solution contains 800 KIE Units of aprotinin, and use this solution as the sample solution. (iii) Apparatus: Use a glass bottle as a reaction reservoir, 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver-silver chloride electrode, a nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature of the bath at $25 \pm 0.1^\circ\text{C}$ by means of a precise thermoregulator. (iv) Procedure: To 5.0 mL of *N*- α -benzoyl-L-arginine ethyl ester TS add 45.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, and use this solution as the substrate solution. Pipet 1 mL of the trypsin solution, add sodium tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution as the test solution I. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution I previously allowed to stand at $25 \pm 0.1^\circ\text{C}$ for 10 minutes, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50- μL micropipet (minimum graduation of 1 μL), while stirring, to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 6 minutes. Separately, pipet 2 mL of the trypsin solution and 1 mL of the sample solution, add sodium tetraborate-calcium chloride buffer solution, pH 8.0 to make exactly 10 mL, and use this solution as the test solution II. Transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add exactly 1 mL of the test solution II, previously allowed to stand at $25 \pm 0.1^\circ\text{C}$ for 10 minutes, and proceed in the same manner. Separately, transfer 10.0 mL of the substrate solution to the reaction reservoir, adjust the pH of the solution to 8.00, while stirring and passing a current of nitrogen, add 1 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, previously allowed to stand at $25 \pm 0.1^\circ\text{C}$ for 10 minutes, and perform a blank determination in the same manner. (v) Calculation: Plot the amount of consumption (μL) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, t_1 and t_2 , designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v_1 and v_2 , respectively, and designate μmol of sodium hydroxide consumed per minute as M .

$$M (\mu\text{mol NaOH}/\text{min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10} \times f$$

f : Factor of 0.1 mol/L sodium hydroxide VS

$$\begin{aligned} &\text{KIE Units per mL of aprotinin to be tested} \\ &= \frac{2(M_A - M_0) - (M_B - M_0)}{L} \times n \times 32.5 \end{aligned}$$

L : Amount (mL) of the sample solution added to the test solution II

n : Dilution coefficient of aprotinin to be tested

M_A : μmol of sodium hydroxide consumed in 1 minute when the test solution I is used

M_B : μmol of sodium hydroxide consumed in 1 minute when the test solution II is used

M_0 : μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

32.5: Equivalent coefficient for calculation of KIE Units from FIP Units

One KIE Unit means an amount of aprotinin making a reduction of 50% off the potency of 2 Units of kallidinogenase at pH 8.0 and room temperature for 2 hours.

Storage—Preserve in a light-resistant, hermetic container and in a cold place.

Aprotinin TS Measure an appropriate amount of aprotinin, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 50 KIE Units per mL.

Aqua regia Add 1 volume of nitric acid to 3 volumes of hydrochloric acid. Prepare before use.

L-Arabinose $\text{C}_5\text{H}_{10}\text{O}_5$ [K 8054: 1991, L(+)-Arabinose, Special class]

Arbutin for component determination Use arbutin for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (280 nm): 70 – 76 [4 mg, previously dried in a desiccator (in vacuum, silica gel), 12 hours, water, 100 mL].

Purity Related substances—Dissolve 40 mg of arbutin for component determination in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than that of arbutin from the sample solution is not larger than the peak area of arbutin from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Component determination under Bearberry Leaf except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add water to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of arbutin obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of arbutin obtained from 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of arbutin beginning after the solvent peak.

Arbutin for thin-layer chromatography $\text{C}_{12}\text{H}_{16}\text{O}_7 \cdot n\text{H}_2\text{O}$ Colorless to white crystals or crystalline powder, and odorless. Freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in ethyl ace-

tate and in chloroform.

Melting point <2.60>: 199 – 201°C

Purity Related substances—Dissolve 1.0 mg of arbutin for thin-layer chromatography in exactly 1 mL of a mixture of ethanol (95) and water (7:3). Perform the test with 20 μ L of this solution as directed in the Identification (2) under Bearberry Leaf: any spot other than the main spot at the *R_f* value of about 0.4 does not appear.

Arecoline hydrobromide for thin-layer chromatography C₈H₁₃NO₂·HBr White crystals. Freely soluble in water, soluble in methanol, and practically insoluble in diethyl ether.

Melting point <2.60>: 169 – 171°C

Purity Related substances—Dissolve 50 mg of arecoline hydrobromide for thin-layer chromatography in exactly 10 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification under Areca: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

L-Arginine C₆H₁₄N₄O₂ White, crystals or crystalline powder. It has a characteristic odor.

Optical rotation <2.49> [α]_D²⁰: +26.9 – +27.9° (After drying, 4 g, 6 mol/L hydrochloric acid TS, 50 mL, 200 mm).

Loss on drying <2.41>: not more than 0.50% (1 g, 105°C, 3 hours).

Content: not less than 98.0% and not more than 102.0%. **Assay**—Weigh accurately about 0.15 g of L-arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes to green through yellow (indicator: 10 drops of *p*-naphtholbenzein TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.710 mg of C₆H₁₄N₄O₂

L-Arginine hydrochloride C₆H₁₄N₄O₂·HCl [Same as the namesake monograph]

Aristolochic acid I for crude drugs purity test

C₁₇H₁₁NO₇ Yellow crystalline powder. Melting point: about 280°C (with decomposition).

Absorbance <2.24> E_{1cm}^{1%} (318 nm): 384 – 424 (1 mg, methanol, 100 mL).

Purity Related substances—Dissolve 1.0 mg of aristolochic acid I for crude drugs purity test in 100 mL of diluted methanol (3 in 4), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than aristolochic acid I obtained from the sample solution is not more than the peak area of aristolochic acid I from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3) under Asiasarum Root.

Time span of measurement: About 3 times as long as the

retention time of aristolochic acid I beginning after the solvent peak.

System suitability

Proceed as directed in the system suitability in the Purity (3) under Asiasarum Root.

Arsenazo III C₂₂H₁₈As₂N₄O₁₄S₂ [K 9524]

Arsenazo III TS Dissolve 0.1 g of arsenazo III in water to make 50 mL.

Arsenic-free zinc See zinc for arsenic analysis.

Arsenic (III) trioxide As₂O₃ [K 8044, Arsenic (III) trioxide, Special class]

Arsenic (III) trioxide TS Add 1 g of arsenic (III) trioxide to 30 mL of a solution of sodium hydroxide (1 in 40), dissolve by heating, cool, and add gently acetic acid (100) to make 100 mL.

Arsenic trioxide See arsenic (III) trioxide.

Arsenic trioxide TS See arsenic (III) trioxide TS.

Ascorbic acid See L-ascorbic acid.

L-Ascorbic acid C₆H₈O₆ [K 9502, L(+)-Ascorbic Acid, Special class]

Ascorbic acid for iron limit test See L-ascorbic acid.

0.012 g/dL L-Ascorbic acid-hydrochloric acid TS Dissolve 15 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

0.02 g/dL L-Ascorbic acid-hydrochloric acid TS Dissolve 25 mg of L-ascorbic acid in 25 mL of methanol, add carefully 100 mL of hydrochloric acid, and mix. Prepare before use.

0.05 g/dL L-Ascorbic acid-hydrochloric acid TS Dissolve 0.05 g of L-ascorbic acid in 30 mL of methanol, add carefully hydrochloric acid to make 100 mL. Prepare before use.

DL-Aspartic acid C₄H₇NO₄ A white crystalline powder that is sparingly soluble in water. Melting point: 270 to 271°C.

L-Aspartic acid C₄H₇NO₄ [K 9045, Special class]

Aspartic acid See L-aspartic acid.

Aspirin C₉H₈O₄ [Same as the namesake monograph]

Astragaloside IV for thin-layer chromatography

C₄₁H₆₈O₁₄ A white powder. Sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in water.

Optical rotation <2.49> [α]_D²⁰: +19 – +26° (10 mg dried with silica gel for 24 hours, methanol, 2 mL, 50 mm).

Purity Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 5 μ L of the standard solution as directed in the Identification (4) under Hochuekkito Extract: no spot appears other than the principal spot of around *R_f* 0.5.

Atractylenolide III for thin-layer chromatography C₁₅H₂₀O₃ White crystals or crystalline powder. Very soluble in methanol, freely soluble in ethanol (99.5), and practically insoluble in water. Melting point: 193 – 196°C

Identification—Determine the absorption spectrum of a

solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 217 nm and 221 nm.

Purity Related substances—Dissolve 1 mg in 10 mL of methanol. Proceed the test with 2 μ L of the standard solution as directed in the Identification (3) under Kamishoyosan Extract: no spot appears other than the principal spot of around Rf 0.5

Atropine sulfate ($C_{17}H_{23}NO_3$)₂·H₂SO₄·H₂O [Same as the monograph Atropin sulfate Hydrate]

Atropine sulfate for assay [Same as the monograph Atropine Sulfate Hydrate. When dried, it contains not less than 99.0% of atropine sulfate [($C_{17}H_{23}NO_3$)₂·H₂SO₄].]

Atropine sulfate for thin-layer chromatography Use atropine sulfate for assay meeting the following additional specifications. Weigh accurately about 50 mg of atropine sulfate for assay, dissolve in ethanol (95) to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L of the solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and diethylamine (9:1) to a distance of about 10 cm, air-dry the plate, and spray evenly chloroplatinic acid-potassium iodide TS on the plate: any spot other than the spot at the Rf value of about 0.4 does not appear.

A-type erythrocyte suspension Prepare a suspension containing 1 vol% of erythrocyte separated from human A-type blood in isotonic sodium chloride solution.

Baicalin for thin-layer chromatography $C_{21}H_{18}O_{11}$ ·H₂O Light yellow odorless powder. Slightly soluble in methanol, and practically insoluble in water and in diethyl ether. Melting point: about 206°C (with decomposition).

Purity Related substance—Dissolve 1.0 mg of baicalin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification (2) under Scutellaria Root: any spot other than the principal spot at the Rf value of about 0.4 does not appear.

Balsam Canada balsam for microscopy. Before use, dilute to a suitable concentration with xylene.

Bamethan sulfate ($C_{12}H_{19}NO_2$)₂·H₂SO₄ [Same as the namesake monograph]

Barbaloin for component determination Use barbaloin for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (360 nm): 260 – 290 [10 mg dried in a desiccator (in vacuum, phosphorus (V) oxide) for not less than 24 hours, methanol, 500 mL.]

Purity Related substances—Dissolve 10 mg of the substance to be tested in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20 μ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration

method: the total area of the peaks other than barbaloin from the sample solution is not larger than the peak area of barbaloin from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Component determination under Aloe except wavelength, detection sensitivity and time span of measurement.

Wavelength: 300 nm

Detection sensitivity: Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of barbaloin obtained from 20 μ L of the standard solution (2) can be measured by the automatic integration method and the peak height of barbaloin obtained from 20 μ L of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of barbaloin beginning after the solvent peak.

Barbaloin for thin-layer chromatography $C_{21}H_{22}O_9$ Light yellow, crystalline powder. Freely soluble in methanol, practically insoluble in water and in diethyl ether.

Melting point <2.60>: 148°C

Purity Related substances—Dissolve 1.0 mg of barbaloin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 20 μ L of this solution as directed in the Identification (2) under Aloe: any spot other than the principal spot at the Rf value of about 0.6 does not appear.

Barbital $C_8H_{12}N_2O_3$ [Same as the namesake monograph]

Barbital buffer solution Dissolve 15 g of barbital sodium in 700 mL of water, adjust the pH to 7.6 with dilute hydrochloric acid, and filter.

Barbital sodium $C_8H_{11}N_2NaO_3$ White, odorless crystals of crystalline powder, having a bitter taste. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

pH <2.54>—The pH of a solution of barbital sodium (1 in 200) is between 9.9 and 10.3.

Loss on drying <2.41>: not more than 1.0% (1 g, 105°C, 4 hours).

Content: not less than 98.5%. **Assay**—Weigh accurately about 0.5 g of barbital sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform. Then extract with three 25-mL portions of chloroform, combine the total extract, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Combine the chloroform extracts, and filter into a conical flask. Wash the filter paper with three 5-mL portions of chloroform, combine the filtrate and the washings, add 10 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow to purple through light purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 20.62 mg of $C_8H_{11}N_2NaO_3$

Barium chloride See barium chloride dihydrate.

Barium chloride dihydrate $BaCl_2 \cdot 2H_2O$ [K 8155, Spec-

ial class]

Barium chloride TS Dissolve 12 g of barium chloride dihydrate in water to make 100 mL (0.5 mol/L).

Barium hydroxide See barium hydroxide octahydrate.

Barium hydroxide octahydrate $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$
[K 8577, Special class] Store in tightly stoppered containers.

Barium hydroxide TS Saturate barium hydroxide octahydrate in freshly boiled and cooled water (0.25 mol/L). Prepare before use.

Barium nitrate $\text{Ba}(\text{NO}_3)_2$ [K 8565, Special class]

Barium nitrate TS Dissolve 6.5 g of barium nitrate in water to make 100 mL (0.25 mol/L).

Barium oxide BaO A white to yellowish or grayish white, powder.

Identification—(1) Dissolve 0.5 g in 15 mL of water and 5 mL of hydrochloric acid, and add 10 mL of dilute sulfuric acid: white precipitates appear.

(2) Perform the test as directed under Flame Coloration Test (1) <1.04>: a green color appears.

Barium perchlorate $\text{Ba}(\text{ClO}_4)_2$ [K 9551, Special class]

Becanamycin sulfate [Same as the namesake monograph]

Beclometasone dipropionate $\text{C}_{28}\text{H}_{37}\text{ClO}_7$ [Same as the namesake monograph]

Benidipine hydrochloride $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$ [Same as the namesake monograph]

Benidipine hydrochloride for assay $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$ [Same as the monograph Benidipine Hydrochloride. When dried, it contains not less than 99.5% of benidipine hydrochloride ($\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_6 \cdot \text{HCl}$)]

Benzaldehyde $\text{C}_6\text{H}_5\text{CHO}$ [K 8857, First class]

Benzalkonium chloride [Same as the namesake monograph]

Benzalphthalide $\text{C}_{15}\text{H}_{10}\text{O}_2$ Yellow crystalline powder. Melting point: 99 – 102°C.

Benzene C_6H_6 [K 8858, Special class]

Benzethonium chloride for assay $\text{C}_{27}\text{H}_{42}\text{ClNO}_2$ [Same as the monograph Benzethonium Chloride. When dried, it contains not less than 99.0% of benzethonium chloride ($\text{C}_{27}\text{H}_{42}\text{ClNO}_2$)]

Benzoic acid $\text{C}_6\text{H}_5\text{COOH}$ [K 8073, Special class]

Benzoin $\text{C}_6\text{H}_5\text{CH}(\text{OH})\text{COC}_6\text{H}_5$ White to pale yellow, crystals or powder.

Melting point <2.60>: 132 – 137°C

Benzophenone $\text{C}_6\text{H}_5\text{COC}_6\text{H}_5$ Colorless crystals, having a characteristic odor.

Melting point <2.60>: 48 – 50°C

p-Benzoquinone $\text{C}_6\text{H}_4\text{O}_2$ Yellow to yellow-brown, crystals or crystalline powder, having a pungent odor. Soluble in ethanol (95) and in diethyl ether, slightly soluble in water. It is gradually changed to a blackish brown color by light.

Melting point <2.60>: 111 – 116°C

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.1 g of p-benzoquinone, place in an iodine bottle, add exactly 25 mL of water and 25 mL of diluted sulfuric acid (1 in 15), dissolve 3 g of potassium iodide by shaking, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 5.405 mg of $\text{C}_6\text{H}_4\text{O}_2$

p-Benzoquinone TS Dissolve 1 g of p-benzoquinone in 5 mL of acetic acid (100), and add ethanol (95) to make 100 mL.

N- α -Benzoyl-L-arginine ethyl ester hydrochloride
 $\text{C}_{15}\text{H}_{22}\text{N}_4\text{O}_3 \cdot \text{HCl}$ White crystals or crystalline powder. Freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Melting point <2.60>: 129 – 133°C

Optical rotation <2.49> $[\alpha]_D^{20}$: –15.5 – –17.0° (2.5 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.1 g of N- α -benzoyl-L-arginine ethyl ester hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Related substances—Weigh 0.10 g of N- α -benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 6 mL of water, add 4 mL of hydrochloric acid, heat in a boiling water bath for 5 minutes to decompose, and use this solution as the sample solution. Perform the test with the sample solution as directed under Paper Chromatography. Spot 5 μL of the sample solution on a chromatographic filter paper. Develop with a mixture of water, acetic acid (100) and 1-butanol (5:4:1) to a distance of about 30 cm, and air-dry the paper. Spray evenly a solution of ninhydrin in acetone (1 in 50) upon the paper, and heat at 90°C for 10 minutes: only one purple spot appears.

Content: not less than 99.0%. **Assay**—Weigh accurately about 0.6 g of N- α -benzoyl-L-arginine ethyl ester hydrochloride, dissolve in 50 mL of water, neutralize with 0.1 mol/L sodium hydroxide VS, if necessary, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 4 drops of dichlorofluorescein TS).

Each mL of 0.1 mol/L silver nitrate VS
= 34.28 mg of $\text{C}_{15}\text{H}_{22}\text{N}_4\text{O}_3 \cdot \text{HCl}$

N- α -Benzoyl-L-arginine ethyl ester TS Dissolve 0.07 g of N- α -benzoyl-L-arginine ethyl ester hydrochloride in freshly boiled and cooled water to make exactly 10 mL.

N- α -Benzoyl-L-arginine-4-nitroanilide hydrochloride
 $\text{C}_{19}\text{H}_{22}\text{N}_6\text{O}_4 \cdot \text{HCl}$ Light yellow crystalline powder.

Optical rotation <2.49> $[\alpha]_D^{20}$: +45.5 – +48.0° (after drying, 0.5 g, N,N-dimethylformamide, 25 mL, 100 mm).

Purity Related substances—Dissolve 0.20 g of N- α -benzoyl-L-arginine-4-nitroanilide hydrochloride in 10 mL of N,N-dimethylformamide, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Exposure the plate to a vapor of iodine: only one spot appears.

N- α -Benzoyl-L-arginine-4-nitroanilide TS Dissolve 0.1 g

of *N*- α -benzoyl-L-arginine-4-nitroanilide hydrochloride in water to make 100 mL.

Benzoyl chloride C_6H_5COCl [K 8158, Special class]

***N*-Benzoyl-L-isoleucyl-L-glutamyl(γ -OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride** An equal amount mixture of two components, R = H and R = CH₃. A white powder. Slightly soluble in water.

Absorbance $\langle 2.24 \rangle E_{1\text{cm}}^{1\%}$ (316 nm): 166 – 184 (10 mg, water, 300 mL).

Benzoylmesaconine hydrochloride for thin-layer chromatography $C_{31}H_{43}NO_{10} \cdot HCl \cdot xH_2O$ White, crystals or crystalline powder. Soluble in water and in ethanol (99.5). Melting point: about 250°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in exactly 10 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Proceed the test as directed in the Identification under Processed Aconite Root: no spot other than the principal spot at around *R_f* 0.4 appears.

Benzoyl peroxide, 25% water containing $(C_6H_5CO)_2O_2$ White moist crystals or powder. Soluble in diethyl ether and in chloroform, and very slightly soluble in water and in ethanol (95). Melting point: 103 – 106°C (dried substance) (with decomposition).

Loss on drying $\langle 2.41 \rangle$: not more than 30% (0.1 g, in vacuum, silica gel, constant mass).

Benzyl alcohol $C_6H_5CH_2OH$ [K 8854, Special class]

Benzyl benzoate $C_6H_5COOCH_2C_6H_5$ [K 8079, Special class]

Benzyl parahydroxybenzoate $HOC_6H_4COOCH_2C_6H_5$ White, odorless, fine crystals or crystalline powder. Freely soluble in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

Melting point $\langle 2.60 \rangle$: 109 – 112°C

Residue on ignition $\langle 2.44 \rangle$: not more than 0.1%.

Content: not less than 99.0%. **Assay**—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS
= 228.2 mg of $C_{14}H_{12}O_3$

Benzylpenicillin Bezyathine $C_{48}H_{64}N_6O_{12}S_2$ [same as the monograph Benzylpenicillin Benzathine Hydrate]

Benzylpenicillin potassium $C_{16}H_{17}KN_2O_4S$ [Same as the monograph Benzylpenicillin Potassium]

Benzyl *p*-hydroxybenzoate See benzyl parahydroxybenzoate.

***p*-Benzylphenol** $C_6H_5CH_2C_6H_4OH$ White to pale yellowish white crystals or crystalline powder.

Melting point $\langle 2.60 \rangle$: 80 – 85°C

Berberine chloride $C_{20}H_{18}ClNO_4 \cdot xH_2O$ [Same as the monograph Berberine chloride Hydrate]

Berberine chloride for thin-layer chromatography [Same as the monograph Berberine Chloride Hydrate. Use the berberine chloride meeting the following additional spec-

ifications.]

Purity Related substances—Dissolve 10 mg of berberine chloride for thin-layer chromatography in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification (2) under Phellodendron Bark: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Bergenin for thin-layer chromatography

$C_{14}H_{16}O_9 \cdot xH_2O$ White crystals or crystalline powder. Freely soluble in ethanol (95), soluble in water, and insoluble in diethyl ether.

Melting point $\langle 2.60 \rangle$: 131 – 133°C, 234 – 236°C (double melting points).

Purity Related substances—Dissolve 1.0 mg of bergenin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 20 μL of this solution as directed in the Identification under Mallotus Bark: any spot other than the principal spot at the *R_f* value of about 0.5 does not appear.

Betahistine mesilate $C_8H_{12}N_2 \cdot 2CH_4O_3S$ [Same as the namesake monograph]

Betahistine mesilate for assay [Same as the monograph Betahistine Mesilate. When dried, it contains not less than 99.0% of betahistine mesilate ($C_8H_{12}N_2 \cdot 2CH_4O_3S$).]

Betanidine sulfate for assay $(C_{10}H_{15}N_3)_2 \cdot H_2SO_4$ [Same as the monograph Betanidine Sulfate. Calculated on the dried basis, it contains not less than 99.0% of betanidine sulfate ($C_{10}H_{15}N_3)_2 \cdot H_2SO_4$.]

Bezafibrate for assay $C_{19}H_{20}ClNO_4$ [Same as the monograph Bezafibrate. When dried it contains not less than 99.0% of bezafibrate ($C_{19}H_{20}ClNO_4$).]

BGLB Dissolve 10 g of peptone and 10 g of lactose monohydrate in 500 mL of water, add 200 mL of fresh ox bile or a solution prepared by dissolving 20 g of dried ox bile powder in 200 mL of water and adjusted the pH to between 7.0 and 7.5, then add water to make 975 mL, and again adjust to pH 7.4. Then add 13.3 mL of a solution of brilliant green (1 in 1000) and water to make 1000 mL in total volume, and filter through absorbent cotton. Dispense 10 mL portions of the filtrate into tubes for fermentation, and sterilize by autoclaving at 121°C for not more than 20 minutes, then cool quickly, or sterilize fractionally on each of three successive days for 30 minutes at 100°C.

α -BHC (α -Hexachlorocyclohexane) $C_6H_6Cl_6$

Melting point $\langle 2.60 \rangle$: 157 – 159°C

Purity Related substances—Dissolve 10 mg of α -BHC in 5 mL of acetone for purity of crude drug, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, and measure each peak area from these

solutions by the automatic integration method: the total peak area other than α -BHC from the sample solution is not larger than the peak area of α -BHC from the standard solution (1).
Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make 20 mL, and use this solution as standard solution (2). Adjust the detection sensitivity so that the peak area of α -BHC obtained from 1 mL of the standard solution (2) can be measured by the automatic integration method, and the peak height of α -BHC from 1 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of α -BHC beginning after the peak of solvent.

β -BHC (β -Hexachlorocyclohexane) $C_6H_6Cl_6$

Melting point <2.60>: 308 – 310°C

Purity Related substances—Proceed as directed in the Purity under α -BHC using the following standard solution (1).

Standard solution (1): Pipet 2 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

γ -BHC (γ -Hexachlorocyclohexane) $C_6H_6Cl_6$

Melting point <2.60>: 112 – 114°C

Purity Related substances—Proceed as directed in the Purity under α -BHC.

δ -BHC (δ -Hexachlorocyclohexane) $C_6H_6Cl_6$

Melting point <2.60>: 137 – 140°C

Purity Related substances—Proceed as directed in the Purity under α -BHC using the following standard solution (1).

Standard solution (1): Pipet 5 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

2-(4-Biphenyl)propionic acid $C_{15}H_{14}O_2$ Light yellowish white powder.

Melting point <2.60>: 145 – 148°C

Purity—Dissolve 1 mg of 2-(4-biphenyl) propionic acid in a mixture of water and acetonitrile (11:9) to make 50 mL. Perform the test with 20 μ L of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions of the Related substances in the Purity (3) under Flurbiprofen. Determine each peak area of the solution in about twice as long as the retention time of the main peak by the automatic integration method, and calculate the amount of 2-(4-biphenyl)propionic acid by the area percentage method: it is not less than 98.0%.

Content: not less than 98.0%. Assay—Weigh accurately about 0.5 g of 2-(4-biphenyl)propionic acid, previously dried in vacuum over silica gel for 4 hours, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 22.63 mg of $C_{15}H_{14}O_2$

2,2'-Bipyridyl $C_{10}H_8N_2$ [K 8486, Special class]

4,4'-Bis(diethylamino)benzophenone

(C_2H_5)₂NC₆H₄)₂CO Light yellow crystals.

Content: not less than 98%. Assay—Weigh accurately 0.25 g of 4,4'-bis(diethylamino)benzophenone, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank titration in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.22 mg of $C_{21}H_{28}N_2O$

***N,N'*-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide** $C_{16}H_{20}I_3N_3O_8$

White crystalline powder.

Identification—(1) Heat 0.1 g of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide over free flame: a purple colored gas evolves.

(2) Determine the infrared absorption spectrum of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390 cm^{-1} , 3230 cm^{-1} , 2882 cm^{-1} , 1637 cm^{-1} , 1540 cm^{-1} , 1356 cm^{-1} and 1053 cm^{-1} .

Purity—Dissolve 0.10 g of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total area of the peaks other than the peak of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide obtained from the sample solution is not more than 3 times of the peak area of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetylamino-2,4,6-triiodoisophthalamide obtained from the standard solution.

Operating conditions

Proceed the operating conditions in the Purity (6) under Iopamidol.

System suitability

Proceed the system suitability in the Purity (6) under Iopamidol.

Bismuth nitrate See bismuth nitrate pentahydrate.

Bismuth nitrate pentahydrate $Bi(NO_3)_3 \cdot 5H_2O$
[K 8566, Special class]

Bismuth nitrate-potassium iodide TS Dissolve 0.35 g of bismuth nitrate pentahydrate in 4 mL of acetic acid (100) and 16 mL of water (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). To 20 mL of a mixture of solution A and solution B (1:1) add 80 mL of dilute sulfuric acid and 0.2 mL of hydrogen peroxide (30). Prepare before use.

Bismuth nitrate TS Dissolve 5.0 g of bismuth nitrate pentahydrate in acetic acid (100) to make 100 mL.

Bismuth potassium iodide TS Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate,

shake for 1 hour, add 20 mL of a solution of potassium iodide (2 in 5), shake thoroughly, allow to stand for 24 hours, and filter (solution A). Separately, dissolve 10 g of L-tartaric acid in 50 mL of water, add 5 mL of solution A, and preserve in a light-resistant, glass-stoppered bottle.

Bismuth sodium trioxide NaBiO_3 [K 8770, Special class]

Bismuth subnitrate [Same as the namesake monograph]

Bismuth subnitrate TS Dissolve 10 g of L-tartaric acid in 40 mL of water, add 0.85 g of bismuth subnitrate, stir for 1 hour, then add 20 mL of a solution of potassium iodide (2 in 5), and shake well. After standing for 24 hours, filter, and preserve the filtrate in a light-resistant bottle.

Bismuth sulfite indicator Prepared for microbial test.

Bis-(1-phenyl-3-methyl-5-pyrazolone) $\text{C}_{20}\text{H}_{18}\text{B}_4\text{O}_2$
White to pale yellow crystals or crystalline powder. It dissolves in mineral acids and in alkali hydroxides, and it does not dissolve in water, in ammonia TS, or in organic solvents. Melting point: not below 300°C .

Nitrogen content <1.08>: 15.5 – 16.5%

Residue on ignition <2.44>: not more than 0.1%.

Bis-trimethyl silyl acetamide $\text{CH}_3\text{CON}[\text{Si}(\text{CH}_3)_2]$
Colorless liquid.

Boiling point <2.57>: $71 - 73^\circ\text{C}$

Refractive index <2.45> n_D^{20} : 1.414 – 1.418

Specific gravity <2.56> d_{20}^{20} : 0.825 – 0.835

Block buffer solution Dissolve 4 g of blocking agent in 100 mL of water, and add 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4.

Blocking agent Powder whose main ingredient is bovine-derived lactoprotein. For immunological research purposes.

Blue tetrazolium $\text{C}_{40}\text{H}_{32}\text{Cl}_2\text{N}_8\text{O}_2$ 3,3'-Dianisole-bis-[4,4'-(3,5-diphenyl) tetrazolium chloride] Light yellow crystals. Freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in water, and practically insoluble in acetone and in ether. Melting point: about 245°C (with decomposition).

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (252 nm): not less than 826 (methanol).

Blue tetrazolium TS, alkaline To 1 volume of a solution of blue tetrazolium in methanol (1 in 200) add 3 volumes of a solution of sodium hydroxide in methanol (3 in 25). Prepare before use.

Borate-hydrochloric acid buffer solution, pH 9.0 Dissolve 19.0 g of sodium borate in 900 mL of water, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Borax See sodium tetraborate decahydrate.

Boric acid H_3BO_3 [K 8863, Special class]

Boric acid-methanol buffer solution Weigh exactly 2.1 g of boric acid, dissolve in 28 mL of sodium hydroxide TS, and dilute with water to exactly 100 mL. Mix equal volumes of this solution and methanol, and shake.

Boric acid-potassium chloride-sodium hydroxide buffer so-

lution, pH 9.0 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 21.30 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.2 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 26.70 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.6 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 36.85 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

Boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0 To 50 mL of 0.2 mol/L boric acid-0.2 mol/L potassium chloride TS for buffer solution add 43.90 mL of 0.2 mol/L sodium hydroxide VS and water to make 200 mL.

0.2 mol/L Boric acid-0.2 mol/L potassium chloride TS for buffer solution Dissolve 12.376 g of boric acid and 14.911 g of potassium chloride in water to make 1000 mL.

Boric acid-sodium hydroxide buffer solution, pH 8.4 Dissolve 24.736 g of boric acid in 0.1 mol/L sodium hydroxide VS to make exactly 1000 mL.

Boron trifluoride BF_3 Colorless gas, having an irritating odor.

Boiling point <2.57>: -100.3°C

Melting point <2.60>: -127.1°C

Boron trifluoride-methanol TS A solution containing 14 w/v% of boron trifluoride (BF_3 ; 67.81) in methanol.

Bovine activated blood coagulation factor X A protein obtained from bovine plasma. It has an activity to decompose prothrombin specifically and limitedly and produce thrombin. It does not contain thrombin and plasmin. It contains not less than 500 Units per mg protein. One unit indicates an amount of the factor X which hydrolyzes $1\ \mu\text{mol}$ of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ -OR)-glycyl-L-arginyl-*p*-nitroanilide in 1 minute at 25°C .

Bovine serum Serum obtained from blood of bovine. Interleukin-2 dependent cell growth suppression substance is removed by heat at 56°C for 30 min before use

Bovine serum albumin Obtained from cattle serum as Cohn's fifth fraction. Contains not less than 95% of albumin.

Bovine serum albumin for assay White or yellowish crystals or crystalline powder.

Take 50 mg of bovine serum albumin containing 99% or more albumin in glass ampoules and put them in the desiccator, whose humidity is adjusted to 31%RH at 25°C with calcium chloride-saturated solution, for 2 weeks, and then take out and seal them immediately.

Protein content: 88% or more. Assay—Weigh accurately about 0.1 g of bovine serum albumin for assay, dissolve in water, and add water to make exactly 20 mL. Put exactly 3 mL of the solution in the Kjeldahl flask, and determine protein content following Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.8754 mg protein

Storage—Store at 4°C or lower.

Bovine serum albumin for test of ulinastatin White crystalline powder obtained from bovine serum by a purification method which does not denature albumin and other serum proteins. It contains not less than 99% of albumin.

Bovine serum albumin-isotonic sodium chloride solution Dissolve 0.1 g of bovine serum albumin in isotonic sodium chloride solution to make 100 mL. Prepare before use.

1 w/v% Bovine serum albumin-phosphate buffer-sodium chloride TS Dissolve 1 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4.

Bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2 Dissolve 10.75 g of disodium hydrogen phosphate dodecahydrate, 7.6 g of sodium chloride and 1.0 g of bovine serum albumin in water to make 1000 mL. Adjust to pH 7.2 with dilute sodium hydroxide TS or diluted phosphoric acid (1 in 10) before use.

Bovine serum albumin TS for secretin Dissolve 0.1 g of bovine serum albumin, 0.1 g of L-cysteine hydrochloride monohydrate, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

Bovine serum albumin TS for Secretin Reference Standard Dissolve 0.1 g of bovine serum albumin, 0.8 g of L-alanine, 0.01 g of citric acid monohydrate, 0.14 g of disodium hydrogen phosphate dodecahydrate and 0.45 g of sodium chloride in 100 mL of water for injection.

Bradykinin $C_{50}H_{73}N_{15}O_{11}$ A white powder. Freely soluble in water and in acetic acid (31), and practically insoluble in diethyl ether.

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: $-80 - -90^\circ$ (15 mg, water, 5 mL, 100 mm).

Purity Related substances—Dissolve 2.0 mg of bradykinin in 0.2 mL of water, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography $\langle 2.03 \rangle$. Spot 5 μ L of the sample solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (31) (15:12:10:3) to a distance of about 10 cm, and dry the plate at 60°C. Spray evenly a solution of ninhydrin in 1-butanol (1 in 1000) on the plate, and heat at 60°C for 30 to 60 minutes: any spot other than the principal spot arisen from bradykinin does not appear.

Brilliant green $C_{27}H_{34}N_2O_4S$ Fine, glistening, yellow crystals. It dissolves in water and in ethanol (95). The wavelength of absorption maximum: 623 nm.

Bromine Br [K 8529, Special class]

Bromine-acetic acid TS Dissolve 10 g of sodium acetate trihydrate in acetic acid (100) to make 100 mL, add 5 mL of bromine, and shake. Preserve in light-resistant containers, preferably in a cold place.

Bromine-carbon tetrachloride TS To 0.1 g of bromine add carbon tetrachloride to make 100 mL, and dilute a 2 mL portion of this solution with carbon tetrachloride to make 100 mL. Prepare before use.

Bromine-cyclohexane TS Dissolve 0.1 g of bromine in cy-

clohexane to make 100 mL. To 2 mL of this solution add cyclohexane to make 10 mL. Prepare before use.

Bromine-sodium hydroxide TS To 100 mL of a solution of sodium hydroxide (3 in 100) add 0.2 mL of bromine. Prepare before use.

Bromine TS Prepare by saturating water with bromine as follows: Transfer 2 to 3 mL of bromine to a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum, add 100 mL of cold water, insert the stopper, and shake. Preserve in light-resistant containers, preferably in a cold place.

Bromocresol green $C_{21}H_{14}Br_4O_5S$ [K 8840, Special class]

Bromocresol green-crystal violet TS Dissolve 0.3 g of bromocresol green and 75 mg of crystal violet in 2 mL of ethanol (95), and dilute with acetone to make 100 mL.

Bromocresol green-methyl red TS Dissolve 0.15 g of bromocresol green and 0.1 g of methyl red in 180 mL of ethanol (99.5), and add water to make 200 mL.

Bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS To 0.25 g of bromocresol green add 15 mL of water and 5 mL of dilute sodium hydroxide TS, then add a small quantity of acetic acid-sodium acetate buffer solution, pH 4.5, dissolve while shaking, and add acetic acid-sodium acetate buffer solution, pH 4.5, to make 500 mL. Wash 250 mL of the solution with two 100 mL portions of dichloromethane. Filter if necessary.

Bromocresol green-sodium hydroxide-ethanol TS Dissolve 50 mg of bromocresol green in 0.72 mL of 0.1 mol/L sodium hydroxide VS and ethanol (95), and add water to make 100 mL.

Bromocresol green-sodium hydroxide TS Triturate 0.2 g of bromocresol green with 2.8 mL of 0.1 mol/L sodium hydroxide VS in a mortar, add water to make 200 mL, and filter if necessary.

Bromocresol green TS Dissolve 0.05 g of bromocresol green in 100 mL of ethanol (95), and filter if necessary.

Bromocresol purple $C_{21}H_{16}Br_2O_5S$ [K 8841, Special class]

Bromocresol purple-dipotassium hydrogenphosphate-citric acid TS Mix 30 mL of bromocresol purple-sodium hydroxide TS and 30 mL of dibasic potassium phosphate-citric acid buffer solution, pH 5.3, and wash with three 60-mL portions of chloroform.

Bromocresol purple-sodium hydroxide TS Triturate 0.4 g of bromocresol purple with 6.3 mL of dilute sodium hydroxide TS in a mortar, add water to make 250 mL, and filter if necessary.

Bromocresol purple TS Dissolve 0.05 g of bromocresol purple in 100 mL of ethanol (95), and filter if necessary.

Bromophenol blue $C_{19}H_{10}Br_4O_5S$ [K 8844, Special class]

Bromophenol blue-potassium biphthalate TS Dissolve 0.1 g of bromophenol blue in potassium biphthalate buffer solution, pH 4.6, to make 100 mL.

Bromophenol blue TS Dissolve 0.1 g of bromophenol blue in 100 mL of dilute ethanol, and filter if necessary.

0.05% Bromophenol blue TS Dissolve 0.01 g of bromophenol blue in water to make 20 mL.

Bromophenol blue TS, dilute Dissolve 0.05 g of bromophenol blue in 100 mL of ethanol (99.5). Prepare before use.

Bromophenol blue TS, pH 7.0 Mix 10 mL of bromophenol blue TS and 10 mL of ethanol (95), and adjust the pH to 7.0 with dilute sodium hydroxide TS.

***N*-Bromosuccinimide** $C_4H_4BrNO_2$ [K 9553, Special class]

***N*-Bromosuccinimide TS** Dissolve 1 g of *N*-bromosuccinimide in 1000 mL of water.

Bromothymol blue $C_{27}H_{28}Br_2O_5S$ [K 8842, Special class]

Bromothymol blue-sodium hydroxide TS To 0.2 g of powdered bromothymol blue add 5 mL of dilute sodium hydroxide TS and a small quantity of water, dissolve by shaking in a water bath at 50°C, then add water to make 100 mL.

Bromothymol blue TS Dissolve 0.1 g of bromothymol blue in 100 mL of dilute ethanol, and filter if necessary.

Bromovalerylurea $C_6H_{11}BrN_2O_2$ [Same as the namesake monograph]

Brucine See brucine dihydrate.

Brucine dihydrate $C_{23}H_{26}N_2O_4 \cdot 2H_2O$ [K 8832, Brucine *n*-hydrate, Special class]

B-type erythrocyte suspension Prepare a suspension containing 1 vol% of erythrocyte separated from human B-type blood in isotonic sodium chloride solution.

Bucillamine $C_7H_{13}NO_3S_2$ [Same as the namesake monograph]

Bufalin for component determination $C_{24}H_{34}O_4 \cdot xH_2O$ White, odorless, crystalline powder.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (300 nm): 143 – 153 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

Purity Related substances—Dissolve 40 mg of bufalin for component determination in 5 mL of chloroform and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and chloroform (4:3:3) to a distance of about 14 cm, and air-dry. Spray evenly sulfuric acid, and heat at 100°C for 2 to 3 minutes: any spot other than the principal spot obtained from the sample solution is not larger and not more intense than the spot from the standard solution.

Content: not less than 99.0%. Component determination—Weigh accurately about 10 mg of bufalin for component determination, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform

the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure the peak area by the automatic integration method, and calculate the amount of bufalin by the area percentage method.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of bufalin is about 6 minutes.

Selection of column: Dissolve 0.01 g each of bufalin for component determination, cinobufagin for component determination and resibufogenin for component determination in methanol to make 200 mL. Proceed with 20 μL of this solution according to the above conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order and completely resolving these peaks.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of bufalin obtained from 20 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of bufalin from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of bufalin beginning after the solvent peak.

Bufexamac for assay $C_{12}H_{17}NO_3$ [Same as the monograph Bufexamac. When dried, it contains not less than 99.0% of $C_{12}H_{17}NO_3$. Proceed as directed in the Identification under Bufexamac Ointment: any peak other than the principal peak does not appear.]

Buffer solution for celmoleukin Combine 12.5 mL of 0.5 mol/L tris buffer solution, pH 6.8, 10 mL of sodium lauryl sulfate solution (1→10), 10 mL of glycerin, and 17.5 mL of water, shake, and then add and dissolve 5 mg of bromophenol blue.

Storage—Store in a cool place, shielded from light.

***n*-Butanol** See 1-butanol.

***sec*-Butanol** See 2-butanol.

***t*-Butanol** $(CH_3)_3COH$ [K 8813, Special class]

***tert*-Butanol** See *t*-butanol.

1-Butanol $CH_3(CH_2)_2CH_2OH$ [K 8810, Special class]

2-Butanol $CH_3CH_2CH(OH)CH_3$ [K 8812, Special class]

2-Butanone $CH_3COC_2H_5$ [K 8900, Special class]

***N*-*t*-Butoxycarbonyl-L-glutamic acid- α -phenyl ester** $C_{16}H_{21}NO_6$ White powder.

Melting point <2.60>: 95–104°C

Purity Related substances—Dissolve 10 mg of *N*-*t*-butox-

ycarnonyl-L-glutamic acid- α -phenyl ester in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on three plates of silica gel with fluorescent indicator for thin-layer chromatography. Develop the first plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (25:25:1), the second plate with a mixture of benzene, 1,4-dioxane and acetic acid (100) (95:25:4), and the third plate with a mixture of chloroform, methanol and acetic acid (100) (45:4:1) to a distance of about 12 cm, and air-dry these plates. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot obtained from the sample solution are not more intense than the spot from the standard solution in all plates.

n-Butyl acetate $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ [K 8377, Special class]

n-Butylamine $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$ A colorless liquid, having an amine-like, characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. The solution in water shows alkalinity and rapidly absorbs carbon dioxide from the air.

Specific gravity <2.56> d_{20}^{20} : 0.740 – 0.747

Distilling range <2.57>: 76.5 – 79°C, not less than 96 vol%.

n-Butyl chloride $\text{CH}_3(\text{CH}_2)_3\text{Cl}$ Clear and colorless liquid, miscible with ethanol (95) and with diethyl ether, practically insoluble in water.

Boiling point <2.57>: about 78°C

Refractive index <2.45> n_D^{20} : 1.401 – 1.045

Specific gravity <2.56> d_{20}^{20} : 0.884 – 0.890

n-Butyl formate $\text{HCOO}(\text{CH}_2)_3\text{CH}_3$ Clear and colorless liquid, having a characteristic odor.

Specific gravity <2.56> d_{20}^{20} : 0.884 – 0.904

tert-Butyl methyl ether $(\text{CH}_3)_3\text{COCH}_3$ Clear colorless liquid, having a specific odor.

Refractive index <2.45> n_D^{20} : 1.3689

Specific gravity <2.56> d_4^{20} : 0.7404

Butyl parahydroxybenzoate
 $\text{HOC}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$ [Same as the namesake monograph]

Butyrolactone $\text{C}_4\text{H}_6\text{O}_2$ Clear, colorless to practically colorless liquid.

Boiling point <2.57>: 198 – 208°C

Specific gravity <2.56> d_4^{25} : 1.128 – 1.135

Cadmium acetate See cadmium acetate dihydrate.

Cadmium acetate dihydrate $\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ [K 8362, Special class]

Cadmium ground metal Cd [H 2113, First class]

Cadmium-ninhydrin TS Dissolve 0.05 g of cadmium acetate dihydrate in 5 mL of water and 1 mL of acetic acid (100), add 2-butanone to make 50 mL, and dissolve 0.1 g of ninhydrin in this solution. Prepare before use.

Caffeine $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2 \cdot \text{H}_2\text{O}$ [Same as the monograph

Caffeine Hydrate]

Caffeine, anhydrous $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ [Same as the namesake monograph]

Calcium carbonate CaCO_3 [K 8617, Special class]

Calcium chloride See calcium chloride dihydrate.

Calcium chloride dihydrate $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ [K 8122, Special class]

Calcium chloride for drying CaCl_2 [K 8124, For drying]

Calcium chloride for Karl Fischer method CaCl_2 [K 8125, For water determination]

Calcium chloride TS Dissolve 7.5 g of calcium chloride dihydrate in water to make 100 mm (0.5 mol/L).

Calcium gluconate for thin-layer chromatography [Same as the monograph Calcium Gluconate Hydrate. When the test is performed as directed in the Identification (1) under Calcium Gluconate Hydrate, no spot other than the principal spot appears.]

Calcium hydroxide $\text{Ca}(\text{OH})_2$ [K 8575, Special class]

Calcium hydroxide for pH determination Calcium hydroxide prepared for pH determination.

Calcium hydroxide pH standard solution See pH Determination <2.54>.

Calcium hydroxide TS To 3 g of calcium hydroxide add 1000 mL of cold distilled water, and occasionally shake the mixture vigorously for 1 hour. Allow to stand, and use the supernatant liquid (0.04 mol/L).

Calcium nitrate See calcium nitrate tetrahydrate.

Calcium nitrate tetrahydrate $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ [K 8549, Special class]

Calcium oxide CaO [K 8410, Special class]

Camphor $\text{C}_{10}\text{H}_{16}\text{O}$ [Same as the monograph *d*-Camphor or *dl*-Camphor]

***d*-Camphorsulfonic acid** $\text{C}_{10}\text{H}_{16}\text{O}_4\text{S}$ White crystals or crystalline powder, having a characteristic odor. Very soluble in water, and soluble in chloroform.

Purity Clarity and color of solution—Dissolve 1.0 g of *d*-camphorsulfonic acid in 10 mL of water: the solution is clear and colorless or pale yellow.

Loss on drying <2.41>: not more than 2.0% (1 g, 105°C, 5 hours).

Content: not less than 99.0%, calculated on the dried basis. Assay—Weigh accurately about 4 g of *d*-camphorsulfonic acid, dissolve in 50 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS
= 232.3 mg of $\text{C}_{10}\text{H}_{16}\text{O}_4\text{S}$

Caprylic acid $\text{CH}_3(\text{CH}_2)_6\text{COOH}$ A clear and colorless oily liquid, having a slight unpleasant odor. Freely soluble in ethanol (95) and in chloroform, and very slightly soluble in water.

Distilling range <2.57>: 238 – 242°C, not less than 95 vol%.

Refractive index <2.45>: n_D^{20} 1.426 – 1.430

Specific gravity <2.56> d_4^{20} : 0.908 – 0.912

Capsaicin for component determination Use capsaicin for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (281 nm): 97 – 105 (10 mg, methanol, 200 mL). Use the sample dried in a desiccator (in vacuum, phosphorus (v) oxide, 40°C) for 5 hours for the test.

Purity Related substances—Dissolve 10 mg of capsaicin for component determination in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total area of the peaks other than capsaicin from the sample solution is not larger than the peak area of capsaicin from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed the operating conditions in the Component determination under Capsicum.

Time span of measurement: About 3 times as long as the retention time of capsaicin beginning after the solvent peak. System suitability

System performance, and system repeatability: Proceed the system suitability in the Component determination under Capsicum.

Test for required detectability: Pipet 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of capsaicin from 20 μL of this solution is equivalent to 3.5 to 6.5% of that of capsaicin from the standard solution.

Capsaicin for thin-layer chromatography $\text{C}_{18}\text{H}_{27}\text{NO}_3$ White crystals, having a strong irritative odor. Very soluble in methanol, freely soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 64.5 – 66.5°C

Purity Related substances—Dissolve 20 mg of capsaicin for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed in the Identification under Capsicum: any spot other than the principal spot at the R_f value of about 0.5 from the sample solution is not more intense than the spot from the standard solution.

Carbazochrome $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$ Yellow-red to red crystals or crystalline powder. Melting point: about 222°C (with decomposition).

Content: not less than 98.0%. **Assay**—Dissolve about 0.2 g of carbazochrome, previously weighed accurately, in 20 mL of acetic acid (100) by heating, add 80 mL of acetic anhydride, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.62 mg of $\text{C}_{10}\text{H}_{12}\text{N}_4\text{O}_3$

Carbazochrome sodium sulfonate for component determination [Same as the monograph Carbazochrome Sodium Sulfonate Hydrate. It contains not less than 14.0% and not more than 15.0% of water, and not less than 99.0% of carbazochrome sodium sulfonate ($\text{C}_{10}\text{H}_{11}\text{N}_4\text{NaO}_5\text{S}$), calculated on the dehydrated basis.]

0.1 mol/L Carbonate buffer solution, pH 9.6 Dissolve 3.18 g of anhydrous sodium carbonate and 5.88 g of sodium hydrogen carbonate in water to make 1000 mL.

Carbon dioxide CO_2 [Same as the namesake monograph]

Carbon disulfide CS_2 [K 8732, Special class] Preserve in tightly stoppered containers in a dark, cold place, remote from fire.

Carbonic anhydrase White powder. Derived from bovine RBC. Molecular weight about 29,000.

Carbon monoxide CO A toxic, colorless gas. Prepare by passing the gas generated by reacting formic acid with sulfuric acid through a layer of sodium hydroxide TS. Carbon monoxide from a metal cylinder may be used.

Carbon tetrachloride CCl_4 [K 8459, Special class]

Casein, milk [K 8234, Special class]

Casein peptone See peptone, casein.

Castor oil [Same as the namesake monograph]

Catechol $\text{C}_6\text{H}_4(\text{OH})_2$ [K 8240, Special class]

Cefadroxil $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$ [Same as the namesake monograph]

Cefcapene pivoxil hydrochloride $\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_8\text{S}_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$ [Same as the monograph Cefcapene Pivoxil Hydrochloride Hydrate]

Cefdinir lactam ring-cleavage lactones $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O}_6\text{S}_2$ A white to yellow powder. A mixture of 4 diastereoisomers.

Identification—Determine the infrared absorption spectrum of cefdinir lactam ring-cleavage lactones as directed in the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1743 cm^{-1} , 1330 cm^{-1} , 1163 cm^{-1} and 1047 cm^{-1} .

Content: not less than 90%. **Assay**—Dissolve about 5 mg of cefdinir lactam ring-cleavage lactones in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed in the operating conditions of the Purity (2) Related substances under Cefdinir, and calculate the areas of each peak by the automatic integration method. Determine the percent of the total peak area of 4 cefdinir lactam ring-cleavage lactones to the total area of all peaks.

Cefoselis-3-ene-isomer $\text{C}_{19}\text{H}_{22}\text{N}_8\text{O}_6\text{S}_2$ A white to yellowish white powder.

Identification—After drying under reduced pressure at 60°C for 3 hours, determine the infrared absorption spectrum of cefoselis-3-ene-isomer according to the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3300 cm^{-1} , 1768 cm^{-1} , 1618 cm^{-1} , 1520 cm^{-1} and 865 cm^{-1} .

Content: not less than 90%. **Assay**—Dissolve about 2.5 mg

of cefoselis-3-ene-isomer in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0 and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed in the Assay under Cefoselis Sulfate, and calculate the percentage of the peak area of cefoselis-3-ene-isomer to the total peak area by the automatic integration method.

Cell suspension solution for teceleukin Centrifuge for 5 minutes at 1000 r.p.m culture medium of NK-7 cells that have been cultured statically for 2 to 4 hours. Remove the supernatant by aspiration, and add culture medium for assay of teceleukin to a cell concentration of 2 to 4×10^5 cells/mL.

Celmoleukin for liquid chromatography

$C_{693}H_{1118}N_{178}O_{203}S_7$ [Same as the monograph Celmoleukin (Genetical Recombination). However, contains 0.5 to 1.5 mg of protein per mL, polymers amount for 0.5% or less, and conforms to the following test].

Identification: (1) When the amino acid sequence is investigated using the Edman technique and liquid chromatography, the amino acids are detected in the following sequence: alanine, proline, threonine, serine, serine, serine, threonine, lysine, lysine, threonine, glutamine, leucine, glutamine, leucine, and glutamic acid. Also, based on the results of the protein content determination test, place an amount of celmoleukin equivalent to about 0.3 mg in a hydrolysis tube, evaporate to dryness under vacuum, and then add 100 μ L of hydrazine anhydride for amino acid sequence analysis. Reduce the internal pressure of the hydrolysis tube by heating for 6 hours at about 100°C. After evaporating to dryness under vacuum, add 250 μ L of water to dissolve the residue. To this add 200 μ L of benzaldehyde, shake occasionally, leave for one hour, centrifuge, and remove the aqueous layer. Add 250 μ L of water to the benzaldehyde layer, shake, centrifuge, combine the aqueous layers, and evaporate to dryness under vacuum. Threonine is detected when amino acid analysis is conducted using the postcolumn technique with ninhydrin on a solution of the residue dissolved by adding 100 μ L of 0.02 mol/L hydrochloric acid TS.

(2) Add 1 mL of protein digestive enzyme solution to 1 mL of celmoleukin, shake, and leave for 18 to 24 hours at 37°C. Pipet 1 mL of this solution and add 25 μ L of trifluoroacetic acid (1 in 10). To another 1 mL, add 10 μ L of 2-mercaptoethanol, leave for 30 minutes at 37°C, and then add 25 μ L of trifluoroacetic acid (1 in 10). Perform Liquid Chromatography <2.01> on these two solutions separately under the conditions outlined in Celmoleukin (Genetical Recombination), Identification (4). Repeatedly pipet the celmoleukin derived peak fraction that elutes and when the test is performed according to Celmoleukin (Genetical Recombination), Identification (2), except for the lysines in positions 9 and 49 from the amino terminal amino acid, a peptide estimated from the complete primary structure is detected.

Cephaeline hydrobromate $C_{28}H_{38}N_2O_4 \cdot 2HBr \cdot xH_2O$
A white or light-yellow crystalline powder.

Purity—Dissolve 10 mg in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed in the Component determination under Ipecac: when measure the peak areas 2 times as long as the retention time of emetine, the total area of the

peaks other than cephaeline is not larger than the peak area of cephaeline from the standard solution.

Ceric ammonium sulfate See cerium (IV) tetraammonium sulfate dihydrate.

Ceric ammonium sulfate-phosphoric acid TS See cerium (IV) tetraammonium sulfate-phosphoric acid TS.

Ceric ammonium sulfate TS See cerium (IV) tetraammonium sulfate TS.

Cerium (III) nitrate hexahydrate $Ce(NO_3)_3 \cdot 6H_2O$ A colorless or light yellow, crystalline powder. It dissolves in water.

Purity (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: not more than 0.120%.

Content: not less than 98.0%. **Assay**—To about 1.5 g of cerous nitrate, accurately weighed, add 5 mL of sulfuric acid, and heat it until white fumes are evolved vigorously. After cooling, add 200 mL of water, 0.5 mL of 0.1 mol/L silver nitrate VS, dissolve 5 g of ammonium peroxodisulfate, dissolve, and boil it for 15 minutes. After cooling, add 2 drops of 1,10-phenanthroline TS, and titrate <2.50> with 0.1 mol/L ferrous ammonium sulfate VS until the pale blue color of the solution changes to red.

Each mL of 0.1 mol/L ferrous ammonium sulfate VS
= 43.42 mg of $Ce(NO_3)_3 \cdot 6H_2O$

Cerium (III) nitrate TS Dissolve 0.44 g of cerium (III) nitrate hexahydrate in water to make 1000 mL.

Cerium (IV) diammonium nitrate $Ce(NH_4)_2(NO_3)_6$ [K 8556, Special class]

Cerium (IV) diammonium nitrate TS Dissolve 6.25 g of cerium (IV) diammonium nitrate in 160 mL of diluted dilute nitric acid (9 in 50). Use within 3 days.

Cerium (IV) sulfate tetrahydrate $Ce(SO_4)_2 \cdot 4H_2O$ [K8976, Special class]

Cerium (IV) tetraammonium sulfate dihydrate
 $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 2H_2O$ [K 8977, Special class]

Cerium (IV) tetraammonium sulfate-phosphoric acid TS Dissolve 0.1 g of cerium (IV) tetraammonium sulfate in diluted phosphoric acid (4 in 5) to make 100 mL.

Cerium (IV) tetraammonium sulfate TS Dissolve 6.8 g of cerium (IV) tetraammonium sulfate in diluted sulfuric acid (3 in 100) to make 100 mL.

Cerous nitrate See cerium (III) nitrate hexahydrate.

Cerous nitrate TS See cerium (III) nitrate TS.

Cetanol [Same as the namesake monograph]

Cetrimide $C_{17}H_{38}BrN$ White to pale yellowish white powder, having a faint, characteristic odor.

Purity Clarity of solution—Dissolve 1.0 g of cetrimide in 5 mL of water: the solution is clear.

Content: not less than 96.0%. **Assay**—Weigh accurately about 2 g of cetrimide, previously dried, and dissolve in water to make exactly 100 mL. Pipet 25 mL of this solution into a separator, add 25 mL of chloroform, 10 mL of 0.1 mol/L sodium hydroxide VS and 10 mL of a freshly prepared solution of potassium iodide (1 in 20), shake well, allow to stand, and remove the chloroform layer. Wash the solution with three

10-mL portions of chloroform, take the water layer, and add 40 mL of hydrochloric acid. After cooling, titrate with 0.05 mol/L potassium iodide VS until the deep brown color of the solution almost disappears, add 2 mL of chloroform, and titrate <2.50> again until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the chloroform layer is decolorized. Perform a blank determination with 20 mL of water, 10 mL of a solution of potassium iodide (1 in 20) and 40 mL of hydrochloric acid.

Each mL of 0.05 mol/L potassium iodate VS
= 33.64 mg of $C_{17}H_{38}BrN$

Chenodeoxycholic acid for thin-layer chromatography $C_{24}H_{40}O_4$ White crystals or crystalline powder. Very soluble in methanol and in acetic acid (100), freely soluble in ethanol (95), soluble in acetone, sparingly soluble in ethyl acetate, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 119°C (recrystallize from ethyl acetate).

Purity Related substances—Dissolve 25 mg of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 250 mL. Perform the test with 10 μ L of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.5 g of chenodeoxycholic acid for thin-layer chromatography, previously dried under reduced pressure (phosphorus (V) oxide) at 80°C for 4 hours, and dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS. Near the end point add 100 mL of freshly boiled and cooled water, and titrate again.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Chikusetsusaponin IV for thin-layer chromatography $C_{47}H_{74}O_{18} \cdot nH_2O$ White crystalline powder. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether. Melting point: about 215°C (with decomposition).

Purity Related substances—Dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and perform the test with 5 μ L of this solution as directed in the Identification under Panax Rhizome: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Chloral hydrate $CCl_3CH(OH)_2$ [Same as the namesake monograph]

Chloral hydrate TS Dissolve 5 g of chloral hydrate in 3 mL of water.

Chloramine See sodium toluenesulfonchloramide trihydrate.

Chloramine TS See sodium toluenesulfonchloramide TS.

Chloramphenicol $C_{11}H_{12}Cl_2N_2O_5$ [Same as the monograph Chloramphenicol]

Chlorauric acid See hydrogen tetrachloroaurate (III)

tetrahydrate.

Chlorauric acid TS See hydrogen tetrachloroaurate (III) tetrahydrate TS.

Chlordiazepoxide $C_{16}H_{14}ClN_3O$ [Same as the namesake monograph]

Chlordiazepoxide for assay $C_{16}H_{14}ClN_3O$ [Same as the monograph Chlordiazepoxide. When dried, it contains not less than 99.0% of $C_{16}H_{14}ClN_3O$].

Chlorinated lime [Same as the namesake monograph]

Chlorinated lime TS Triturate 1 g of chlorinated lime with 9 mL of water, and filter. Prepare before use.

Chlorine Cl_2 A yellow-green gas, having a suffocating odor. It is heavier than air, and dissolves in water. Prepare from chlorinated lime with hydrochloric acid. Chlorine from a metal cylinder may be used.

Chlorine TS Use a saturated solution of chlorine in water. Preserve this solution in fully filled, light-resistant, glass-stoppered bottles, preferably in a cold place.

Chloroacetic acid $C_2H_3ClO_2$ [K 8899, Special class]

p-Chloroaniline See 4-chloroaniline

4-Chloroaniline $H_2NC_6H_4Cl$ White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in hot water.

Melting point <2.60>: 70 – 72°C

Residue on ignition <2.44>: not more than 0.1% (1 g).

4-Chlorobenzenediazonium TS Dissolve 0.5 g of 4-chloroaniline in 1.5 mL of hydrochloric acid, and add water to make 100 mL. To 10 mL of this solution add 10 mL of sodium nitrite TS and 5 mL of acetone. Prepare before use.

p-Chlorobenzene sulfonamide See 4-chlorobenzene sulfonamide.

4-Chlorobenzene sulfonamide $ClC_6H_4SO_2NH_2$ White to pale yellow, odorless, crystalline powder. Dissolves in acetone.

Purity Related substances—Dissolve 0.60 g of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL, and perform the test with 5 μ L of this solution as directed in the Purity (5) under Chlorpropamide: any spot other than the principal spot at the *R_f* value of about 0.5 does not appear.

p-Chlorobenzoic acid See 4-chlorobenzoic acid.

4-Chlorobenzoic acid ClC_6H_4COOH White crystals or powder. Sparingly soluble in ethanol (95), slightly soluble in chloroform, and practically insoluble in water.

Melting point <2.60>: 238 – 242°C

Content: not less than 99.0%. **Assay**—Weigh accurately about 0.3 g of 4-chlorobenzoic acid, dissolve in 30 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.66 mg of $C_7H_5ClO_2$

Chlorobutanol $C_4H_7Cl_3O$ [Same as the namesake monograph]

1-Chloro-2,4-dinitrobenzene $C_6H_3(NO_2)_2Cl$ [K 8478, Special class]

Chloroform CHCl_3 [K 8322, Special class]

Chloroform, ethanol-free Mix 20 mL of chloroform with 20 mL of water, gently shake for 3 minutes, separate the chloroform layer, wash the layer again with two 20-mL portions of water, and filter it through dry filter paper. To the filtrate add 5 g of anhydrous sodium sulfate, shake for 5 minutes, allow the mixture to stand for 2 hours, and filter through dry filter paper. Prepare before use.

Chloroform for Karl Fischer method See Water Determination <2.48>.

Chlorogenic acid for thin-layer chromatography $\text{C}_{16}\text{H}_{18}\text{O}_9 \cdot x\text{H}_2\text{O}$ A white powder. Freely soluble in methanol and in ethanol (99.5), and sparingly soluble in water. Melting point: about 205°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot other than the principal spot at around R_f 0.5 appears.

p-Chlorophenol See 4-Chlorophenol.

4-Chlorophenol $\text{ClC}_6\text{H}_4\text{OH}$ Colorless or pale red crystals or crystalline mass, having a characteristic odor. Very soluble in ethanol (95), in chloroform, in diethyl ether and in glycerin, and sparingly soluble in water. Melting point: about 43°C.

Content: not less than 99.0%. **Assay**—Weigh accurately about 0.2 g of 4-chlorophenol, and dissolve in water to make 100 mL. Measure exactly 25 mL of this solution into an iodine flask, add exactly 20 mL of 0.05 mol/L bromine VS and then 5 mL of hydrochloric acid, stopper immediately, shake occasionally for 30 minutes, and allow to stand for 15 minutes. Add 5 mL of a solution of potassium iodide (1 in 5), stopper immediately, shake well, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 3.214 mg of $\text{C}_6\text{H}_5\text{ClO}$

Preserve in tight, light-resistant containers.

(2-Chlorophenyl)-diphenylmethanol for thin-layer chromatography $\text{C}_{19}\text{H}_{15}\text{ClO}$ To 5 g of clotrimazole add 300 mL of 0.2 mol/L hydrochloric acid TS, boil for 30 minutes, cool, and extract with 100 mL of diethyl ether. Wash the diethyl ether extract with two 10 mL portions of 0.2 mol/L hydrochloric acid TS, then with two 10-mL portions of water. Shake the diethyl ether extract with 5 g of anhydrous sodium sulfate, and filter. Evaporate the diethyl ether of the filtrate, dissolve the residue in 200 mL of methanol by warming, and filter. Warm the filtrate, and add gradually 100 mL of water by stirring. Cool in an ice bath, filter the separated crystals, and dry in a desiccator (phosphorus (V) oxide) for 24 hours. A white crystalline powder. Very soluble in dichloromethane, freely soluble in diethyl ether, soluble in

methanol, and practically insoluble in water.

Melting point <2.60>: 92 – 95°C

Purity Related substances—Dissolve 10 mg of (2-chlorophenyl)-diphenylmethanol in dichloromethane to make exactly 20 mL, and perform the test with 10 μL of this solution as directed in the Purity (7) under Clotrimazole: any spot other than the principal spot does not appear.

Chloroplatinic acid See hydrogen hexachloroplatinate (IV) hexahydrate.

Chloroplatinic acid-potassium iodide TS See hydrogen hexachloroplatinate (IV)-potassium iodide TS.

Chloroplatinic acid TS See hydrogen hexachloroplatinate (IV) TS.

Chlorpheniramine maleate $\text{C}_{16}\text{H}_{19}\text{ClN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$ [Same as the namesake monograph]

Chlorpromazine hydrochloride for assay $\text{C}_{17}\text{H}_{19}\text{ClN}_2\text{S} \cdot \text{HCl}$ [Same as the monograph Chlorpromazine Hydrochloride]

Chlorpropamide for assay $\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$ [Same as the monograph Chlorpropamide. When dried, it contains not less than 99.0% of chlorpropamide ($\text{C}_{10}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$).]

Cholesterol $\text{C}_{27}\text{H}_{45}\text{OH}$ [Same as the namesake monograph]

Cholesterol benzoate $\text{C}_{34}\text{H}_{50}\text{O}_2$ White crystalline powder.

Melting point <2.60>: 145 – 152°C

Cholic acid for thin-layer chromatography $\text{C}_{24}\text{H}_{40}\text{O}_5$ White, crystals or crystalline powder. Soluble in acetic acid (100), sparingly soluble in acetone and in ethanol (95), and very slightly soluble in water. Melting point: about 198°C

Purity Related substances—Dissolve 25 mg in acetone to make exactly 250 mL. Proceed with 10 μL of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot, having R_f about 0.1, does not appear.

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.5 g, previously dried at 80°C for 4 hours (in vacuum, phosphorous (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water, add 2 drops of phenolphthalein TS, and titrate with 0.1 mol/L sodium hydroxide VS until immediately before the end-point has been reached. Then add 100 mL of freshly boiled and cooled water, and continue the titration <2.50>. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.86 mg of $\text{C}_{24}\text{H}_{40}\text{O}_5$

Choline chloride $[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{OH}]\text{Cl}$ White crystalline powder.

Melting point <2.60>: 303 – 305°C (decomposition).

Water <2.48>: less than 0.1%.

Chromic acid-sulfuric acid TS Saturate chromium (VI) trioxide in sulfuric acid.

Chromium trioxide See chromium (VI) trioxide.

Chromium trioxide TS See chromium (VI) trioxide TS.

Chromium (VI) trioxide CrO_3 A dark red-purple thin

needle-shaped or inner prism-like crystals, or light masses.

Identification—To 5 mL of a solution (1 in 50) add 0.2 mL of lead (II) acetate TS; yellow precipitates appear which does not dissolve on the addition of acetic acid.

Chromium (VI) trioxide TS Dissolve 3 g of chromium (VI) trioxide in water to make 100 mL.

Chromogenic synthetic substrate Equal amount mixture of *N*-benzoyl-L-isoleucyl-L-glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride and *N*-benzoyl-L-isoleucyl- γ -methoxy glutamyl-glycyl-L-arginyl-*p*-nitroanilid hydrochloride. White or pale yellow masses or powder. It is slightly soluble in water.

Identification: Perform the test with the solution (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorption maximum at about 316 nm is observed.

Purity: Free 4-nitroaniline (not more than 0.5%)

Loss on drying <2.41>: not more than 5% (0.2 g, reduced pressure (0.3 kPa), calcium chloride, between 30 and 40°C, 18 hours)

Content: not less than 95% and not more than 105% of the label.

Chromophore TS for teceleukin Mix 0.1 mL of diluted hydrogen peroxide (30) (1 in 20) with 10 mL of 0.2 mol/L citric acid buffer, pH 3.8, containing 0.2 mmol/L 3,3',5,5'-tetramethylbenzidine dihydrochloride dehydrate, and use immediately.

Chromotropic acid See disodium chromotropate dihydrate.

Chromotropic acid TS Dissolve 0.05 g of disodium chromotropate dihydrate in the solution prepared by cautiously adding 68 mL of sulfuric acid to 30 mL of water, cooling, then adding water to make 100 mL. Preserve in light-resistant containers.

Chromotropic acid TS, concentrated Suspend 0.5 g of disodium chromotropate dihydrate in 50 mL of sulfuric acid, centrifuge, and use the supernatant liquid. Prepare before use.

Cilastatin ammonium for assay $C_{16}H_{29}N_3O_5S$: 375.48 A white crystalline powder.

Water <2.48>: not more than 0.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44>: not more than 0.5% (1 g)

Purity Related substances—Dissolve 40 mg of the substance to be examined in 25 mL of water, and use this as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the each peak area by the automatic integration method. Separately, perform the test with 20 μ L of water in the same manner to correct any variance of the peak area caused the variation of the baseline: the total area of the peaks other than cilastatin is not larger than 1/6 times the peak area of cilastatin from the standard solution.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of the mobile phase: Control the gradient by mixing the mobile phase A and B directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	15→100	85→0
30 – 40	100	100

Flow rate: 2.0 mL per minute.

Time span of measurement: 40 minutes.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 μ L of this solution is equivalent to 2.3 to 4.5% of that with 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions: the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 3.0%.

Residual solvent—Weigh accurately about 1 g, dissolve in water to make exactly 100 mL, and use this as the sample solution. Separately, weigh accurately about 0.10 g of ethanol (99.5), add water to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak areas, A_T and A_S , of ethanol by the automatic integration method, and calculate the amount of ethanol (C_2H_5OH): not more than 0.5%.

Amount (%) of ethanol (C_2H_5OH)

$$= \frac{W_S}{W_T} \times \frac{A_T}{A_S} \times 100$$

W_S : Amount (mg) of ethanol (99.5)

W_T : Amount (mg) of the sample

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.5 mm in inside diameter and 30 m in length, coated the inside with 5% diphenyl-95% dimethylpolysiloxane for gas chromatography in thickness of 5 μ m.

Column temperature: Inject the sample at a constant temperature of about 50°C, keep on for 150 seconds, then raise

to 70°C at the rate of 8°C per minute, and keep this for 30 seconds.

Carrier gas: Helium

Flow rate: Adjust so that the retention time of ethanol is about 1 minute.

Sprit ratio: 5:1

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add water to make exactly 10 mL, and designate this the solution for system suitability test. To exactly 1 mL of the solution for system suitability test add water to make exactly 10 mL. Confirm that the peak area of ethanol obtained with 1 μ L of this solution is equivalent to 7 to 13% of that with 1 μ L of the solution for system suitability test.

System performance: When the procedure is run with 1 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethanol are not less than 1500 and not more than 3.0, respectively.

System repeatability: When determine the peak area of methanol by repeating 6 times with 1 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area is not more than 2.0%.

Content: not less than 99.0% of cilastatin ammonium ($C_{16}H_{29}N_3O_5S$), calculated on the anhydrous basis and corrected on the amount of ethanol. **Assay**—Weigh accurately about 0.5 g, dissolve in 30 mL of methanol, and add 5 mL of water. Adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 37.55 mg of $C_{16}H_{29}N_3O_5S$

Cinchonidine $C_{19}H_{22}N_2O$ White crystals or crystalline powder. Soluble in ethanol (95), in methanol and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water. A solution of cinchonidine in ethanol (95) (1 in 100) is levorotatory. Melting point: about 207°C

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.3 g of cinchonidine, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 14.72 mg of $C_{19}H_{22}N_2O$

Cinchonine $C_{19}H_{22}N_2O$ White crystals or powder.

Identification—Dissolve 1 g in 20 mL of diluted hydrochloric acid (1 in 4), and add 2 mL of potassium hexacyanoferrate (II) TS: yellow precipitates appear, which are dissolved by heating, and crystals are formed after allowing to cool.

Purity Cinchonidine and quinine—To 1 g add 30 mL of water, add diluted hydrochloric acid (2 in 3) dropwise until the substance to be tested dissolves, and neutralize with ammonia TS. To this solution add 10 mL of a solution of sodium tartrate dihydrate (1 in 2), boil, and allow to stand for 1 hour: no precipitates appear.

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.3 g, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same man-

ner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.72 mg of $C_{19}H_{22}N_2O$

Cineol for assay $C_{10}H_{18}O$ Clear and colorless liquid, having a characteristic aroma.

Refractive index <2.45> n_D^{20} : 1.457 – 1.459

Specific gravity <2.56> d_{20}^{20} : 0.920 – 0.930

Purity (1) Related substances (i)—Dissolve 0.20 g of cineol for assay in 10 mL of hexane and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:1) to a distance of about 10 cm, and air-dry. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS, and heat at 105°C for 5 minutes: any spot other than the principal spot does not appear.

(2) Related substances (ii)—Dissolve 0.10 g of cineol for assay in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cineol by the area percentage method: it is not less than 99.0%.

Operating conditions

Proceed the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Measure 1 mL of the sample solution and add hexane to make 100 mL. Adjust the detection sensitivity so that the peak height of cineol obtained from 2 μ L of this solution is 40% to 60% of the full scale.

Time span of measurement: About 3 times as long as the retention time of cineol beginning after the solvent peak.

Cinnamaldehyde for thin-layer chromatography C_9H_8O A colorless or light yellow liquid, having a characteristic aromatic odor. Very soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (285 nm): 1679 – 1943 (5 mg, methanol, 2000 mL)

Purity Related substances—Dissolve 10 mg in 2 mL of methanol. Perform the test with 1 μ L of this solution as directed in the Identification (3) under Kakkonto Extract: no spot other than the principal spot (R_f value is about 0.4) appears.

Cinnamic acid $C_9H_8O_2$ White crystalline powder, having a characteristic odor.

Melting point <2.60>: 132 – 135°C

(E)-Cinnamic acid for component determination (*E*)-Cinnamic acid for thin-layer chromatography. It meets the following requirements.

Purity Related substances—Conduct this procedure without exposure to day-light, using light-resistant vessels. Dissolve 10 mg in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> ac-

ording to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than (*E*)-cinnamic acid and the solvent is not more than the peak area of (*E*)-cinnamic acid obtained with the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Ryokeijutsukanto Extract.

Time span of measurement: About 6 times as long as the retention time of (*E*)-cinnamic acid.

System suitability

Test for required detectability: To exactly measured 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of (*E*)-cinnamic acid obtained with 10 μ L of this solution is equivalent to 3.5 to 6.5 % of that with 10 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Ryokeijutsukanto Extract.

(*E*)-Cinnamic acid for thin-layer chromatography

$C_9H_8O_2$ White crystals or crystalline powder, having a characteristic aromatic odor. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point <2.60>: 132 – 136°C

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (273 nm): 1307 – 1547 (5 mg dried with silica gel for 24 hours, methanol, 1000 mL).

Purity Related substances—Conduct this procedure without exposure to day-light, using light-resistant vessels. Dissolve 10 mg in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Proceed the test with 10 μ L each of the sample solution and standard solution as directed in the Identification (1) under Ryokeijutsukanto Extract: the spot other than the principal spot of around *Rf* 0.5 is not more intense than the spot obtained with the standard solution.

Cinobufagin for component determination

$C_{26}H_{34}O_6 \cdot nH_2O$ White crystalline odorless powder.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (295 nm): 125 – 127 (10 mg, methanol, 250 mL). Use the sample dried in a desiccator (silica gel) for 24 hours for the test.

Purity Related substances—Proceed with 40 mg of cinobufagin for component determination as directed in the Purity under bufalin for component determination.

Content: not less than 98.0%. Content determination—Weigh accurately about 10 mg of cinobufagin for component determination, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of cinobufagin by the area percentage method.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 295 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in

particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of cinobufagin is about 7 minutes.

Selection of column: Dissolve 10 mg each of bufalin for component determination, cinobufagin for component determination and resibufogenin for component determination in methanol to make 200 mL. Proceed with 20 μ L of this solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of cinobufagin obtained from 20 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of cinobufagin from 20 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of cinobufagin beginning after the solvent peak.

Cisplatin $Cl_2H_6N_2Pt$ [Same as the namesake monograph]

Citric acid See citric acid monohydrate.

Citric acid-acetic acid TS To 1 g of citric acid monohydrate add 90 mL of acetic anhydride and 10 mL of acetic acid (100), and dissolve under shaking.

Citric acid-acetic anhydride TS To 1 g of citric acid monohydrate add 50 mL of acetic anhydride, and dissolve by heating. Prepare before use.

Citric acid monohydrate $C_6H_8O_7 \cdot H_2O$ [K 8283, or same as the monograph Citric Acid Hydrate]

Citric acid-phosphate-acetonitrile TS Dissolve 2.1 g of citric acid monohydrate, 13.4 g of dipotassium hydrogen phosphate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1).

0.01 mol/L Citric acid TS Dissolve 2.1 g of citric acid monohydrate in water to make 1000 mL.

1 mol/L Citric acid TS for buffer solution Dissolve 210.14 g of citric acid monohydrate in water to make 1000 mL.

Clotrimazole $C_{22}H_{17}ClN_2$ [Same as the namesake monograph]

Cloxacolam $C_{17}H_{14}Cl_2N_2O_2$ [Same as the namesake monograph]

Cobalt (II) chloride-ethanol TS Dissolve 0.5 g of cobalt (II) chloride hexahydrate, previously dried at 105°C for 2 hours, in ethanol (99.5) to make 100 mL.

Cobalt (II) chloride hexahydrate $CoCl_2 \cdot 6H_2O$ [K 8129, Special class]

Cobalt (II) chloride TS Dissolve 2 g of cobalt (II) chlo-

ride hexahydrate in 1 mL of hydrochloric acid and water to make 100 mL (0.08 mol/L).

Cobalt (II) nitrate hexahydrate $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$
[K 8552, Special class]

Cobaltous chloride See cobalt (II) chloride hexahydrate.

Cobaltous nitrate See cobalt (II) nitrate hexahydrate.

Codeine phosphate for assay $\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$
[Same as the monograph Codeine Phosphate Hydrate. It contains not less than 99.0% of codeine phosphate ($\text{C}_{18}\text{H}_{21}\text{NO}_3 \cdot \text{H}_3\text{PO}_4$), calculated on the anhydrous basis.]

Collodion Clear, colorless, viscous liquid, having a diethyl ether-like odor.

pH <2.54>: 5.0–8.0

Stir 5 g of collodion while warming, add 10 mL of water gradually, and dry at 110°C after evaporating to dryness: mass of the residue is 0.250–0.275 g.

Concentrated chromotropic acid TS See chromotropic acid, concentrated.

Concentrated diazobenzenesulfonic acid TS See diazobenzenesulfonic acid TS, concentrated.

Congo red $\text{C}_{32}\text{H}_{22}\text{N}_6\text{Na}_2\text{O}_6\text{S}_2$ [K 8352, Special class]

Congo red TS Dissolve 0.5 g of congo red in 100 mL of a mixture of ethanol (95) and water (1:9).

Control anti-interleukin-2 antiserum solution Anti-interleukin-2 antiserum is diluted with culture media for celmoleukin, so that the diluted antiserum solution neutralizes the same volume of about 800 unit/mL solution of Celmoleukin (Genetical Recombination).

Coomassie brilliant blue G-250 $\text{C}_{47}\text{H}_{48}\text{N}_3\text{NaO}_7\text{S}_2$
A deep violet powder. A solution in ethanol (99.5) (1 in 100,000) exhibits an absorption maxima at a wavelength of 608 nm.

Coomassie brilliant blue R-250 $\text{C}_{45}\text{H}_{44}\text{N}_3\text{NaO}_7\text{S}_2$ Deep blue-purple powder. Odorless.

Content: not less than 50%.

Coomassie staining TS Dissolve 125 mg of Coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Copper Cu [K 8660, Special class]

Copper (II) acetate monohydrate $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$
[K 8370, Special class]

Copper (II) acetate TS, strong Dissolve 13.3 g of copper (II) acetate monohydrate in a mixture of 195 mL of water and 5 mL of acetic acid.

Copper (II) chloride-acetone TS Dissolve 0.3 g of copper (II) chloride dihydrate in acetone to make 10 mL.

Copper (II) chloride dihydrate $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ [K 8145, Special class]

Copper (II) disodium ethylenediamine tetraacetate tetrahydrate $\text{C}_{10}\text{H}_{12}\text{CuN}_2\text{Na}_2\text{O}_8 \cdot 4\text{H}_2\text{O}$ A blue powder.

pH <2.54>: 7.0–9.0

Purity Clarity and color of solution—Add 0.10 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate

to 10 mL of freshly boiled and cooled water: the solution is blue in color and clear.

Content: not less than 98.0%. Assay—Weigh accurately about 0.45 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH of the mixture to about 1.5 by adding 100 mL of water and dilute nitric acid, then add 5 mL of a solution of 1,10-phenanthroline monohydrate in methanol (1 in 20), and titrate <2.50> with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS
= 4.698 mg of $\text{C}_{10}\text{H}_{12}\text{CuN}_2\text{Na}_2\text{O}_8 \cdot 4\text{H}_2\text{O}$

Copper (II) hydroxide $\text{Cu}(\text{OH})_2$ Light blue powder. Practically insoluble in water.

Content: not less than 95.0% as $\text{Cu}(\text{OH})_2$. Assay—Weigh accurately about 0.6 g of Copper (II) hydroxide, and dissolve in 3 mL of hydrochloric acid and water to make exactly 500 mL. Pipet 25 mL of this solution, add 75 mL of water, 10 mL of a solution of ammonium chloride (3 in 50), 3 mL of diluted ammonia solution (28) (1 in 10) and 0.05 g of murexide-sodium chloride indicator, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the liquid is changed from yellow-green to red-purple.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.9756 mg of $\text{Cu}(\text{OH})_2$

Copper (II) sulfate (anhydrous) CuSO_4
[K 8984, First class]

Copper (II) sulfate pentahydrate $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
[K 8983, Special class]

Copper (II) sulfate-pyridine TS Dissolve 4 g of copper (II) sulfate pentahydrate in 90 mL of water, then add 30 mL of pyridine. Prepare before use.

Copper (II) sulfate solution, alkaline Dissolve 150 g of potassium bicarbonate, 101.4 g of potassium carbonate and 6.93 g of copper (II) sulfate pentahydrate in water to make 1000 mL.

Copper (II) sulfate TS Dissolve 12.5 g of copper (II) sulfate pentahydrate in water to make 100 mL (0.5 mol/L).

Copper (standard reagent) Cu [K 8005, Standard reagent for quantitative analysis]

Corn oil [Same as the namesake monograph]

Cortisone acetate $\text{C}_{23}\text{H}_{30}\text{O}_6$ [Same as the namesake monograph]

Cottonseed oil A refined, nonvolatile fatty oil obtained from the seed of plants of *Gossypium hirsutum* Linné (*Gossypium*) or of other similar species. A pale yellow, odorless, oily liquid. Miscible with diethyl ether, and with hexane. Slightly soluble in ethanol (95).

Refractive index <2.45> n_D^{20} : 1.472–1.474

Specific gravity <2.56> d_{25}^{25} : 0.915–0.921

Acid value <1.13>: not more than 0.5.

Saponification value <1.13>: 190–198

Iodine value <1.13>: 103–116

Cresol $\text{CH}_3\text{C}_6\text{H}_4(\text{OH})$ [Same as the namesake monograph]

m-Cresol $\text{CH}_3\text{C}_6\text{H}_4(\text{OH})$ [K 8305, Special class]

m-Cresol purple $\text{C}_{21}\text{H}_{18}\text{O}_5\text{S}$ [K8889, Special grade]

m-Cresol purple TS Dissolve 0.10 g of m-cresol purple in 13 mL of 0.01 mol/L sodium hydroxide TS, and add water to make 100 mL.

Cresol red $\text{C}_{21}\text{H}_{18}\text{O}_5\text{S}$ [K 8308, Special class]

Cresol red TS Dissolve 0.1 g of cresol red in 100 mL of ethanol (95), and filter if necessary.

Crystalline trypsin for ulinastatin assay A proteolytic enzyme prepared from bovine pancreas. White to light yellow crystalline powder. Odorless. Sparingly soluble in water, and dissolves in 0.001 mol/L hydrochloric acid TS.

Content: not less than 3200 trypsin Units per mg. Assay—(i) Sample solution: Weigh accurately about 20 mg of crystalline trypsin for ulinastatin assay, and dissolve in 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 3000 trypsin Units. Dilute this solution with 0.001 mol/L hydrochloric acid TS so that each mL of the solution contains about 40 trypsin Units, and use this solution as the sample solution. (ii) Diluent: Dissolve 4.54 g of potassium dihydrogen phosphate in water to make exactly 500 mL (Solution I). Dissolve 4.73 g of anhydrous disodium hydrogen phosphate in water to make exactly 500 mL (Solution II). To 80 mL of Solution II add a suitable amount of Solution I to adjust to pH 7.6. (iii) Substrate solution: Dissolve 85.7 mg of *N*- α -benzoyl-L-arginine ethyl ester hydrochloride in water to make exactly 100 mL, and use this solution as the substrate stock solution. Pipet 10 mL of the stock solution, add the diluent to make exactly 100 mL, and use this solution as the substrate solution. The absorbance of the substrate solution determined at 253 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank is between 0.575 and 0.585. If the absorbance of the substrate solution is not in this range, adjust with the diluent or the substrate stock solution. (iv) Procedure: Pipet 3 mL of the substrate solution, previously warmed at $25 \pm 0.1^\circ\text{C}$, into a 1-cm quartz cell, add exactly 0.2 mL of the sample solution, and start the determination of the absorbance change at 253 nm for 5 minutes at $25 \pm 0.1^\circ\text{C}$ using a solution prepared by adding exactly 0.2 mL of 0.001 mol/L hydrochloric acid TS to exactly 3 mL of the substrate solution as the blank. Determine the difference of the absorbance change per minute, *A*, when the difference has been constant for at least 3 minutes. (v) Calculation: Trypsin Units per mg is obtained by use of the following equation. One trypsin Unit is an amount of the enzyme which gives 0.003 change in absorbance per minute under the conditions described above.

$$\text{Trypsin Units per mg} = \frac{A}{0.003 \times W}$$

W: Amount (mg) of the substance to be assayed in 0.2 mL of the sample solution

Storage—Preserve in a cold place.

Crystallized trypsin To trypsin obtained from bovine pancreas gland add an appropriate amount of trichloroacetic acid to precipitate the trypsin, and recrystallize in ethanol (95). White to yellowish white crystals or powder. It is odor-

less. Freely soluble in water and in sodium tetraborate-calcium chloride buffer solution, pH 8.0.

Content: not less than 45 FIP Units of trypsin per mg. Assay—(i) Sample solution: Weigh accurately an appropriate amount of crystallized trypsin according to the labeled Units, dissolve in 0.001 mol/L hydrochloric acid TS to prepare a solution containing 50 FIP Units per mL, and use this solution as the sample solution. Prepare before use, and preserve in ice. (ii) Apparatus: Use a glass bottle as a reaction reservoir 20 mm in inside diameter and 50 mm in height, equipped with a rubber stopper for attachment to a glass/silver-silver chloride electrode for pH determination, nitrogen-induction tube and an exhaust port. Fix the reaction reservoir in a thermostat, and keep the temperature at $25 \pm 0.1^\circ\text{C}$ by means of a precise thermoregulator. (iii) Procedure: Pipet 1.0 mL of *N*- α -benzoyl-L-arginine ethyl ester TS, transfer to the reaction reservoir, and add 9.0 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0. Allow to stand in the thermostat for 10 minutes to make the temperature of the contents reach to $25 \pm 0.1^\circ\text{C}$, adjust the pH of the solution to 8.00 by adding dropwise 0.1 mol/L sodium hydroxide VS while stirring and passing a current of nitrogen, add exactly 0.05 mL of the sample solution previously allowed to stand at $25 \pm 0.1^\circ\text{C}$, then immediately add dropwise 0.1 mol/L sodium hydroxide VS by a 50 μL -micropipet (minimum graduation of 1 μL) while stirring to keep the reaction solution at pH 8.00, and read the amount of 0.1 mol/L sodium hydroxide VS consumed and the reaction time when the pH reached 8.00. Continue this procedure up to 8 minutes. Separately, transfer 10 mL of sodium tetraborate-calcium chloride buffer solution, pH 8.0, and perform a blank determination in the same manner. (iv) Calculation: Plot the amount of consumption (μL) of 0.1 mol/L sodium hydroxide VS against the reaction time (minutes), select linear reaction times, t_1 and t_2 , designate the corresponding consumption amount of 0.1 mol/L sodium hydroxide VS as v_1 and v_2 , respectively, and designate μmol of sodium hydroxide consumed per minute as *M* (FIP Unit).

$$M (\mu\text{mol NaOH}/\text{min}) = \frac{v_2 - v_1}{t_2 - t_1} \times \frac{1}{10} \times f$$

f: Factor of 0.1 mol/L sodium hydroxide VS

FIP Units per mg of crystallized trypsin to be tested

$$= \frac{(M_1 - M_0) \times T}{L \times W}$$

*M*₁: μmol of sodium hydroxide consumed in 1 minute when the sample solution is used

*M*₀: μmol of sodium hydroxide consumed in 1 minute when the solution for blank determination is used

W: Amount (mg) of crystallized trypsin sampled

L: Amount (mL) of the sample solution put in the reaction reservoir

T: Total volume (mL) of the sample solution prepared by dissolving in 0.001 mol/L hydrochloric acid TS

One FIP Unit is an amount of enzyme which decomposes 1 μmol of *N*- α -benzoyl-L-arginine ethyl ester per minute under the conditions described in the Assay.

Storage—Preserve in a cold place.

Crystal violet $\text{C}_{25}\text{H}_{30}\text{CN}_3 \cdot 9\text{H}_2\text{O}$ [K 8294, Special class]

Crystal violet TS Dissolve 0.1 g of crystal violet 10 mL of acetic acid (100).

Culture medium for assay of teceleukin Add 100 mL of fetal calf serum to 1000 mL of medium for float culture. Store at 4°C.

Culture medium for celmoleukin Take a specified amount of RPMI-1640 powdered medium that contains glutamate but does not contain sodium hydrogen carbonate, add water to dissolve, and add *N*-2-hydroxyethylpiperidine-*N*-2-ethansulfonic acid as a buffering agent to a concentration of 0.025 mol/L. To 1000 mL of this solution add 0.1 g (potency) of streptomycin sulfate, 100,000 units of potassium benzylpenicillin, and 2 g of sodium hydrogen carbonate, adjust the pH to 7.1 to 7.2 with sodium hydroxide TS, and then sterilize by filtration. To this solution add fetal calf serum heated at 56°C for 30 minutes to 20 vol%.

Cu-PAN Prepare by mixing 1 g of 1-(2-pyridylazo)-2-naphthol (free acid) with 11.1 g of copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

A grayish orange-yellow, grayish red-brown or light grayish purple powder.

Absorbance—Dissolve 0.50 g of Cu-PAN in diluted 1,4-dioxane (1 in 2) to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL. Read the absorbance of this solution at 470 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution: the absorbance is not less than 0.48.

Purity Clarity and color of solution—Dissolve 0.50 g of Cu-PAN in 50 mL of diluted 1,4-dioxane (1 in 2): the solution is clear and yellow-brown.

Cu-PAN TS Dissolve 1 g of Cu-PAN in 100 mL of diluted 1,4-dioxane (1 in 2).

Cupferron C₆H₉N₃O₂ [K 8289, Special class]

Cupferron TS Dissolve 6 g of cupferron in water to make 100 mL. Prepare before use.

Cupric acetate See copper (II) acetate monohydrate.

Cupric acetate TS, strong See copper (II) acetate monohydrate TS, strong.

Cupric carbonate See cupric carbonate monohydrate.

Cupric carbonate monohydrate CuCO₃·Cu(OH)₂·H₂O A blue to blue-green powder. It is insoluble in water, and dissolves foamingly in dilute acid. It dissolves in ammonia TS and shows a deep blue color.

Purity (1) Chloride <1.03>: not more than 0.036%.

(2) Sulfate <1.14>: not more than 0.120%.

(3) Iron—Dissolve 5.0 g of cupric carbonate monohydrate in excess ammonia TS and filter. Wash the residue with ammonia TS, dissolve in dilute hydrochloric acid, add excess ammonia TS and filter. Wash the residue with ammonia TS, and dry to constant mass: the residue is not more than 10 mg.

Cupric chloride See copper (II) chloride dihydrate.

Cupric chloride-acetone TS See copper (II) chloride-acetone TS.

Cupric sulfate See copper (II) sulfate pentahydrate.

Cupric sulfate, anhydrous See copper (II) sulfate (anhydrous).

Cupric sulfate-pyridine TS See copper (II) sulfate-pyri-

dine TS.

Cupric sulfate solution, alkaline See copper (II) sulfate solution, alkaline.

Cupric sulfate TS See copper (II) sulfate TS.

1 mol/L Cupriethylenediamine TS Put 100 g of copper (II) hydroxide in a 1-L thick-walled bottle marked a 500-mL line, and add water to make 500 mL. Connect the bottle with a liquid introducing funnel, a nitrogen introducing glass tube and a gas removing glass tube. Adjust so that the lower end of the nitrogen introducing tube is located at about 1.3 cm above of the bottom of the bottle. Introduce the nitrogen for about 3 hours to replacing the inside gas by adjusting the pressure (about 14 kPa) to get a mild bubbling. Then add gradually 160 mL of ethylenediamine TS through the funnel while introducing the nitrogen and cooling the bottle with the running water, and replace the funnel with a glass rod to close tightly. After introducing the nitrogen for further 10 minutes, replace the gas removing tube with a glass rod to close tightly. Keep the inside pressure with the nitrogen to about 14 kPa. After allowing the bottle to stand for about 16 hours while occasional shaking, filter the content if necessary using a glass-filter under reducing pressure, and reserve under nitrogen atmosphere. The concentration of copper (II) ion of this solution is about 1.3 mol/L. Determine the concentration of ethylenediamine of this solution X (mol/L) and copper (II) ion Y (mol/L) by the following Assays, and adjust to that X is 1.96–2.04, Y is 0.98–1.02 and X/Y is 1.96–2.04 by adding water, copper (II) hydroxide or ethylenediamine TS, then determine X and Y again in the same manner, and use this solution as the test solution.

Assay (1) Ethylenediamine—Pipet 1 mL (*V*₁) of the solution to be assayed, add 60 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (pH Determination <2.54>); End point is about pH 8.4).

$$X = \frac{N_1 a}{V_1}$$

X: Concentration of ethylenediamine (mol/L)

a: Volume of 0.1 mol/L hydrochloric acid VS consumed for the titration (mL)

N₁: Concentration of 0.1 mol/L hydrochloric acid VS (mol/L)

(2) Copper (II) ion—Pipet 2 mL (*V*₂) of the solution to be assayed, add 20 mL of water, about 3 g of potassium iodide and 50 mL of 2 mol/L sulfuric acid TS, shake for 5 minutes, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS. When the solution turns light yellow at near the end point add 3 mL of starch TS and 10 mL of a solution of ammonium thiocyanate (2 in 10), and then titrate until the blue color disappears.

$$Y = \frac{N_2 b}{V_2}$$

Y: Concentration of copper (II) ion (mol/L)

b: Volume of 0.1 mol/L sodium thiosulfate VS consumed for the titration (mL)

N₂: Concentration of 0.1 mol/L sodium thiosulfate VS (mol/L)

Curcumin C₂₁H₂₀O₆ [K 8297, Special class]

Curcumin TS Dissolve 0.125 g of curcumin in acetic acid (100) to make 100 mL. Prepare before use.

Cyanoacetic acid $C_3H_3NO_2$ White to light yellow crystals. Very soluble in water.

Content: not less than 99%. *Assay*—Weigh accurately about 300 mg of cyanoacetic acid, add 25 mL of water and 25 mL of ethanol (95) to dissolve, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 85.06 mg of $C_3H_3NO_2$

Cyanocobalamin $C_{63}H_{88}CoN_{14}O_{14}P$ [Same as the namesake monograph]

Cyanogen bromide TS To 100 mL of ice-cold water add 1 mL of bromine, shake vigorously, and add ice-cold potassium cyanide TS dropwise until the color of bromine just disappears. Prepare this test solution in a hood before use.

On handling this solution, be careful not to inhale its vapors, which are very toxic.

1-Cyanoguanidine $NH_2C(NH)NHCN$ A white crystalline powder. Freely soluble in water.

Melting point <2.60>: 209 – 212°C

Loss on drying <2.41>: not more than 0.1% (1 g, 105°C, 3 hours)

Nitrogen content <1.08>: 66.0 – 67.3% (after drying)

6% Cyanopropyl-6% phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

7% Cyanopropyl-7% phenylmethylsilicone polymer for gas chromatography Prepared for gas chromatography.

Cyclohexane C_6H_{12} [K 8464, Special class]

Cyclohexylamine $C_6H_{11}NH_2$ A clear and colorless liquid, having a characteristic amine-like odor. Miscible with water, with *N,N*-dimethylformamide and with acetone.

Purity Related substances—Use cyclohexylamine as the sample solution. Separately, pipet 1 mL of cyclohexylamine, add hexane to make exactly 100 mL, and use this as the standard solution. Perform the test as directed in Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol, ammonia water (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

Cyclohexylmethanol $C_7H_{14}O$ A liquid having slight camphor odor. Soluble in ethanol (99.5).

Boiling point <2.57>: about 185°C

Refractive index <2.45> n_D^{20} : about 1.464

Cyclosporine U $C_{81}H_{109}N_{11}O_{12}$ White powder.

Optical rotation <2.49> $[\alpha]_D^{20}$: about -190° (0.1 g, methanol, 20 mL 100 mm)

L-Cysteic acid $C_3H_7NO_3S$ White powder.

Melting point <2.60>: about 260°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+7.5 - +9.0^\circ$ (1.5 g, water, 20 mL, 100 mm)

L-Cysteine hydrochloride See L-cysteine hydrochloride

monohydrate.

L-Cysteine hydrochloride monohydrate
 $HSCH_2CH(NH_2)COOH.HCl.H_2O$ [K 8470, Special class]

L-Cystine $HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$
[K 9048, L(-)-Cystine, Special class]

Cytochrome c An oxidase (molecular weight: 8000 – 13,000) derived from bovine cardiac muscle

Cytosine $C_4H_5N_3O$ White, crystalline powder or powder.

Absorbance <2.24> $E_{1cm}^{1\%}$ (276 nm): not less than 800 (after drying, 40 mg, 10,000 mL of 0.1 mol/L hydrochloric acid TS).

Dacuronium Bromide for thin-layer chromatography
 $C_{33}H_{58}Br_2N_2O_3$ White crystalline powder. Very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether. Hygroscopic.

Identification—Determine the infrared absorption spectrum of dacuronium bromide for thin-layer chromatography according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits the absorptions at the wave numbers at about 2938 cm^{-1} , 1737 cm^{-1} , 1630 cm^{-1} , 1373 cm^{-1} , 1233 cm^{-1} and 1031 cm^{-1} .

Purity Related substances—Dissolve 10 mg of dacuronium bromide for thin-layer chromatography in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed in the Purity (2) Related substances under Pancuronium Bromide: the spots other than the principal spot from the sample solution do not show more intense color than the spot from the standard solution.

Water <2.48>: not more than 1.0% (1 g, volumetric titration, direct titration).

Content: not less than 98.0%, calculated on the dehydrated basis. *Assay*—Weigh accurately about 0.2 g of dacuronium bromide for thin-layer chromatography, dissolve in 50 mL of acetic anhydride by warming, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.53 mg of $C_{33}H_{58}Br_2N_2O_3$

***p,p'*-DDD** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethane)
 $C_{14}H_{10}Cl_4$

Melting point <2.60>: 108 – 110°C

Purity Related substances—Dissolve 10 mg of *p,p'*-DDD in hexane for purity of crude drug to make exactly 100 mL, pipet 1 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 1 μ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than *p,p'*-DDD from the sample solution is not larger than the peak area of *p,p'*-DDD

from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of *p,p'*-DDD obtained from 1 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of *p,p'*-DDD from 1 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of *p,p'*-DDD beginning after the solvent peak.

***p,p'*-DDE** (2,2-Bis(4-chlorophenyl)-1,1-dichloroethylene) $C_{14}H_8Cl_4$

Melting point <2.60>: 88 – 90°C

Purity Related substances—Proceed as directed in the Purity of *p,p'*-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

***o,p'*-DDT** (1,1,1-Trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane) $C_{14}H_9Cl_5$

Melting point <2.60>: 73 – 75°C

Purity Related substances—Proceed as directed in the Purity of *p,p'*-DDD.

***p,p'*-DDT** (1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane) $C_{14}H_9Cl_5$

Melting point <2.60>: 108 – 110°C

Purity Related substances—Proceed as directed in the Purity of *p,p'*-DDD using the following standard solution (1).

Standard solution (1): Pipet 1 mL of the sample solution, and add hexane for purity of crude drug to make exactly 100 mL.

DEAE-Cross-linking dextran anion exchanger (Cl type), slightly alkaline Slightly alkaline anion exchanger prepared by introducing diethylaminoethyl group into cross-linking dextran of gel filtration carrier.

Decolorized fuchsin TS Add 1 g of fuchsin in 100 mL of water, heat at about 50°C, then cool with occasional shaking. After standing for 48 hours, mix and filter. To 4 mL of the filtration add 6 mL of hydrochloric acid and water to make 100 mL. Use after standing for at least 1 hour. Prepare before use.

***n*-Decyl trimethylammonium bromide** $C_{13}H_{30}NBr$
White powder. Melting point: about 232°C (with decomposition).

Content: not less than 99%. Assay—Weigh accurately about 0.5 g of *n*-decyl trimethylammonium bromide, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 28.03 mg of $C_{13}H_{30}NBr$

0.005 mol/L *n*-Decyl trimethylammonium bromide TS
Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.40 g of *n*-decyl trimethylammonium bromide in water to make 1000 mL.

Defibrinated blood of rabbit Transfer 100 mL of blood obtained from rabbit to a flask, put in about 20 glass balls 8 mm in diameter, shake for 5 minutes gently, and filter through gauze. Prepare before use.

Dehydrated ethanol See ethanol (99.5).

Dehydrated ether See diethyl ether.

Dehydrated pyridine See pyridine, dehydrated.

Dehydrocorydaline nitrate for component determination $C_{22}H_{24}N_2O_7$ Yellow, crystals or crystalline powder. It is sparingly soluble in methanol, slightly soluble in water, in ethanol (95) and in acetonitrile, and practically insoluble in diethyl ether. Melting point: about 240°C (with decomposition).

Absorbance <2.24> $E_{1cm}^{1\%}$ (333 nm): 577 – 642 [3 mg after drying in a desiccator (silica gel) for not less than 1 hour, water, 500 mL].

Purity Related substances—(1) Dissolve 5.0 mg of dehydrocorydaline nitrate for component determination in 1 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add a mixture of water and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop immediately with a mixture of methanol, water and acetic acid (100) (20:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm) and then spray Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in either case.

(2) Dissolve 5.0 mg of dehydrocorydaline nitrate for component determination in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method in each solution: the total area of peaks other than dehydrocorydaline and the solvent from the sample solution is not larger than the peak area of dehydrocorydaline from the standard solution.

Operating conditions

Column, column temperature, mobile phase, and flow rate: Proceed the operating conditions in the Component determination under *Corydalis* Tuber.

Detector: Ultraviolet absorption photometer (wavelength: 230 nm)

Time span of measurement: About 3 times as long as the retention time of dehydrocorydaline beginning after the solvent peak.

System suitability

System performance and system repeatability: Proceed as directed in the system suitability in the Component determination under *Corydalis Tuber*.

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of dehydrocorydaline obtained from 5 μ L of this solution can be measured by the automatic integration method, and that the peak height of dehydrocorydaline obtained from 5 μ L of the standard solution is equivalent to around 20% of the full scale.

N-Demethylroxithromycin $C_{40}H_{74}N_2O_{15}$ White powder.

Identification—Determine the infrared absorption spectrum of a solution of the substance to be tested in chloroform (1 in 20) as directed in the solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm cell made of potassium bromide: it exhibits absorption at the wave numbers of about 3600 cm^{-1} , 3520 cm^{-1} , 3450 cm^{-1} , 3340 cm^{-1} , 1730 cm^{-1} and 1627 cm^{-1} .

N-Demethylerythromycin $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

2'-Deoxyuridine for liquid chromatography $C_9H_{12}N_2O_5$ White, crystalline powder.

Melting point <2.60>: $162 - 166^\circ\text{C}$

Purity—Dissolve 3.0 mg of 2'-deoxyuridine for liquid chromatography in diluted methanol (1 in 25) to make 50 mL. Perform the test with 10 μ L of this solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Purity under Idoxuridine Ophthalmic Solution. Measure each peak area by the automatic integration method to the range about twice the retention time of 2'-deoxyuridine, and calculate the amount of 2'-deoxyuridine by the area percentage method: it shows a purity of not less than 98.5%.

Content: not less than 98.5%. **Assay**—Weigh accurately about 5 mg of 2'-deoxyuridine for liquid chromatography, previously dried in vacuum at 60°C for 3 hours, and dissolve in water to make exactly 250 mL. Pipet 10 mL of this solution, dilute with water to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine absorbance A at the maximum wavelength at about 262 nm.

$$\begin{aligned} &\text{Amount (mg) of deoxyuridine (C}_9\text{H}_{12}\text{N}_2\text{O}_5\text{)} \\ &= \frac{A}{447} \times 5000 \end{aligned}$$

Deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy $(\text{CD}_3)_2\text{SO}$ Prepared for nuclear magnetic resonance spectroscopy.

Deuterated formic acid for nuclear magnetic resonance spectroscopy DCOOD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy DCl Prepared for nuclear magnetic resonance spectroscopy.

Deuterated methanol for nuclear magnetic resonance spectroscopy CD_3OD Prepared for nuclear magnetic resonance spectroscopy.

Deuterated NMR solvents Prepared for nuclear magnetic resonance spectroscopy. For example: deuterated dimethyl-

sulfoxide $[(\text{CD}_3)_2\text{SO}]$, deuterated pyridine $(\text{C}_5\text{D}_5\text{N})$, deuteriochloroform (CDCl_3) , heavy water (D_2O) , etc.

Deuterated pyridine for nuclear magnetic resonance spectroscopy $\text{C}_5\text{D}_5\text{N}$ Prepared for nuclear magnetic resonance spectroscopy.

Deuteriochloroform for nuclear magnetic resonance spectroscopy CDCl_3 Prepared for nuclear magnetic resonance spectroscopy.

Devarda's alloy [K 8653, Special class]

Diacetyl $\text{CH}_3\text{COCOCH}_3$ A yellow to yellow-green, clear liquid, having a strong, pungent odor. Miscible with ethanol (95) and with diethyl ether, and freely soluble in water.

Boiling point <2.57>: $85 - 91^\circ\text{C}$

Congearing point <2.42>: $-2.0 - -5.5^\circ\text{C}$

Refractive index <2.45> n_D^{20} : $1.390 - 1.398$

Specific gravity <2.56> d_{20}^{20} : $0.98 - 1.00$

Purity Clarity of solution—Dissolve 1.0 g of diacetyl in 10 mL of water: the solution is clear.

Content: not less than 95.0%. **Assay**—Weigh accurately about 0.4 g of diacetyl, add exactly 75 mL of hydroxylamine TS, and heat on a water bath for 1 hour under a reflux condenser. After cooling, titrate <2.50> the excess hydroxylamine with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from blue to yellow-green through green (indicator: 3 drops of bromophenol blue TS). Perform a blank determination in the same manner.

$$\begin{aligned} &\text{Each mL of 0.5 mol/L hydrochloric acid VS} \\ &= 21.52\text{ mg of C}_4\text{H}_6\text{O}_2 \end{aligned}$$

Diacetyl TS Dissolve 1 mL of diacetyl in water to make 100 mL, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

2,3-Diaminonaphthalene $\text{C}_{10}\text{H}_{10}\text{N}_2$ Light yellow-brown crystals or powder. Slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: $193 - 198^\circ\text{C}$

Sensitivity—Pipet separately 40 mL each of the selenium standard solution and diluted nitric acid (1 in 60) as the blank solution into beakers, and to these solutions add ammonia solution (28) to adjust the pH to between 1.8 and 2.2. Dissolve 0.2 g of hydroxylammonium chloride in each of these solutions under gentle shaking, add 5 mL of 2,3-diaminonaphthalene TS, mix by shaking, and allow to stand for 100 minutes. Transfer these solutions to separators separately, rinse the beakers with 10 mL of water, add these rinsings to the separators, extract each with 5.0 mL of cyclohexane by thorough shaking for 2 minutes, and centrifuge the cyclohexane layers to remove moisture. When the absorbance at 378 nm of cyclohexane extract obtained from selenium standard solution is determined using the solution obtained from the blank solution as the reference solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, it is not less than 0.08.

Selenium standard solution—Weigh accurately 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), by heating on water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. Pipet 2 mL of this solution, and add

diluted nitric acid (1 in 60) to make exactly 50 mL. Prepare before use. This solution contains 0.04 μg of selenium (Se) per mL.

2,3-Diaminonaphthalene TS Dissolve 0.10 g of 2,3-diaminonaphthalene and 0.5 g of hydroxylammonium chloride in 0.1 mol/L hydrochloric acid TS to make 100 mL.

2,4-Diaminophenol hydrochloride $\text{C}_6\text{H}_8\text{N}_2\text{O}\cdot 2\text{HCl}$
Pale yellow-brown to grayish yellow-green crystalline powder. Freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Purity Clarity of solution—Dissolve 1.0 g of 2,4-diaminophenol hydrochloride in 20 mL of water: the solution is clear or a slight turbidity is produced.

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44>: not more than 0.5% (1 g).

Content: not less than 98.0%. *Assay*—Weigh accurately about 0.2 g of 2,4-diaminophenol hydrochloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 9.854 mg of $\text{C}_6\text{H}_8\text{N}_2\text{O}\cdot 2\text{HCl}$

2,4-Diaminophenol hydrochloride TS Dissolve 1 g of 2,4-diaminophenol hydrochloride and 20 g of sodium bisulfite in 100 mL of water, and filter, if necessary.

Diammonium hydrogen citrate $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_7$ [K 8284, Special class]

Diammonium hydrogenphosphate $(\text{NH}_4)_2\text{HPO}_4$
[K 9016, Special class]

Diazobenzenesulfonic acid TS Weigh 0.9 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 10 mL of dilute hydrochloric acid by heating, and add water to make 100 mL. Pipet 3.0 mL of this solution, add 2.5 mL of sodium nitrite TS, and allow to stand for 5 minutes while cooling with ice. Then add 5 mL of sodium nitrite TS and water to make 100 mL, and allow to stand in ice water for 15 minutes. Prepare before use.

Diazobenzenesulfonic acid TS, concentrated Weigh 0.2 g of sulfanilic acid, previously dried at 105°C for 3 hours, dissolve it in 20 mL of 1 mol/L hydrochloric acid TS by warming. Cool this solution with ice, and add 2.2 mL of a solution of sodium nitrite (1 in 25) dropwise under stirring. Allow to stand in ice water for 10 minutes, and add 1 mL of a solution of sulfaminic acid (1 in 20). Prepare before use.

Diazo TS Weigh accurately 0.9 g of sulfanilic acid, add 0.9 mL of hydrochloric acid and 20 mL of water, and dissolve by heating. After cooling, filter, and dilute the filtrate with water to make exactly 100 mL. Pipet 1.5 mL of this solution, cool in an ice bath, and add exactly 1 mL of sodium nitrite solution (1 in 20) dropwise, while shaking. Cool in an ice bath for 10 minutes, add cold water to make exactly 50 mL. Store in a cold place, and use within 8 hours.

Dibasic ammonium phosphate See diammonium hydrogen phosphate.

Dibasic potassium phosphate See dipotassium hydrogen phosphate.

Dibasic potassium phosphate-citric acid buffer solution, pH 5.3 See dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3.

1 mol/L Dibasic potassium phosphate TS for buffer solution See 1 mol/L dipotassium hydrogen phosphate TS for buffer solution.

Dibasic sodium ammonium phosphate See ammonium sodium hydrogen phosphate tetrahydrate.

Dibasic sodium phosphate See disodium hydrogen phosphate dodecahydrate.

Dibasic sodium phosphate TS See disodium hydrogen phosphate TS.

Dibasic sodium phosphate, anhydrous See disodium hydrogen phosphate.

Dibasic sodium phosphate, anhydrous, for pH determination See disodium hydrogen phosphate for pH determination.

Dibasic sodium phosphate-citric acid buffer solution, pH 4.5 See disodium hydrogen phosphate-citric acid buffer solution, pH 4.5.

Dibasic sodium phosphate-citric acid buffer solution, pH 5.4 See disodium hydrogen phosphate-citric acid buffer solution, pH 5.4.

Dibasic sodium phosphate-citric acid buffer solution, pH 6.0 See disodium hydrogen phosphate-citric acid buffer solution, pH 6.0.

0.05 mol/L Dibasic sodium phosphate TS See 0.05 mol/L disodium hydrogen phosphate TS.

0.5 mol/L Dibasic sodium phosphate TS See 0.5 mol/L disodium hydrogen phosphate TS.

Dibekacin sulfate [Same as the namesake monograph]

Dibenzyl $\text{C}_{14}\text{H}_{14}$ White crystals, freely soluble in diethyl ether, soluble in methanol and in ethanol (95), and practically insoluble in water.

Melting point <2.60>: 50 – 54°C

Purity—Dissolve 32 mg of dibenzyl in methanol to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Assay under Vinblastine Sulfate for Injection: any peak other than the principal peak does not appear. Adjust the detection sensitivity so that the peak height of dibenzyl obtained from 20 μL of the solution prepared by adding methanol to 10 mL of the sample solution to make 20 mL, is 3 to 5 cm, and the time span of measurement is about 1.2 times as long as the retention time of dibenzyl after the solvent peak.

***N,N'*-Dibenzylethylenediamine diacetate** A white to slightly pale yellow crystalline powder.

Identification—Determine the infrared absorption spectrum of the substance to be examined as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1530 cm^{-1} , 1490 cm^{-1} , 1460 cm^{-1} , 1400 cm^{-1} and 1290 cm^{-1} .

Content: not less than 99.0%. **Assay**—Weigh accurately about 25 mg of *N,N'*-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 8 mg of acetic acid (100), add 25 mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the control solution. Perform the test with exactly 20 μ L each of the sample solution and control solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. After making correction for the peak areas based on the valiance of the base-line and the peak of acetic acid on the chromatogram obtained with the sample solution, calculate the amount of *N,N'*-dibenzylethylenediamine by the area percentage method.

Operating conditions

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of *N,N'*-dibenzylethylenediamine is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of *N,N'*-dibenzylethylenediamine.

System suitability

System performance: Dissolve an amount of Benzylpenicillin Benzathine, equivalent to about 85,000 Units, in 25 mL of methanol, add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, *N,N'*-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of *N,N'*-dibenzylethylenediamine is not more than 2.0%.

2,6-Dibromo-*N*-chloro-1,4-benzoquinone monoimine
C₆H₂Br₂:ClNO [K 8491, Special class]

2,6-Dibromo-*N*-chloro-1,4-benzoquinone monoimine TS
Dissolve 0.5 g of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

2,6-Dibromo-*N*-chloro-1,4-benzoquinone monoimine TS, dilute
Dissolve 0.2 g of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in methanol to make 100 mL.

2,6-Dibromoquinone chlorimide See 2,6-dibromo-*N*-chloro-1, 4-benzoquinone monoimine.

2,6-Dibromoquinone chlorimide TS See 2,6-dibromo-*N*-chloro-1, 4-benzoquinone monoimine TS.

Dibucaine hydrochloride C₂₀H₂₉N₃O₂.HCl [Same as the namesake monograph]

Di-*n*-butyl ether (C₄H₉)₂O Clear colorless, water-non-miscible liquid.

Specific gravity <2.56> d_4^{20} : 0.768 ~ 0.771

Di-*n*-butyl phthalate C₆H₄(COOC₄H₉)₂ Clear colorless liquid.

Purity Related substances—Dissolve 0.5 g of di-*n*-butyl phthalate in 50 mL of methanol, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed in the Assay under Nicardipine Hydrochloride Injection, and determine the peak area by the automatic integration method. Calculate the amount of di-*n*-butyl phthalate by the area percentage method: the amount of di-*n*-butyl phthalate is not less than 98.0%, and no peak appears at the same position as nicardipine. Adjust the detection sensitivity so that the peak height of di-*n*-butyl phthalate obtained from 10 μ L of the sample solution is 50 to 100% of the full scale, and measure about 2 times as long as the retention time of di-*n*-butyl phthalate after the solvent peak.

1,2-Dichlorobenzene C₆H₄Cl₂ A colorless liquid.

Boiling point <2.57>: 180 – 181°C

Specific gravity <2.56> d_4^{20} : 1.306

1,2-Dichloroethane ClCH₂CH₂Cl [K 8465, Special class]

Dichlorofluorescein C₂₀H₁₀Cl₂O₅ Orange to red-brown powder.

Identification—(1) Dissolve 0.1 g in 10 mL of sodium hydroxide TS: the solution is an orange-red color, and red-orange precipitates appear by the addition of 10 mL of dilute hydrochloric acid.

(2) Dissolve 0.1 g in 10 mL of sodium hydroxide TS, and add 40 mL of water: a green-yellow fluorescence is exhibited.

Dichlorofluorescein TS Dissolve 0.1 g of dichlorofluorescein in 60 mL of ethanol (95), add 2.5 mL of 0.1 mol/L sodium hydroxide VS, and dilute with water to make 100 mL.

2,6-Dichloroindophenol sodium dihydrate
C₁₂H₆Cl₂NNaO₂.2H₂O [K 8469, Special class]

2,6-Dichloroindophenol sodium TS Add 0.1 g of 2,6-dichloroindophenol sodium dihydrate to 100 mL of water, warm, and filter. Use within 3 days.

2,6-Dichloroindophenol sodium TS for titration See the monograph Ascorbic Acid Powder.

Dichloromethane CH₂Cl₂ [K 8161, Special class]

2,6-Dichlorophenol C₆H₄Cl₂O White to purplish white

crystals.

Melting point <2.60>: 65 – 67°C

2,6-Dichlorophenol-indophenol sodium See 2,6-dichloroindophenol sodium dihydrate.

2,6-Dichlorophenol-indophenol sodium TS See 2,6-dichloroindophenol sodium TS.

2,6-Dichlorophenol-indophenol sodium TS for titration See 2,6-dichloroindophenol sodium TS for titration.

Dicyclohexyl C₁₂H₂₂

Boiling point <2.57>: about 227°C

Melting point <2.60>: about 4°C

Specific gravity <2.56> d_{20}^{20} : about 0.864

***N,N'*-Dicyclohexylcarbodiimide** C₆H₁₁N=C=NC₆H₁₁
Colorless or white crystals or crystalline mass. Dissolves in ethanol (95), but decomposes in water to produce a white precipitate.

Melting point <2.60>: 35 – 36°C

***N,N'*-Dicyclohexylcarbodiimide-dehydrated ethanol TS** See *N,N'*-dicyclohexylcarbodiimide-ethanol (99.5) TS.

***N,N'*-Dicyclohexylcarbodiimide-ethanol (99.5) TS** Dissolve 6 g of *N,N'*-dicyclohexylcarbodiimide in ethanol (99.5) to make 100 mL.

Storage—Preserve in tight containers, in a cold place.

Dicyclohexyl phthalate C₆H₄(COOC₆H₁₁)₂ A white, crystalline powder.

Melting point <2.60>: 63 – 66°C

Purity Clarity and color of solution—Dissolve 1.0 g of dicyclohexyl phthalate in 20 mL of ethanol (95): the solution is clear and colorless.

Dicyclohexylurea C₆H₁₁NHCONHC₆H₁₁ A white crystalline powder, having no odor.

Purity Related substances—Dissolve 50 mg of dicyclohexylurea in methanol to make 100 mL. Pipet 10 mL of this solution, and add methanol to make 100 mL. Pipet 20 mL of this solution, add 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add 5 mL of diluted hydrochloric acid (1 in 10), and shake. Perform the test with 50 μL of this solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the area of each peak by the automatic integration method, and calculate the amount by the area percentage method: the total amount of the peaks other than dicyclohexylurea is not more than 3.0%.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (5) (ii) under Acetohexamide.

Time span of measurement: About 5 times as long as the retention time of dicyclohexylurea beginning after the solvent peak.

System suitability

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 200 mL. Confirm that the peak area of dicyclohexylurea obtained with 50 μL of this solution is equivalent to 1.8 to 3.3% of that with 50 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Purity (5) (ii) under Acetohexamide.

Diethanolamine C₄H₁₁NO₂ Colorless viscous liquid.

Melting point <2.60>: 27 – 30°C

Water <2.48>: less than 0.1%.

Diethanolamine hydrochloride C₄H₁₁NO₂.HCl A pale yellow liquid.

Refractive index <2.45> n_D^{20} : 1.515 – 1.519

Specific gravity <2.56> d_{20}^{20} : 1.259 – 1.263

Water <2.48>: less than 0.1%.

Diethylamine (C₂H₅)₂NH A clear, colorless liquid, having an amine-like odor. Miscible with water and with ethanol (95). The solution in water is alkaline, and readily absorbs carbon dioxide in air.

Distilling range <2.57>: 54 – 58°C; not less than 96 vol%.

Specific gravity <2.56> d_4^{10} : 0.702 – 0.708

Content: not less than 99.0%. Assay—Weigh accurately about 1.5 g of diethylamine in a flask containing exactly 30 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> the excess of sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS
= 73.14 mg of (C₂H₅)₂NH

Diethylene glycol HO(CH₂CH₂O)₂H Colorless and odorless liquid. Miscible with water and with ethanol (95).

Specific gravity <2.56> d_{20}^{20} : 1.118 – 1.120

Diethylene glycol adipate for gas chromatography Prepared for gas chromatography.

Diethylene glycol dimethyl ether (CH₃OCH₂CH₂)₂O Clear and colorless liquid, miscible with water.

Specific gravity <2.56> d_4^{20} : 0.940 – 0.950

Distilling range <2.57>: 158 – 160°C, not less than 95 vol%.

Diethylene glycol monoethyl ether

C₂H₅(OCH₂CH₂)₂OH [2-(2-ethoxyethoxy)ethanol] Clear, colorless liquid, of which boiling point is about 203°C. It freely mixed with water.

Acid (as CH₃COOH): less than 0.01%.

Refractive index <2.45> n_D^{20} : 1.425 – 1.429

Specific gravity <2.56> d_{20}^{20} : 0.990 – 0.995

Diethylene glycol monoethyl ether for water determination See Water Determination <2.48>.

Diethylene glycol succinate ester for gas chromatography Prepared for gas chromatography.

Diethylene glycol succinate polyester for gas chromatography Prepared for gas chromatography.

Diethyl ether C₂H₅OC₂H₅ [K 8103, Special class]

Diethyl ether, dehydrated C₂H₅OC₂H₅ [K 8103, Special class. The water content is not more than 0.01%.]

Diethyl ether for purity of crude drug [K 8103, Special class] Use diethyl ether meeting the following additional specification. Evaporate 300.0 mL of diethyl ether for purity of crude drug in vacuum at a temperature not higher than 40°C, add the diethyl ether to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg

of γ -BHC in hexane for purity of crude drug to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane for purity of crude drug to make exactly 100 mL. Pipet 2 mL of this solution, add hexane for purity of crude drug to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with 1 μ L each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the solvent peak from the sample solution is not larger than the peak area of γ -BHC from the standard solution I.

Operating conditions

Proceed the operating conditions in the Purity (2) under the Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane for purity of crude drug to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of γ -BHC obtained from 1 μ L of the standard solution II can be measured by the automatic integration method, and the peak height of γ -BHC from 1 μ L of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ -BHC beginning after the peak of solvent.

***N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate**
C₁₈H₂₄N₂O₄ A white crystalline powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3340 cm⁻¹, 2940 cm⁻¹, 1581 cm⁻¹, 1536 cm⁻¹, 1412 cm⁻¹, 789 cm⁻¹, 774 cm⁻¹ and 721 cm⁻¹.

Purity Clarity of solution—To 0.1 g add 20 mL of water, and dissolve by warming: the solution is clear.

***N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS** Dissolve 1 g of *N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate in 100 mL of a mixture of acetone and water (1:1). Prepare before use.

***N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate TS** Dissolve 1 g of *N,N*-Diethyl-*N'*-1-naphthylethylenediamine oxalate in water to make 1000 mL.

Diethyl phthalate C₆H₄(COOC₂H₅)₂ A colorless, clear liquid.

Refractive index <2.45> n_D^{20} : 1.500 – 1.505

Purity Related substances—To 1 mL of diethyl phthalate add a solution of tetra *n*-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 100 mL. To 6 mL of this solution add a solution of tetra *n*-heptylammonium bromide in a mixture of water, acetonitrile and methanol (137:80:23) (2 in 625) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed in the Assay under Cefetamet Pivoxil Hydrochloride: any peaks other than peaks of diethyl phthalate and the solvent are not observed.

Diethyl terephthalate C₆H₄(COOC₂H₅)₂ White to pale brownish white, crystalline or mass.

Melting point <2.60>: 44 – 46°C

Content: not less than 99%. Assay—Dissolve 100 mg of diethyl terephthalate in 10 mL of methanol. Perform the test with 2 μ L of this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the area of each peak by the automatic integration method.

$$\text{Content} = \frac{\text{peak area of diethyl terephthalate}}{\text{total of all peak areas}} \times 100$$

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass tube 4 mm in inside diameter and 2 m in length, packed with Shimalite W(AW, DMCS) coated with SE-30 in 10% (177 – 250 μ m in particle diameter).

Column temperature: A constant temperature of about 200°C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of diethyl terephthalate is between 6 and 7 minutes.

Time span of measurement: About 5 times as long as the retention time of diethyl terephthalate beginning after the solvent peak.

Digitonin C₅₆H₉₂O₂₉ [K 8452, Special class]

Digoxin C₄₁H₆₄O₁₄ [Same as the namesake monograph]

Dihydrocodeine phosphate for assay

C₁₈H₂₃NO₃·H₃PO₄ [Same as the monograph Dihydrocodeine Phosphate. It contains not less than 99.0% of dihydrocodeine phosphate (C₁₈H₂₃NO₃·H₃PO₄), calculated on the dried basis.

Dihydroergocristine mesilate for thin-layer chromatography C₃₅H₄₁N₅O₅·CH₄O₃S A pale yellowish white powder. Freely soluble in methanol, in ethanol (95) and in chloroform, soluble in acetonitrile, sparingly soluble in water, and practically insoluble in diethyl ether. Melting point: about 190°C (with decomposition).

Purity Related substances—Dissolve 6 mg of dihydroergocristine mesilate for thin-layer chromatography in exact 100 mL of a mixture of chloroform and methanol (9:1), and perform the test with 5 μ L of this solution as directed in the Purity (3) under Dihydroergocristine Mesilate: any spot other than the principal spot at the R_f value around 0.4 does not appear.

3,4-Dihydro-6-hydroxy-2(1H)-quinolinone

C₉H₉NO₂ A white to light brown powder or granule. Melting point: about 240°C (with decomposition).

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3210 cm⁻¹, 1649 cm⁻¹, 1502 cm⁻¹, 1252 cm⁻¹ and 816 cm⁻¹.

2,4-Dihydroxybenzoic acid C₇H₆O₄ White to pale brown powder.

Purity Clarity of solution—Dissolve 1.0 g of 2,4-dihydroxybenzoic acid in 20 mL of ethanol (95): the solution is clear.

Content: not less than 95%. Assay—Weigh accurately about 1 g of 2,4-dihydroxybenzoic acid, dissolve in 50 mL of ethanol (95) and 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS.

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.41 mg of $C_7H_6O_4$

1,3-Dihydroxynaphthalene $C_{10}H_8(OH)_2$ Purple-brown, crystals or powder. Freely soluble in water and in ethanol (95).

Melting point <2.60>: about 125°C

2,7-Dihydroxynaphthalene $C_{10}H_8(OH)_2$
Purity: not less than 97.0%.

2,7-Dihydroxynaphthalene TS Dissolve 0.10 g of 2,7-dihydroxynaphthalene in 1000 mL of sulfuric acid, and allow to stand until the yellow color initially developed disappears. If the solution is blackened notably, prepare freshly.

Diltiazem hydrochloride $C_{22}H_{26}N_2O_4S.HCl$ [Same as the namesake monograph]

Dilute acetic acid See acetic acid, dilute.

Dilute bismuth subnitrate-potassium iodide TS for spray Dissolve 10 g of L-tartaric acid in 50 mL of water, and add 5 mL of bismuth subnitrate TS.

Dilute bromophenol blue TS See bromophenol blue TS, dilute.

Diluted ethanol See ethanol, diluted.

Dilute ethanol See ethanol, dilute.

Dilute ferric ammonium sulfate TS See ammonium iron (III) sulfate TS, dilute.

Dilute ferric chloride TS See iron (III) chloride TS, dilute.

Dilute hydrochloric acid See hydrochloric acid, dilute.

Dilute hydrogen peroxide TS See hydrogen peroxide TS, dilute.

Dilute iodine TS See iodine TS, dilute.

Dilute iron-phenol TS See iron-phenol TS, dilute.

Dilute lead subacetate TS See lead subacetate TS, dilute.

Dilute methyl red TS See methyl red TS, dilute.

Dilute nitric acid See nitric acid, dilute.

Dilute *p*-dimethylaminobenzaldehyde-ferric chloride TS See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide $C_{18}H_{16}BrN_5S$ Yellow crystals. *Melting point*: about 195°C (with decomposition).

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide $C_{18}H_{16}N_5SBr$ Yellowish crystals, Melting point: 195°C

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide TS Dissolve 5 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide in phosphate-buffered sodium chloride TS to make 1000 mL.

Dilute potassium hydroxide-ethanol TS See potassium hydroxide-ethanol TS, dilute.

Dilute sodium hydroxide TS See sodium hydroxide TS,

dilute.

Dilute sulfuric acid See sulfuric acid, dilute.

Dilute thymol blue TS See thymol blue TS, dilute.

Dilute vanadium pentoxide TS See vanadium (V) oxide TS, dilute.

Dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

Dimedon $C_8H_{12}O_2$ White to pale yellow, crystalline powder.

Melting point <2.60>: 145–149°C

***N,N*-Dimethylacetamide** $CH_3CON(CH_3)_2$ Clear and colorless liquid.

Boiling point <2.57>: 163 – 165°C

Specific gravity <2.56> *d*: 0.938 – 0.945 (Method 3).

Water <2.48>: not more than 0.2% (0.1 g, Coulometric titration).

Purity—Perform the test with 3 μ L of *N,N*-dimethylacetamide as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of *N,N*-dimethylacetamide by the area percentage method: not less than 98.0%.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A fused silica column 0.25 mm in inside diameter and 30 m in length, coated the inside surface 0.5 μ m in thickness with polyethylene glycol 20 M for gas chromatography.

Column temperature: The sample is injected at a constant temperature of about 70°C, keep this temperature for 1 minute, then raise to 200°C in a rate of 10°C per minute, and keep 200°C for 3 minutes.

Carrier gas: Helium

Flow rate (linear velocity): About 30 cm/sec.

Time span of measurement: About 2 times as long as the retention time of *N,N*-dimethylacetamide.

System suitability

Test for required detection: To exactly 1.0 g of *N,N*-dimethylacetamide add acetone to make exactly 100 mL. Pipet 5 mL of this solution, and add acetone to make exactly 50 mL. Confirm that the peak area of *N,N*-dimethylacetamide obtained from 3 μ L of this solution is equivalent to 40 to 60% of the full-scale.

System repeatability: When the test is repeated with 3 μ L of *N,N*-dimethylacetamide under the above operating conditions, the relative standard deviation of the peak area of *N,N*-dimethylacetamide is not more than 2.0%.

Dimethylamine $(CH_3)_2NH$ Colorless, clear liquid, having amine-like, characteristic odor. It is miscible with water and with ethanol (99.5). It is alkaline.

Specific gravity <2.56> d_{20}^{20} : 0.85 – 0.93

Content: 38.0 – 45.0%. Assay—Weigh accurately about 1.0 g of dimethylamine, transfer to a flask containing exactly 20 mL of 0.5 mol/L sulfuric acid VS, and titrate <2.50> the excess sulfuric acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of methyl red TS). Perform a blank determination in the same manner.

Each mL of 0.5 mol/L sulfuric acid VS

= 45.08 mg of $(\text{CH}_3)_2\text{NH}$

4-Dimethylaminoantipyrine $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}$ Colorless or white crystals, or a white crystalline powder.

Purity—Proceed the test with 5 μL of a solution of 4-dimethylaminoantipyrine (1 in 2000) as directed in the Assay under Cefpiramide Sodium, determine each peak area in a range of about 2 times as long as the retention time of 4-dimethylaminoantipyrine after the solvent peak by the automatic integration method, and calculate the total amount of the peaks other than 4-dimethylaminoantipyrine by the area percentage method: not more than 1.0%.

***p*-Dimethylaminobenzaldehyde** See 4-dimethylaminobenzaldehyde.

4-Dimethylaminobenzaldehyde $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CHO}$ [K 8496, *p*-Dimethylaminobenzaldehyde, Special class]

***p*-Dimethylaminobenzaldehyde-ferric chloride TS** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS.

***p*-Dimethylaminobenzaldehyde-ferric chloride TS, dilute** See 4-dimethylaminobenzaldehyde-iron (III) chloride TS, dilute.

***p*-Dimethylaminobenzaldehyde-hydrochloric acid TS** See 4-dimethylaminobenzaldehyde-hydrochloric acid TS.

4-Dimethylaminobenzaldehyde-hydrochloric acid TS Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 50 mL of hydrochloric acid while cooling, and add 50 mL of ethanol (95).

4-Dimethylaminobenzaldehyde-iron (III) chloride TS Dissolve 0.125 g of 4-dimethylaminobenzaldehyde in a cold mixture of 65 mL of sulfuric acid and 35 mL of water, then add 0.05 mL of iron (III) chloride TS. Use within 7 days.

4-Dimethylaminobenzaldehyde-iron (III) chloride TS, dilute To 80 mL of water add carefully 100 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS and 0.15 mL of iron (III) chloride TS, while cooling with ice.

***p*-Dimethylaminobenzaldehyde TS** See 4-dimethylaminobenzaldehyde TS.

4-Dimethylaminobenzaldehyde TS Dissolve 10 g of 4-dimethylaminobenzaldehyde in a cold mixture of 90 mL of sulfuric acid and 10 mL of water. Prepare before use.

***p*-Dimethylaminobenzaldehyde TS for spraying** See 4-dimethylaminobenzaldehyde TS for spraying.

4-Dimethylaminobenzaldehyde TS for spraying Dissolve 1.0 g of 4-dimethylaminobenzaldehyde in 20 mL of dilute sulfuric acid. Prepare before use.

***p*-Dimethylaminobenzylidene rhodanine** See 4-dimethylaminobenzylidene rhodanine.

4-Dimethylaminobenzylidene rhodanine $\text{C}_{12}\text{H}_{12}\text{N}_2\text{OS}_2$ [K 8495, Special class]

***p*-Dimethylaminobenzylidene rhodanine TS** See 4-dimethylaminobenzylidene rhodanine TS.

4-Dimethylaminobenzylidene rhodanine TS Dissolve 0.02 g of 4-dimethylaminobenzylidene rhodanine in acetone to make 100 mL.

***p*-Dimethylaminocinnamaldehyde** See 4-dimethylamino-

cinnamaldehyde.

4-Dimethylaminocinnamaldehyde $\text{C}_{11}\text{H}_{13}\text{NO}$ Orange crystals or crystalline powder, having a characteristic odor. Freely soluble in dilute hydrochloric acid, sparingly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 140 – 142°C

Purity Clarity of solution—Dissolve 0.20 g of 4-dimethylaminocinnamaldehyde in 20 mL of ethanol (95); the solution is clear.

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44>: not more than 0.1% (1 g).

Nitrogen content <1.08>: 7.8 – 8.1% (105°C, 2 hours, after drying).

***p*-Dimethylaminocinnamaldehyde TS** See 4-dimethylaminocinnamaldehyde TS.

4-Dimethylaminocinnamaldehyde TS Before use, add 1 mL of acetic acid (100) to 10 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 2000).

Dimethylaminophenol $(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{OH}$ Dark purple, crystals or crystalline mass.

Melting point <2.60>: 85°C

Dimethylaniline See *N,N*-dimethylaniline.

***N,N*-Dimethylaniline** $\text{C}_6\text{H}_5\text{N}(\text{CH}_3)_2$ Colorless or light yellow liquid, having a characteristic odor.

Specific gravity <2.56> d_{20}^{20} : 0.955 – 0.960

Distilling range <2.57>: 192 – 195°C, not less than 95 vol%.

Dimethylformamide See *N,N*-dimethylformamide.

***N,N*-Dimethylformamide** $\text{HCON}(\text{CH}_3)_2$ [K 8500, Special class]

***N,N*-Dimethylformamide for liquid chromatography** [K 8500, *N,N*-Dimethylformamide, Special class] Read absorbance as directed under Ultraviolet-visible Spectrophotometry <2.24> (in a 1-cm cell, using water as the blank): the absorbance is not more than 0.60 at 270 nm, not more than 0.15 at 280 nm, and not more than 0.05 at 300 nm.

Dimethylglyoxime $\text{C}_4\text{H}_8\text{N}_2\text{O}_2$ [K 8498, Special class]

Dimethylglyoxime-thiosemicarbazide TS Solution A: Dissolve 0.5 g of dimethylglyoxime in hydrochloric acid to make 100 mL. Prepare before use. Solution B: Dissolve 0.1 g of thiosemicarbazide in 50 mL of water with the acid of warming if necessary, and add diluted hydrochloric acid (1 in 2) to make 100 mL. Prepare before use.

Mix 10 mL each of solution A and solution B, add diluted hydrochloric acid (1 in 2) to make 100 mL, and allow the mixture to stand for 1 hour. Use within 24 hours.

Dimethylglyoxime TS Dissolve 1 g of dimethylglyoxime in ethanol (95) to make 100 mL.

Dimethyl malonate $\text{C}_5\text{H}_8\text{O}_4$ Clear, colorless or pale yellow liquid.

Specific gravity <2.56> d_4^{20} : 1.152 – 1.162

Water <2.48>: not more than 0.3%.

Residue on ignition <2.44>: not more than 0.1%.

***N,N*-Dimethyl-*n*-octylamine** $\text{C}_{10}\text{H}_{23}\text{N}$ Colorless liquid.

Refractive index <2.45> n_D^{20} : 1.424

Dimethyl phthalate $C_{16}H_{22}O_4$ Colorless, clear liquid, having a slight aroma.

Refractive index <2.45> n_D^{20} : 1.491 – 1.493

Purity—To 6.0 mL of a solution of Dimethyl phthalate in isoctane (1 in 100) add a solution of *n*-amyl alcohol in hexane (3 in 1000) to make 50 mL, and perform the test with 10 μ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay under Ergocalciferol or Cholecalciferol: any peak other than the principal peak does not appear.

***N,N*-Dimethyl-*p*-phenylenediammonium dichloride**
 $H_2NC_6H_4N(CH_3)_2 \cdot 2HCl$ [K 8193, *N,N*-Dimethyl-*p*-phenylenediammonium dichloride, Special class]

***N,N*-Dimethyl-*p*-phenylenediammonium hydrochloride**
See *N,N*-dimethyl-*p*-phenylenediamine dichloride.

Dimethylsulfoxide $(CH_3)_2SO$ [K 9702, Special class]

Dimethylsulfoxide for ultraviolet-visible spectrophotometry Colorless crystals or clear colorless liquid, having a characteristic odor. It is highly hygroscopic.

Congearing point <2.42>: not less than 18.3°C.

Purity—Read absorbance of dimethylsulfoxide for ultraviolet-visible spectrophotometry, immediately after saturating with nitrogen, using water as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: its value is not more than 0.20 at 270 nm, not more than 0.09 at 275 nm, not more than 0.06 at 280 nm, and not more than 0.015 at 300 nm. It exhibits no characteristic absorption between 260 nm and 350 nm.

Water <2.48>: not more than 0.1%.

2,6-Dimethyl-4-(2-nitrosophenyl)3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography
 $C_{17}H_{16}N_2O_5$ Irradiate xenon light at 50,000 lx of illumination for 8 hours to a methanol solution of nifedipine (1 in 100), and evaporate the methanol on a water bath. Recrystallize the residue 4 times from 1-propanol, and dry in a desiccator (in vacuum, phosphorus pentoxide). Pale blue crystals. Very soluble in chloroform, freely soluble in acetone, and practically insoluble in water.

Melting point <2.60>: 93 – 95°C

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.4 g of 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylic acid dimethyl ester for thin-layer chromatography, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 32.83 mg of $C_{17}H_{16}N_2O_5$

3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide $C_{18}H_{16}BrN_5S$ Yellow crystals. Melting point: about 195°C (with decomposition).

Dimorpholamine for assay [Same as the monograph Dimorpholamine. When dried, it contains not less than 99.0% of dimorpholamine ($C_{20}H_{38}N_4O_4$).

***m*-Dinitrobenzene** See 1,3-dinitrobenzene.

1,3-Dinitrobenzene $C_6H_4(NO_2)_2$ [K 8482, *m*-Dinitrobenzene, Special class]

***m*-Dinitrobenzene TS** See 1,3-dinitrobenzene TS.

1,3-Dinitrobenzene TS Dissolve 1 g of 1,3-dinitrobenzene in 100 mL of ethanol (95). Prepare before use.

***m*-Dinitrobenzene TS, alkaline** See 1,3-dinitrobenzene TS, alkaline.

1,3-Dinitrobenzene TS, alkaline Mix 1 mL of tetramethylammonium hydroxide and 140 mL of ethanol (99.5), titrate a part of the mixture with 0.01 mol/L hydrochloric acid VS, and dilute the remainder with ethanol (99.5) to give a 0.008 mol/L solution. Before use, mix 40 mL of this solution with 60 mL of a solution of 1,3-dinitrobenzene in benzene (1 in 20).

2,4-Dinitrochlorobenzene See 1-chloro-2, 4-dinitrobenzene.

2,4-Dinitrofluorobenzene See 1-fluoro-2, 4-dinitrobenzene.

2,4-Dinitrophenol $C_6H_3OH(NO_2)_2$ Yellow crystals or crystalline powder.

Melting point <2.60>: 110 – 114°C

2,4-Dinitrophenol TS Dissolve 0.5 g of 2,4-dinitrophenol in 100 mL of ethanol (95).

2,4-Dinitrophenylhydrazine $(NO_2)_2C_6H_3NHNH_2$
[K 8480, Special class]

2,4-Dinitrophenylhydrazine-diethylene glycol dimethyl ether TS Dissolve 3 g of 2,4-dinitrophenylhydrazine in 100 mL of diethylene glycol dimethyl ether while heating, cool, and filter if necessary.

2,4-Dinitrophenylhydrazine-ethanol TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add a mixture of 1 volume of aldehyde-free ethanol and 3 volumes of water to make 100 mL, and filter if necessary.

2,4-Dinitrophenylhydrazine TS Dissolve 1.5 g of 2,4-dinitrophenylhydrazine in a cold mixture of 10 mL of sulfuric acid and 10 mL of water, then add water to make 100 mL, and filter if necessary.

Dinonyl phthalate $C_6H_4(COOC_9H_{19})_2$ Colorless to pale yellow, clear liquid.

Acid value <1.13>: not more than 2.

Specific gravity <2.56> d_{20}^{20} : 0.967 – 0.987

Dioxane See 1,4-dioxane.

1,4-Dioxane $C_4H_8O_2$ [K 8461, Special class]

Diphenhydramine $C_{17}H_{21}NO$ [Same as the namesake monograph]

Diphenhydramine tannate [Same as the namesake monograph]

Diphenyl $C_{12}H_{10}$ White crystals or crystalline powder, having a characteristic odor. Freely soluble in acetone and in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

Melting point <2.60>: 68 – 72°C

Purity—Dissolve 0.1 g of diphenyl in 5 mL of acetone and use this solution as the sample solution. Perform the test with

2 μL of this solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of diphenyl by the area percentage method: it shows the purity of not less than 98.0%.

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass tube about 3 mm in inside diameter and about 2 m in length, packed with 150 to 180 μm mesh siliceous earth for gas chromatography coated with 10% of polyethylene glycol 20 M for thin-layer chromatography.

Column temperature: A constant temperature of about 180°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of diphenyl is about 8 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diphenyl obtained from 2 μL of the solution prepared by adding acetone to 1.0 mL of the sample solution to make 100 mL, is 5% to 15% of the full scale.

Time span of measurement: About 3 times as long as the retention time of diphenyl beginning after the solvent peak.

Diphenylamine $(\text{C}_6\text{H}_5)_2\text{NH}$ [K 8487, Special class]

Diphenylamine-acetic acid TS Dissolve 1.5 g of diphenylamine in 1.5 mL of sulfuric acid and acetic acid (100) to make 100 mL.

Diphenylamine-acetic acid (100) TS See diphenylamine-acetic acid TS.

Diphenylamine TS Dissolve 1 g of diphenylamine in 100 mL of sulfuric acid. Use the colorless solution.

9,10-Diphenylanthracene $\text{C}_{26}\text{H}_{18}$ Yellow crystalline powder. Soluble in diethyl ether, and practically insoluble in water.

Melting point <2.60>: about 248°C

1,4-Diphenylbenzene $\text{C}_{18}\text{H}_{14}$ White scaly crystals, having a slight aromatic odor. It is freely soluble in ethanol (99.5), and slightly soluble in water.

Identification—Determine the infrared absorption spectrum of 1,4-diphenylbenzene as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3050 cm^{-1} , 3020 cm^{-1} , 1585 cm^{-1} , 1565 cm^{-1} , 1476 cm^{-1} , 1450 cm^{-1} , 995 cm^{-1} , 834 cm^{-1} , 740 cm^{-1} and 680 cm^{-1} .

Diphenylcarbazine See 1,5-diphenylcarbonohydrazide.

Diphenylcarbazine TS See 1,5-diphenylcarbonohydrazide TS.

Diphenylcarbazone [K 8489, Special class]

Diphenylcarbazone TS Dissolve 1 g of diphenylcarbazone in ethanol (95) to make 1000 mL.

1,5-Diphenylcarbonohydrazide $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}$ [K 8488, Special class]

1,5-Diphenylcarbonohydrazide TS Dissolve 0.2 g of 1,5-diphenylcarbonohydrazide in 100 mL of a mixture of ethanol (95) and acetic acid (100) (9:1).

5% **Diphenyl-95% dimethylpolysiloxane for gas chro-**

matography Prepared for gas chromatography.

Diphenyl ether $\text{C}_{12}\text{H}_{10}\text{O}$ Colorless crystals, having a geranium-like aroma. Dissolves in alcohol (95) and in diethyl ether, and practically insoluble in water.

Boiling point <2.57>: 254 – 259°C

Melting point <2.60>: 28°C

Specific gravity <2.56> d_{20}^{20} : 1.072 – 1.075

Diphenyl imidazole $\text{C}_{15}\text{H}_{12}\text{N}_2$ White crystals or crystalline powder, freely soluble in acetic acid (100), and sparingly soluble in methanol.

Melting point <2.60>: 234 – 238°C

Loss on drying <2.41>: not more than 0.5% (0.5 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Dissolve about 0.3 g of diphenyl imidazole, previously dried and weighed accurately, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS).

Each mL of 0.1 mol/L perchloric acid VS
= 22.03 mg of $\text{C}_{15}\text{H}_{12}\text{N}_2$

Diphenyl phthalate $\text{C}_6\text{H}_4(\text{COOC}_6\text{H}_5)_2$ White crystalline powder.

Melting point <2.60>: 71 – 76°C

Purity Related substances—Dissolve 0.06 g of diphenyl phthalate in 50 mL of chloroform and use this solution as the sample solution. Proceed with 10 μL of the sample solution as directed in the Assay under Tolnaftate Solution: any peak other than the principal peak at the retention time of about 8 minutes and the peak of the solvent does not appear. Adjust the detection sensitivity so that the peak height of diphenyl phthalate obtained from 10 μL of the sample solution is 50 to 100% of the full scale, and the time span of measurement is about twice as long as the retention time of diphenyl phthalate after the solvent peak.

1,1-Diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography $\text{C}_{21}\text{H}_{25}\text{N} \cdot \text{HCl}$ To 1 g of diphenidole hydrochloride add 30 mL of 1 mol/L hydrochloric acid TS, and heat under a reflux condenser for 1 hour. After cooling, extract twice with 30 mL-portions of chloroform, combine the chloroform extracts, wash twice with 10 mL portions of water, and evaporate chloroform under reduced pressure. Recrystallize the residue from a mixture of diethyl ether and ethanol (95) (3:1), and dry in a desiccator (in vacuum, silica gel) for 2 hours. White crystals or crystalline powder.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (250 nm): 386 – 446 (10 mg, water, 1000 mL).

Melting point <2.60>: 176 – 180°C

Content: not less than 99.0%. Assay—Dissolve about 0.2 g of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography, previously weighed accurately, in 20 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 16.40 mg of $\text{C}_{21}\text{H}_{25}\text{N} \cdot \text{HCl}$

Dipotassium hydrogen phosphate K_2HPO_4 [K 9017,

Special class]

Dipotassium hydrogen phosphate-citric acid buffer solution, pH 5.3 Mix 100 mL of 1 mol/L dipotassium hydrogen phosphate TS for buffer solution and 38 mL of 1 mol/L citric acid TS for buffer solution, and add water to make 200 mL.

1 mol/L Dipotassium hydrogen phosphate TS for buffer solution Dissolve 174.18 g of dipotassium hydrogen phosphate in water to make 1000 mL.

Diprophylline $C_{10}H_{14}N_4O_4$ A white, powder or grain. Freely soluble in water, and slightly soluble in ethanol (95).

Identification—Determine the infrared absorption spectrum of the substance to be examined, previously dried at 105 °C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3460 cm^{-1} , 3330 cm^{-1} , 1651 cm^{-1} , 1242 cm^{-1} , 1059 cm^{-1} and 1035 cm^{-1} .

α, α' -Dipyridyl See 2,2'-bipyridyl.

Disodium chromotropate dihydrate

$C_{10}H_6Na_2O_8S_2 \cdot 2H_2O$ [K 8316, Special class] Preserve in light-resistant containers.

Disodium dihydrogen ethylenediamine tetraacetate dihydrate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ [K 8107, Special class]

0.1 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS Dissolve 37.2 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

0.04 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS Dissolve 14.890 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 1000 mL.

0.4 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5 Dissolve 148.9 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in about 800 mL of water, adjust to pH 8.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Disodium ethylenediaminetetraacetate See disodium dihydrogen ethylenediamine tetraacetate dihydrate.

Disodium ethylenediaminetetraacetate copper See copper (II) disodium ethylenediamine tetraacetate tetrahydrate.

0.1 mol/L Disodium ethylenediaminetetraacetate TS See 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Disodium hydrogen phosphate, anhydrous Na_2HPO_4 [K 9020, Special class]

Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0 Dissolve 35.8 g of disodium hydrogen phosphate 12-water in water to make 500 mL. To this solution add a solution of citric acid monohydrate (21 in 1000) to adjust the pH to 3.0.

Disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 Dissolve 21.02 g of citric acid monohydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 35.82 g of disodium hydrogen phosphate 12-water in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution,

pH 5.4 Dissolve 1.05 g of citric acid monohydrate and 2.92 g of disodium hydrogen phosphate dodecahydrate in 200 mL of water, and adjust the pH with phosphoric acid or sodium hydroxide TS, if necessary.

Disodium hydrogen phosphate-citric acid buffer solution, 0.05 mol/L, pH 6.0 To 1000 mL of 0.05 mol/L disodium hydrogen phosphate TS add a solution prepared by dissolving 5.25 g of citric acid monohydrate in water to make 1000 mL to adjust pH 6.0.

Disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL. To this solution add a solution, prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL, until the pH becomes 6.0 (ratio of volume: about 63:37).

Disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 Dissolve 7.1 g of disodium hydrogen phosphate in water to make 1000 mL. Adjust this solution to pH 7.2 with a solution prepared by dissolving 5.3 g of citric acid monohydrate in water to make 1000 mL.

Disodium hydrogen phosphate-citric acid buffer solution for penicillium origin β -galactosidase, pH 4.5 Dissolve 71.6 g of disodium hydrogen phosphate dodecahydrate in water to make 1000 mL, and adjust the pH to 4.5 with a solution prepared by dissolving 21.0 g of citric acid monohydrate in water to make 1000 mL (volume ratio: about 44:56).

Disodium hydrogen phosphate for pH determination Na_2HPO_4 [K 9020, for pH determination]

Disodium hydrogen phosphate TS Dissolve 12 g of disodium hydrogen phosphate dodecahydrate in water to make 100 mL (0.3 mol/L).

0.05 mol/L Disodium hydrogen phosphate TS Dissolve 7.0982 g of disodium hydrogen phosphate in water to make 1000 mL.

0.5 mol/L Disodium hydrogen phosphate TS Dissolve 70.982 g of disodium hydrogen phosphate in water to make 1000 mL.

Disodium hydrogen phosphate dodecahydrate $Na_2HPO_4 \cdot 12H_2O$ [K 9019, Special class]

Disodium 1-nitroso-2-naphthol-3,6-disulfonate $C_{10}H_5NNa_2O_8S_2$ [K 8714, Special class]

Dissolved acetylene C_2H_2 [K 1902]

Distigmine bromide for assay [Same as the monograph Distigmine Bromide. It contains not less than 99.0% of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$), calculated on the anhydrous basis.]

Distilled water for injection [Same as the monograph Water for Injection. Prepared by distillation.]

2,6-Di-*tert*-butylcresol $[(CH_3)_3C]_2C_6H_2(CH_3)OH$
A white, crystalline powder. Freely soluble in ethanol (95).
Melting point <2.60>: 69 – 71 °C
Residue on ignition <2.44>: not more than 0.05%.

2,6-Di-*tert*-butylcresol TS Dissolve 0.1 g of 2,6-di-*tert*-butylcresol in ethanol (95) to make 10 mL.

2,6-Di-*tert*-butyl-*p*-cresol See 2,6-di-*tert*-butylcresol.

2,6-Di-*tert*-butyl-*p*-cresol TS See 2,6-di-*tert*-butylcresol TS.

1,3-Di (4-pyridyl) propane $C_{13}H_{14}N_2$ A pale yellow powder.

Melting point <2.60>: 61 – 62°C

Water <2.48>: less than 0.1%.

1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-L-diproline $C_{18}H_{28}N_2O_6S_2$ White, crystals or crystalline powder. Sparingly soluble in methanol, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline according to potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2960 cm^{-1} , 1750 cm^{-1} , 1720 cm^{-1} , 1600 cm^{-1} , 1480 cm^{-1} , 1450 cm^{-1} and 1185 cm^{-1} .

Purity Related substances—Dissolve 0.10 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline in exactly 10 mL of methanol. Perform the test with this solution as directed in the Purity (3) under Captopril: any spot other than the principal spot at the *Rf* value of about 0.2 does not appear.

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.3 g of 1,1'-[3,3'-dithiobis (2-methyl-1-oxopropyl)]-L-diproline, dissolve in 20 mL of methanol, add 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through bluish green to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.63 mg of $C_{18}H_{28}N_2O_6S_2$

Dithiothreitol $C_4H_{10}O_2S_2$ Crystals.

Melting point <2.60>: about 42°C

Dithizone $C_6H_5NHNHCSN:NC_6H_5$ [K 8490, Special class]

Dithizone solution for extraction Dissolve 30 mg of dithizone in 1000 mL of chloroform, and add 5 mL of ethanol (95). Store in a cold place. Before use, shake a suitable volume of the solution with one-half of its volume of diluted nitric acid (1 in 100), and use the chloroform layer after discarding the water layer.

Dithizone TS Dissolve 25 mg of dithizone in ethanol (95) to make 100 mL. Prepare before use.

Dopamine hydrochloride for assay $C_8H_{11}NO_2 \cdot HCl$ [Same as the monograph Dopamine hydrochloride. When dried, it contains not less than 99.0% of dopamine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$).

Doxifluridine $C_9H_{11}FN_2O_5$ [Same as the namesake monograph]

Doxifluridine for assay $C_9H_{11}FN_2O_5$ [Same as the monograph Doxifluridine. When dried, it contains not less than 99.5% of doxifluridine ($C_9H_{11}FN_2O_5$).

Dragendorff's TS Dissolve 0.85 g of bismuth subnitrate in 10 mL of acetic acid (100) and 40 mL of water with vigorous shaking (solution A). Dissolve 8 g of potassium iodide in 20 mL of water (solution B). Immediately before use, mix equal volumes of solution A, solution B and acetic acid (100). Store solution A and solution B in light-resistant

containers.

Dragendorff's TS for spraying Add 20 mL of diluted acetic acid (31) (1 in 5) to 4 mL of a mixture of equal volumes of solution A and solution B of Dragendorff's TS. Prepare before use.

Dried human normal plasma powder Freeze-dried normal plasma obtained from healthy human.

Dried sodium carbonate Na_2CO_3 [Same as the namesake monograph]

Dydrogesterone for assay $C_{21}H_{28}O_2$ [Same as the monograph Dydrogesterone. When dried, it contains not less than 99.0% of $C_{21}H_{28}O_2$]

E. coli protein Process *E. coli* cells (*E. coli* N4830/pTB281) retaining a plasmid deficient in the celmoleukin gene according to the celmoleukin purification process in the following order; (i) extraction, (ii) butylated vinyl polymer hydrophobic chromatography, (iii) carboxymethylated vinyl polymer ion-exchange column chromatography, and (iv) sulfopropyl-polymer ion-exchange chromatography, and during process (iv) collect the fractions corresponding to the celmoleukin elution position. Dialyze the fractions obtained in (iv) against 0.01 mol/L acetate buffer solution, pH 5.0, and take the dialysis solution as *E. coli* protein.

Description: Clear and colorless solution

Identification: When the absorption spectrum is determined using UV absorption photometry <2.24>, an absorption maximum is observed in the region of 278 nm.

Protein content: When determining the protein content using the Assay (1) Total protein content under Celmoleukin (Genetical Recombination), the protein content per mL is 0.1 to 0.5 mg.

E. coli protein stock solution A solution obtained by culturing a bacteria that contains a plasmid lacking the teceleukin gene but is otherwise exactly identical to the teceleukin-producing *E. coli* strain in every function except teceleukin production, and then purified using a purification technique that is more simple than that for teceleukin. Determine the amount of protein by Brodford method using bovine serum albumin as the standard substance. Store shielded from light at –70°C.

ECP See *E. coli* protein.

Eleutheroside B for liquid chromatography

$C_{17}H_{24}O_9 \cdot xH_2O$ A white crystalline powder. Sparingly soluble in methanol, slightly soluble in water, and very slightly soluble in ethanol (99.5). Melting point: 190 – 194°C.

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

Purity Related substances—Dissolve 1.0 mg in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of eleutheroside B is not larger than the peak area of eleuthero-

side B obtained with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Identification under *Eleutherococcus Senticosus* Rhizome.

Time span of measurement: About 3 times as long as the retention time of eleutheroside B beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add methanol to make exactly 20 mL. Confirm that the peak area of eleutheroside B obtained with 10 μ L of this solution is equivalent to 3.5 to 6.5% of that with 10 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Identification under *Eleutherococcus Senticosus* Rhizome.

EMB plate medium Melt eosin methylene blue agar medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dish with the cover slightly opened in the incubator to evaporate the inner vapor and water on the plate.

Emetine hydrochloride for component determination

$C_{29}H_{40}N_2O_4 \cdot 2HCl \cdot xH_2O$ A white or light-yellow crystalline powder. Soluble in water.

Melting point <2.60>: about 250°C [with decomposition, after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours].

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (283 nm) : 116 - 127 (10 mg, diluted methanol (1 in 2), 400 mL) [after drying in a desiccator (reduced pressure below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours.]

Purity Related substances—Dissolve 10 mg of emetine hydrochloride for component determination in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of peaks other than emetine from the sample solution is not larger than the peak of emetine from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Component determination under Ipecac except the detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the sensitivity so that the peak area of emetine obtained from 10 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of emetine obtained from 10 μ L of the standard solution (1) is about 20 % of the full scale.

Time span of measurement: About 3 times as long as the retention time of emetine beginning after the solvent peak.

Endo's medium Melt 1000 mL of the ordinary agar medi-

um by heating in a water bath, and adjust the pH to between 7.5 and 7.8. Add 10 g of lactose monohydrate previously dissolved in a small quantity of water, mix thoroughly, and add 1 mL of fuchsin-ethanol (95) TS. After cooling to about 50°C, add dropwise a freshly prepared solution of sodium bisulfite (1 in 10) until a light red color develops owing to reducing fuchsin, requiring about 10 to 15 mL of a solution of sodium sulfite heptahydrate (1 in 10). Dispense the mixture, and sterilize fractionally on each of three successive days for 15 minutes at 100°C.

Endo's plate medium Melt Endo's medium by heating, and cool to about 50°C. Transfer about 20 mL of this medium to a Petri dish, and solidify horizontally. Place the dishes with the cover slightly opened in the incubator to evaporate the inner vapor and water on the surface of the agar.

Enzyme TS The supernatant liquid is obtained as follows: To 0.3 g of an enzyme preparation potent in amylolytic and phosphorolytic activities, obtained from *Aspergillus oryzae*, add 10 mL of water and 0.5 mL of 0.1 mol/L hydrochloric acid TS, mix vigorously for a few minutes, and centrifuge. Prepare before use.

Eosin See eosin Y.

Eosin Y $C_{20}H_6Br_4Na_2O_5$ Red, masses or powder.

Identification—To 10 mL of a solution (1 in 1000) add 1 drop of hydrochloric acid: yellow-red precipitates appear.

Eosin methylene blue agar medium Dissolve by boiling 10 g of casein peptone, 2 g of dipotassium hydrogenphosphate and 25 to 30 g of agar in about 900 mL of water. To this mixture add 10 g of lactose monohydrate, 20 mL of a solution of eosin Y (1 in 50), 13 mL of a solution of methylene blue (1 in 200) and warm water to make 1000 mL. Mix thoroughly, dispense, sterilize by autoclaving at 121°C for not more than 20 minutes, and cool quickly by immersing in cold water, or sterilize fractionally on each of three successive days for 30 minutes at 100°C.

Ephedrine hydrochloride $C_{10}H_{15}NO \cdot HCl$ [Same as the namesake monograph]

Ephedrine hydrochloride for assay $C_{10}H_{15}NO \cdot HCl$ [Same as the monograph Ephedrine Hydrochloride meeting the following additional specifications.]

Purity Related substances—Dissolve 50 mg of ephedrine hydrochloride for assay in 50 mL of the mobile phase and use this solution as the sample solution. Pipet 1 mL of this solution and add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μ L of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than ephedrine from the sample solution is not larger than the peak area of ephedrine from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Ephedra Herb except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection

sensitivity so that the peak area of ephedrine obtained from 10 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of ephedrine from 10 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement : About 3 times as long as the retention time of ephedrine beginning after the solvent peak.

6-Epidoxycycline hydrochloride $C_{22}H_{24}N_2O_8 \cdot HCl$
Yellow to dark yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of 6-epioxycycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of the peaks other than 6-epidoxycycline is not more than 10%.

4-Epioxytetracycline $C_{22}H_{24}N_2O_9$ Green-brown to brown powder.

Purity Related substances—Dissolve 20 mg of 4-epioxytetracycline in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μ L of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than 4-epioxytetracycline is not more than 10%.

Eriochrome black T $C_{20}H_{12}N_3NaO_7S$ [K 8736, Special class]

Eriochrome black T-sodium chloride indicator Mix 0.1 g of eriochrome black T and 10 g of sodium chloride, and triturate until the mixture becomes homogeneous.

Eriochrome black T TS Dissolve 0.3 g of eriochrome black T and 2 g of hydroxylammonium chloride in methanol to make 50 mL. Use within 1 week. Preserve in light-resistant containers.

Erythromycin B $C_{37}H_{67}NO_{12}$ White to light yellowish white powder.

Purity Related substances—Dissolve 10 mg of erythromycin B in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μ L each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin B from the sample solution is not more than the peak area of erythromycin B from the standard solution.

Erythromycin C $C_{36}H_{65}NO_{13}$ White to light yellowish white powder.

Purity Related substances—Dissolve 10 mg of erythromycin C in 1 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make 5 mL,

and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 20 mL, and use this solution as the standard solution. Proceed with exactly 100 μ L each of the sample solution and standard solution as directed in the Purity (3) Related substances under Erythromycin, and determine each peak area from the solutions by the automatic integration method: the total of areas of the peaks other than erythromycin C from the sample solution is not more than the peak area of erythromycin C from the standard solution.

Essential oil Same as the essential oil under the monograph.

Etacrynic acid for assay [Same as the monograph Etacrynic acid. When dried, it contains not less than 99.0% of etacrynic acid ($C_{13}H_{12}Cl_2O_4$).]

Ethanol See ethanol (95).

Ethanol, aldehyde-free Transfer 1000 mL of ethanol (95) to a glass-stoppered bottle, add the solution prepared by dissolving 2.5 g of lead (II) acetate trihydrate in 5 mL of water, and mix thoroughly. In a separate container, dissolve 5 g of potassium hydroxide in 25 mL of warm ethanol (95), cool, and add this solution gently, without stirring, to the first solution. After 1 hour, shake this mixture vigorously, allow to stand overnight, decant the supernatant liquid, and distil the ethanol.

Ethanol, dehydrated See ethanol (99.5).

Ethanol, dilute To 1 volume of ethanol (95) add 1 volume of water. It contains 47.45 to 50.00 vol% of C_2H_5OH .

Ethanol, diluted Prepare by diluting ethanol (99.5).

Ethanol for alcohol number determination See Alcohol Number Determination <I.01>.

Ethanol for disinfection [Same as the namesake monograph]

Ethanol for gas chromatography Use ethanol prepared by distilling ethanol (99.5) with iron (II) sulfate heptahydrate. Preserve in containers, in which the air has been displaced with nitrogen, in a dark, cold place.

Ethanol-free chloroform See chloroform, ethanol-free.

Ethanol-isotonic sodium chloride solution To 1 volume of ethanol (95) add 19 volumes of isotonic sodium chloride solution.

Ethanol, methanol-free See ethanol (95), methanol-free.

Ethanol, neutralized To a suitable quantity of ethanol (95) add 2 to 3 drops of phenolphthalein TS, then add 0.01 mol/L or 0.1 mol/L sodium hydroxide VS until a light red color develops. Prepare before use.

Ethanol (95) C_2H_5OH [K 8102, Special class]

Ethanol (95), methanol-free Perform the test for methanol, by using this methanol-free ethanol (95) in place of the standard solution, as directed in Methanol Test <I.12>: it is practically colorless.

Ethanol (99.5) C_2H_5OH [K 8101, Special class]

Ethenzamide $C_9H_{11}NO_2$ [Same as the namesake mono-

graph].

Ether See diethyl ether.

Ether, anesthetic $C_2H_5OC_2H_5$ [Same as the namesake monograph]

Ether, dehydrated See diethyl ether, dehydrated.

Ether for purity of crude drug See diethyl ether for purity of crude drug.

Ethinylestradiol $C_{20}H_{24}O_2$ [Same as the namesake monograph]

3-Ethoxy-4-hydroxybenzaldehyde $C_9H_{10}O_3$ White to pale yellowish white crystalline. Freely soluble in ethanol (95), and slightly soluble in water.

Melting point <2.60>: 76 – 78°C

Content: not less than 98.0%. *Assay*—Weigh accurately about 0.3 g of 3-ethoxy-4-hydroxybenzaldehyde, previously dried in a desiccator (phosphorous (V) oxide) for 4 hours, dissolve in 50 mL of *N,N*-dimethylacetamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: thymol blue TS).

Each mL of 0.1 mol/L sodium methoxide VS
= 16.62 mg of $C_9H_{10}O_3$

***p*-Ethoxyphenol** See 4-ethoxyphenol.

4-Ethoxyphenol $C_8H_{10}O_2$ White to light yellow-brown crystals or crystalline powder. Freely soluble in ethanol (95), and very slightly soluble in water.

Melting point <2.60>: 62 – 68°C

Purity—Dissolve 0.5 g of 4-Ethoxyphenol in 5 mL of ethanol (95), and use this solution as the sample solution. Perform the test as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of substance other than 4-ethoxyphenol by the area percentage method: it is not more than 2.0%.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 180- to 250- μ m siliceous earth for gas chromatography coated with methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 150°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 4-ethoxyphenol is about 5 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 4-ethoxyphenol obtained from 1 μ L of the sample solution is not less than 50% of the full scale.

Time span of measurement: 3 times as long as the retention time of 4-ethoxyphenol beginning after the solvent peak.

Ethyl acetate $CH_3COOC_2H_5$ [K 8361, Special class]

Ethyl aminobenzoate $C_9H_{11}NO_2$ [Same as the namesake monograph]

Ethylbenzene $C_6H_5C_2H_5$ A colorless liquid. Freely soluble in acetone and in ethanol (99.5), and practically insoluble in water.

Specific gravity <2.56> d_4^{20} : 0.862 ~ 0.872

Boiling point <2.57>: about 135°C

Ethyl benzoate $C_6H_5COOC_2H_5$ Clear, colorless liquid.
Refractive index <2.45> n_D^{20} : 1.502 – 1.507

Specific gravity <2.56> d_{20}^{20} : 1.045 – 1.053

Ethyl carbamate $H_2NCOOC_2H_5$ White crystals or powder.

Melting point <2.60>: 48 – 50°C

Purity Clarity of solution: Dissolve 5 g in 20 mL of water: the solution is clear.

Ethyl cyanoacetate $NCCH_2COOC_2H_5$ Colorless or light yellow, clear liquid, having an aromatic odor. Specific gravity d_{20}^{20} : about 1.08

Identification—To 0.5 mL of a solution in ethanol (99.5) (1 in 10,000) add a mixture of 1 mL of a solution of quinhydrone in diluted ethanol (99.5) (1 in 2) (1 in 20,000) and 1 drop of ammonia solution (28): a light blue color develops.

Ethylenediamine $C_2H_8N_2$ [Same as the namesake monograph]

Ethylenediamine TS Dissolve 70 g of ethylenediamine in 30 g of water.

Ethylene glycol $HOCH_2CH_2OH$ [K 8105, Special class]

Ethylene glycol for Karl Fischer method Distil ethylene glycol, and collect the fraction distilling between 195°C and 198°C. The water content is not more than 1.0 mg per mL.

Ethyl iodide See iodoethane.

***N*-Ethylmaleimide** $C_6H_7NO_2$ White crystals, having a pungent, characteristic odor. Freely soluble in ethanol (95), and slightly soluble in water.

Melting point <2.60>: 43 – 46°C

Purity Clarity and color of solution—Dissolve 1 g of *N*-ethylmaleimide in 20 mL of ethanol (95): the solution is clear and colorless.

Content: not less than 99.0%. *Assay*—Dissolve about 0.1 g of *N*-ethylmaleimide, accurately weighed, in 20 mL of ethanol (95), add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.51 mg of $C_6H_7NO_2$

Ethyl *n*-caprylate $C_{10}H_{20}O_2$ Clear and colorless to almost colorless liquid.

Specific gravity <2.56> d_{20}^{20} : 0.864 – 0.871

Purity Related substances—Dissolve 0.1 g of ethyl *n*-caprylate in 10 mL of dioxane and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 μ L each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak areas other than ethyl *n*-caprylate from the sample solution is not larger than the peak area of ethyl *n*-caprylate from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Menthath Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of ethyl *n*-caprylate obtained from 5 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of ethyl *n*-caprylate from 5 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: 3 times as long as the retention time of ethyl *n*-caprylate beginning after the solvent peak.

Ethyl parahydroxybenzoate $\text{HOC}_6\text{H}_4\text{COOC}_2\text{H}_5$ [Same as the namesake monograph]

Ethyl propionate $\text{CH}_3\text{CH}_2\text{COOC}_2\text{H}_5$ Colorless, clear liquid.

Specific gravity <2.56> d_4^{20} : 0.890 – 0.892

2-Ethyl-2-phenylmalondiamide $\text{C}_{11}\text{H}_{14}\text{O}_2\text{N}_2$ White, odorless crystals. Soluble in ethanol (95), and very slightly soluble in water. Melting point: about 120°C (with decomposition).

Purity Related substances—To 5.0 mg of 2-ethyl-2-phenylmalondiamide add 4 mL of pyridine and 1 mL of bis-trimethylsilylacetamide, shake thoroughly, and heat at 100°C for 5 minutes. After cooling, add pyridine to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the conditions in the Purity (3) under Primidone: any peak other than the peaks of 2-ethyl-2-phenylmalondiamide and the solvent does not appear. Adjust the detection sensitivity so that the peak height of 2-ethyl-2-phenylmalondiamide obtained from 2 μ L of the sample solution is about 80% of the full scale, and the time span of measurement is about twice as long as the retention time of 2-ethyl-2-phenylmalondiamide beginning after the solvent peak.

Etidronate disodium for assay $\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$ [Same as the monograph Etidronate Disodium. When dried, it contains not less than 99.0% of $\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$]

Etilefrine hydrochloride $\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$ [Same as the namesake monograph]

Etilefrine hydrochloride for assay $\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$ [Same as the monograph Etilefrine Hydrochloride. When dried, it contains not less than 99.0% of etilefrine hydrochloride ($\text{C}_{10}\text{H}_{15}\text{NO}_2\cdot\text{HCl}$).]

Factor Xa It is prepared from lyophilization of Factor Xa which has been prepared from bovine plasma. White or pale yellow masses or powder.

Clarity and color of solution: Dissolve 7 *Inkat*_{s-2222} of it in 10 mL water; the solution is clear and colorless or pale yellow.

Content: not less than 75% and not more than 125% of the label.

Factor Xa TS Dissolve 7 *Inkat*_{s-2222} of factor Xa in 10 mL of water.

Famotidine for assay $\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$ [Same as the monograph Famotidine. When dried, it contains not less than 99.0

% of famotidine ($\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$), and when proceed as directed in the Purity (3), the total related substance is not more than 0.4%.]

Fatty oil Same as the fatty oil under the monograph.

Fehling's TS The copper solution—Dissolve 34.66 g of copper (II) sulfate pentahydrate in water to make 500 mL. Keep this solution in a glass-stoppered bottles in well-filled.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make 500 mL. Preserve this solution in a polyethylene container.

Before use, mix equal volumes of both solutions.

Fehling's TS for amyolytic activity test

The copper solution—Dissolve 34.660 g of copper (II) sulfate pentahydrate, accurately weighed, in water to make exactly 500 mL. Preserve this solution in well-filled, glass-stoppered bottles.

The alkaline tartrate solution—Dissolve 173 g of potassium sodium tartrate tetrahydrate and 50 g of sodium hydroxide in water to make exactly 500 mL. Preserve this solution in polyethylene containers.

Before use, mix exactly equal volumes of both solutions.

Ferric ammonium citrate See ammonium iron (III) citrate.

Ferric ammonium sulfate See ammonium iron (III) sulfate dodecahydrate.

Ferric ammonium sulfate TS See ammonium iron (III) sulfate TS.

Ferric ammonium sulfate TS, dilute See ammonium iron (III) sulfate TS, dilute.

Ferric chloride See iron (III) chloride hexahydrate.

Ferric chloride-acetic acid TS See iron (III) chloride-acetic acid TS.

Ferric chloride-iodine TS See iron (III) chloride-iodine TS.

Ferric chloride-methanol TS See iron (III) chloride-methanol TS.

Ferric chloride-pyridine TS, anhydrous See iron (III) chloride-pyridin TS, anhydrous.

Ferric chloride TS See iron (III) chloride TS.

Ferric chloride TS, acidic See iron (III) chloride TS, acidic.

Ferric chloride TS, dilute See iron (III) chloride TS, dilute.

Ferric nitrate See iron (III) nitrate enneahydrate.

Ferric nitrate TS See iron (III) nitrate TS.

Ferric perchlorate See iron (III) perchlorate hexahydrate.

Ferric perchlorate-dehydrated ethanol TS See iron (III) perchlorate-ethanol TS.

Ferric salicylate TS See Iron salicylate TS

Ferric sulfate See iron (III) sulfate *n*-hydrate.

Ferric sulfate TS See iron (III) sulfate TS.

Ferrous ammonium sulfate See ammonium iron (II) sulfate hexahydrate.

Ferrous sulfate See iron (II) sulfate heptahydrate.

Ferrous sulfate TS See iron (II) sulfate TS.

Ferrous sulfide See iron (II) sulfide.

Ferrous tartrate TS See iron (II) tartrate TS.

Ferrous thiocyanate TS See iron (II) thiocyanate TS.

Ferrous trisodium pentacyanoamine TS See iron (II) trisodium pentacyanoamine TS.

(E)-Ferulic acid $C_{10}H_{10}O_4$ White to light yellow, crystals or crystalline powder. Freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 173 – 176°C.

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, between 231 nm and 235 nm, and between 318 nm and 322 nm.

Purity Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2 μ L of this solution as directed in the Identification (11) under Hochuekkito Extract: no spot other than the principle spot of around *Rf* 0.6 appears.

Fetal calf serum Serum obtained from fetal calves. Interleukin-2 dependent cell growth suppression substance is removed by heat at 56°C for 30 min before use.

Fibrinogen Fibrinogen is prepared from human or bovine blood by fractional precipitation with ethanol or ammonium sulfate. It may contain citrate, oxalate and sodium chloride. A white, amorphous solid. Add 1 mL of isotonic sodium chloride solution to 0.01 g of fibrinogen. It, when warmed to 37°C, dissolves with a slight turbidity, and clots on the subsequent addition of 1 unit of thrombin.

1st Fluid for disintegration test See 1st fluid for dissolution test.

1st Fluid for dissolution test Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and water to make 1000 mL. It is clear and colorless, and has a pH of about 1.2.

Fixed oil Same as the vegetable oils under the monograph.

Flopropione [Same as the namesake monograph]

Flopropione for assay [Same as the monograph Flopropione. It contains not less than 99.0% of flopropione ($C_9H_{10}O_4$: 182.17), calculated on the dehydrated basis.]

Fluid thioglycolate medium See the Sterility Test <4.06>.

Fluocinolone acetonide $C_{24}H_{30}F_2O_6$ [Same as the namesake monograph]

Fluorescein $C_{20}H_{12}O_5$ An yellowish red powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1597 cm^{-1} , 1466 cm^{-1} , 1389 cm^{-1} , 1317 cm^{-1} , 1264 cm^{-1} , 1247 cm^{-1} , 1213 cm^{-1} , 1114 cm^{-1} and 849 cm^{-1} .

Fluorescein sodium $C_{20}H_{10}Na_2O_5$ [Same as the name-

sake monograph].

Fluorescein sodium TS Dissolve 0.2 g of fluorescein sodium in water to make 100 mL.

4-Fluorobenzoic acid $C_7H_5FO_2$ White, crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1684 cm^{-1} , 1606 cm^{-1} and 1231 cm^{-1} .

Melting point <2.60>: 182 – 188°C

1-Fluoro-2,4-dinitrobenzene $C_6H_3(NO_2)_2F$ [K 8479, Special class]

Flurazepam for assay $C_{21}H_{23}ClFN_3O$ [Same as the monograph Flurazepam. When dried, it contains not less than 99.0% of flurazepam ($C_{21}H_{23}ClFN_3O$).]

Folic acid $C_{19}H_{19}N_7O_6$ [Same as the namesake monograph]

Folin's TS Place 20 g of sodium tungstate (VI) dihydrate, 5 g of sodium molybdate dihydrate and about 140 mL of water in a 300-mL volumetric flask, add 10 mL of diluted phosphoric acid (17 in 20) and 20 mL of hydrochloric acid, and boil gently using a reflux condenser with ground-glass joints for 10 hours. To the mixture add 30 g of lithium sulfate monohydrate and 10 mL of water, and then add a very small quantity of bromine to change the deep green color of the solution to yellow. Remove the excess bromine by boiling for 15 minutes without a condenser, and cool. Add water to make 200 mL, and filter through a glass filter. Store it free from dust. Use this solution as the stock solution, and dilute with water to the directed concentration before use.

Folin's TS, dilute Titrate <2.50> Folin's TS with 0.1 mol/L sodium hydroxide VS (indicator: phenolphthalein TS), and determine the acid concentration. Prepare by adding water to Folin's TS so the acid concentration is 1 mol/L.

Formaldehyde solution HCHO [K 8872, Special class]

Formaldehyde solution-sulfuric acid TS Add 1 drop of formaldehyde solution to 1 mL of sulfuric acid. Prepare before use.

Formaldehyde solution TS To 0.5 mL of formaldehyde solution add water to make 100 mL.

Formaldehyde TS, dilute See Test Methods for Plastic Containers <7.02>.

Formalin See formaldehyde solution.

Formalin TS See formaldehyde solution TS.

Formalin-sulfuric acid TS See formaldehyde solution-sulfuric acid TS.

Formamide HCONH₂ [K 8873, Special class]

Formamide for Karl Fischer method HCONH₂ [K 8873, Special class; water content per g of formamide for Karl Fischer method should be not more than 1 mg.]

Formic acid HCOOH [K 8264, Special class, specific gravity: not less than 1.21].

2-Formylbenzoic acid $\text{CHOC}_6\text{H}_4\text{COOH}$ White crystals. Melting point: 97 – 99°C

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.3 g of 2-formylbenzoic acid, previously dried (in vacuum, phosphorus (V) oxide, 3 hours), dissolve in 50 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenol red TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 15.01 mg of $\text{C}_8\text{H}_6\text{O}_3$

Freund's complete adjuvant A suspension of 5 mg of mycobacteria of *Corynebacterium butyricum*, killed by heating, in 10 mL of a mixture of mineral oil and aricel A (17:3).

Fructose $\text{C}_6\text{H}_{12}\text{O}_6$ [Same as the namesake monograph]

Fuchsin A lustrous, green, crystalline powder or mass, slightly soluble in water and in ethanol (95).

Loss on drying <2.41>: 17.5 – 20.0% (1 g, 105°C, 4 hours)

Residue on ignition <2.44>: not more than 0.1% (1 g).

Fuchsin-ethanol TS Dissolve 11 g of fuchsin in 100 mL of ethanol (95).

Fuchsin-sulfurous acid TS Dissolve 0.2 g of fuchsin in 120 mL of hot water, and allow the solution to cool. Add a solution prepared by dissolving 2 g of anhydrous sodium sulfite in 20 mL of water, then add 2 mL of hydrochloric acid and water to make 200 mL, and allow to stand for at least 1 hour. Prepare before use.

Fumaric acid for thin-layer chromatography $\text{C}_4\text{H}_4\text{O}_4$ White, crystalline powder, odorless, and has a characteristic acid taste.

Purity—Perform the test as directed in the Identification (5) under Clemastine Fumarate: any spot other than the principal spot at the *R_f* value of about 0.8 does not appear.

Fuming nitric acid See nitric acid, fuming.

Fuming sulfuric acid See sulfuric acid, fuming.

Furfural $\text{C}_5\text{H}_4\text{O}_2$ A clear, colorless liquid.

Specific gravity <2.56> d_{20}^{20} : 1.160 – 1.165

Distilling range <2.57>: 160 – 163°C, not less than 95 vol%.

Galactose See D-galactose.

D-Galactose $\text{C}_6\text{H}_{12}\text{O}_6$ White crystals, granules or powder.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3390 cm^{-1} , 3210 cm^{-1} , 3140 cm^{-1} , 1151 cm^{-1} , 1068 cm^{-1} , 956 cm^{-1} , 836 cm^{-1} , 765 cm^{-1} and 660 cm^{-1} .

Optical rotation <2.49> $[\alpha]_D^{20}$: +79 – +82° (desiccator (silica gel), 2.5 g after drying for 18 hours, diluted ammonia solution (28) (1 in 300), 25 mL, 100 mm).

Gallic acid See gallic acid monohydrate.

Gallic acid monohydrate $\text{C}_6\text{H}_2(\text{OH})_3\text{COOH}\cdot\text{H}_2\text{O}$ White to pale yellowish white, crystals or powder.

Melting point <2.60>: about 260°C (with decomposition).

Gelatin [Same as the namesake monograph]

Gelatin, acid-treated [Same as the monograph Gelatin. Its isoelectric point is at pH between 7.0 and 9.0]

Gelatin peptone See peptone, gelatin.

Gelatin-phosphate buffer solution Dissolve 13.6 g of potassium dihydrogen phosphate, 15.6 g of sodium dihydrogen phosphate dihydrate and 1.0 g of sodium azide in water to make 1000 mL, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75) (solution A). Dissolve 5.0 g of acid-treated gelatin in 400 mL of the solution A by warming, after cooling, adjust the pH to 3.0 with diluted phosphoric acid (1 in 75), and add the solution-A to make 1000 mL.

Gelatin-phosphate buffer solution, pH 7.0 Dissolve 1.15 g of sodium dihydrogen phosphate dihydrate, 5.96 g of disodium hydrogen phosphate dodecahydrate and 5.4 g of sodium chloride in 500 mL of water. Dissolve 1.2 g of gelatin to this solution by heating, and after cooling add water to make 600 mL.

Gelatin-phosphate buffer solution, pH 7.4 To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 39.50 mL of 0.2 mol/L sodium hydroxide VS and 50 mL of water. Dissolve 0.2 g of gelatin to this solution by heating, then after cooling adjust to pH 7.4 with 0.2 mol/L sodium hydroxide TS, and add water to make 200 mL.

Gelatin-tris buffer solution Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 2.22 g of sodium chloride in 700 mL of water. Separately, dissolve 10 g of acid-treated gelatin in 200 mL of water by warming. After cooling, mix these solutions, and adjust the pH to 8.8 with dilute hydrochloric acid, and add water to make 1000 mL.

Gelatin-tris buffer solution, pH 8.0 Dissolve 40 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 5.4 g of sodium chloride in 500 mL of water. Add 1.2 g of gelatin to dissolve by heating, adjust to pH 8.0 with dilute hydrochloric acid after cooling, and add water to make 600 mL.

Gelatin TS Dissolve 1 g of gelatin in 50 mL of water by gentle heating, and filter if necessary. Prepare before use.

Geniposide for component determination Use geniposide for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (240 nm): 249 – 269 [10 mg dried in a desiccator (reduced pressure of not exceeding 0.67 kPa, phosphorus (V) oxide) for 24 hours, diluted methanol (1 in 2), 500 mL].

Purity Related substances—Dissolve 5 mg of geniposide for component determination in 50 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of the both solutions by the automatic integration method: the total area of the peaks other than geniposide from the sample solution is not larger than the peak area of geniposide from the standard solution (1).

Operating conditions

Proceed as directed in the Component determination under

Gardenia Fruit except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of geniposide obtained from 10 μ L of the standard solution (2) can be measured by the automatic integration method and the peak height of geniposide obtained from 10 μ L of the standard solution (1) shows about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of geniposide beginning after the solvent peak.

Geniposide for thin-layer chromatography $C_{17}H_{24}O_{10}$ White crystals or crystalline powder. Melting point: 159 – 163 $^{\circ}$ C.

Purity Related substances—Dissolve 1.0 mg of geniposide for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 20 μ L of this solution as directed in the Identification (2) under Gardenia Fruit: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

Gentamicin B $C_{19}H_{38}N_4O_{10}$ White to pale yellowish white powder. Very soluble in water, and practically insoluble in ethanol (95).

Content: not less than 80.0%. *Assay*—Dissolve a suitable amount of gentamicin B in 0.05 mol/L sulfuric acid TS to make the solution containing 0.1 mg of gentamicin B per mL, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amount of gentamicin B by the area percentage method.

Operating conditions

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed the operating conditions in the Assay under Isepamicin Sulfate.

Time span of measurement: About 3 times as long as the retention time of gentamicin B.

System suitability

Proceed the system suitability in the Assay under Isepamicin Sulfate.

Geniopicroside for thin-layer chromatography

$C_{16}H_{20}O_9$ A white powder. Freely soluble in water and in methanol, and practically insoluble in diethyl ether. Melting point: about 110 $^{\circ}$ C (with decomposition).

Purity Related substances—Dissolve 10 mg of geniopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed in the Identification (2) under Gentian: the spots other than the principal spot at the *Rf* value of about 0.4 from the sample solution are not more intense than the spot from the standard solution.

Giemsa's TS Dissolve 3 g of azure II-eosin Y and 0.8 g of azure II in 250 g of glycerin by warming to 60 $^{\circ}$ C. After cooling, add 250 g of methanol, and mix well. Allow to stand for

24 hours, and filter. Store in tightly stoppered bottles.

Azure II-eosin Y is prepared by coupling eosin Y to azure II. Azure II is the mixture of equal quantities of methylene azure (azure I), prepared by oxidizing methylene blue, and methylene blue.

[6]-Gingerol for thin-layer chromatography $C_{17}H_{26}O_4$ A yellow-white to yellow, liquid or solid. Freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Purity Related substances—Dissolve 1.0 mg of [6]-gingerol for thin-layer chromatography in exactly 2 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification under Ginger: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

Ginsenoside Rc $C_{53}H_{90}O_{22} \cdot xH_2O$ A white crystalline powder. It is odorless.

Purity—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Rc and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

Ginsenoside Re $C_{48}H_{82}O_{18} \cdot xH_2O$ A white crystalline powder. It is odorless.

Purity—Dissolve 1 mg in diluted methanol (3 in 5) to make 10 mL. Perform the test with 10 μ L of this solution as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay under Ginseng: the total area of the peak other than ginsenoside Re and solvent peak is not more than 1/10 times the total peak area excluding the peak area of the solvent.

Ginsenoside Rg₁ for thin-layer chromatography

$C_{42}H_{72}O_{14}$ White, crystalline powder, having a slight, bitter taste. Freely soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether and in chloroform.

Melting point <2.60>: 194 – 196.5 $^{\circ}$ C

Purity Related substances—Dissolve 1 mg of ginsenoside Rg₁ for thin layer chromatography in 1 mL of methanol, and perform the test with 20 μ L of this solution as directed in the Identification (2) under Ginseng: any spot other than the principal spot at the *Rf* value of about 0.4 does not appear.

Glacial acetic acid See acetic acid (100).

Glacial acetic acid for nonaqueous titration See acetic acid for nonaqueous titration.

Glacial acetic acid-sulfuric acid TS See acetic acid (100)-sulfuric acid TS.

γ -Globulin A plasma protein obtained from human serum as Cohn's II and III fractions. White crystalline powder. It contains not less than 98% of γ -globulin in the total protein.

Glucose $C_6H_{12}O_6$ [Same as the namesake monograph]

Glucose detection TS Dissolve 1600 units of glucose oxidase, 16 mg of 4-aminoantipyrine, 145 units of peroxidase and 0.27 g of *p*-hydroxybenzoic acid in tris buffer solution, pH 7.0, to make 200 mL.

Glucose detection TS for penicillium origin β -galactosidase

Dissolve glucose oxidase (not less than 500 units), peroxidase (not less than 50 units), 0.01 g of 4-aminoantipyrine and 0.1 g of phenol in phosphate buffer, pH 7.2 to make 100 mL.

Glucose oxidase Obtained from *Aspergillus nigar*. White powder. It is freely soluble in water. It contains about 200 Units per mg. One unit indicates an amount of the enzyme which produces 1 μ mol of D-glucono- δ -lactone in 1 minute at 25°C and pH 7.0 from glucose used as the substrate.

Glucose-pepton medium for sterility test See soybean-casein digest medium

Glucose TS Dissolve 30 g of glucose in water to make 100 mL. Prepare as directed under Injections.

L-Glutamic acid HOOC(CH₂)₂CH(NH₂)COOH [K 9047, Special class]

L-Glutamine H₂NCO(CH₂)₂CH(NH₂)COOH [K 9103, Special class]

Glutamine TS See Test Methods for Plastic Containers <7.02>.

7-(Glutarylglucyl-L-arginylamino)-4-methylcoumarin C₂₃H₃₀N₆O₇ White powder. It is freely soluble in acetic acid (100), sparingly soluble in dimethylsulfoxide, and practically insoluble in water.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (325 nm): 310 – 350 [2 mg, diluted acetic acid (100) (1 in 500), 200 mL].

Optical rotation <2.49> $[\alpha]_D^{20}$: –50 – –60° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

Purity Related substances—Prepare the sample solution by dissolving 5 mg of 7-(glutarylglucyl-L-arginylamino)-4-methylcoumarin in 0.5 mL of acetic acid (100), and perform the test as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, allow the plate to stand for 30 minutes in a box filled with iodine vapors: any observable spot other than the principal spot at the R_f value of about 0.6 does not appear.

7-(Glutarylglucyl-L-arginylamino)-4-methylcoumarin TS Dissolve 5 mg of 7-(glutarylglucyl-L-arginylamino)-4-methylcoumarin in 0.5 to 1 mL of acetic acid (100), lyophilize, dissolve this in 1 mL of dimethylsulfoxide, and use this solution as solution A. Dissolve 30.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.6 g of sodium chloride in 400 mL of water, adjust the pH to 8.5 with dilute hydrochloric acid, add water to make 500 mL, and use this solution as solution B. Mix 1 mL of the solution A and 500 mL of the solution B before use.

Glycerin C₃H₈O₃ [K 8295, Glycerol, Special class. Same as the monograph Concentrated Glycerin]

85% Glycerin C₃H₈O₃ [Same as the monograph Glycerin]

Glycine C₂H₆NO₂ [K8291, Special class]

Glycolic acid C₂H₄O₃ *Purity*: not less than 98.0%.

Glycyrrhizic acid for thin-layer chromatography

C₄₂H₆₂O₁₆·xH₂O Colorless or white, sweet, crystalline powder. Freely soluble in hot water and in ethanol (95), and practically insoluble in diethyl ether. Melting point: 213 – 218°C (with decomposition).

Purity Related substances—Dissolve 10 mg of glycyrrhizic acid for thin-layer chromatography in 5 mL of dilute ethanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed in the Identification under Glycyrrhiza: the spots other than the principal spot at the R_f value of about 0.3 from the sample solution are not more intense than the spot from the standard solution.

Goat anti-ECP antibody Combine 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and immunize goats subcutaneously in the back region with this solution 5 times at 2 week intervals. Harvest blood on the 10th day after completing the immunization to obtain goat antiserum. Goat anti-ECP antibody is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to sepharose 4B and then purifying by affinity column chromatography.

Description: Clear and colorless solution.

Identification: When sodium lauryl sulfate-supplemented polyacrylamide gel electrophoresis is conducted under non-reducing conditions, the molecular weight of the major band is within the range of 1.30 × 10⁵ to 1.70 × 10⁵.

Protein content: When determining the protein content using Assay (1) under Celmoleukin (Genetical Recombination), the protein content per mL is 0.2 to 1.0 mg.

Goat anti-ECP antibody TS Dilute goat anti-ECP antibody with 0.1 mol/L carbonate buffer solution, pH 9.6 to prepare a solution containing 50 μ g protein per mL.

Griess-Romijn's nitric acid reagent Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 1.5 g of zinc dust in a mortar.

Storage—Preserve in tight, light-resistant containers.

Griess-Romijn's nitrous acid reagent Triturate thoroughly 1 g of 1-naphthylamine, 10 g of sulfanilic acid and 89 g of tartaric acid in a mortar.

Storage—Preserve in tight, light-resistant containers.

Guaiacol CH₃OC₆H₄OH Clear, colorless to yellow liquid or colorless crystals, having a characteristic aroma. Sparingly soluble in water, and miscible with ethanol (95), with diethyl ether and with chloroform. Melting point: about 28°C

Purity—Perform the test with 0.5 μ L of guaiacol as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of guaiacol by the area percentage method: It showed the purity of not less than 99.0%.

Operating conditions

Detector: Hydrogen flame-ionization detector

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 150- to 180- μ m in particle diameter, coated with polyethylene glycol 20 M at the ratio of 20%.

Column temperature: A constant temperature of about 200 °C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of guaiacol is 4 to 6 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of guaiacol obtained from 0.5 μL of guaiacol is about 90% of the full scale.

Time span of measurement: About 3 times as long as the retention time of guaiacol beginning after the solvent peak.

Guaifenesin C₁₀H₁₄O₄ [Same as the namesake monograph]

Haloperidol for assay C₂₁H₂₃ClFNO₂ [Same as the monograph Haloperidol.]

Hanus' TS Dissolve 20 g of iodine monobromide in 1000 mL of acetic acid (100). Preserve in light-resistant, glass-stoppered bottles, in a cold place.

Heavy hydrogenated solvent for nuclear magnetic resonance spectroscopy Prepared for nuclear magnetic resonance spectroscopy. Heavy hydrogenated chloroform (CDCl₃), heavy hydrogenated dimethyl sulfoxide [(CD₃)₂SO], heavy water (D₂O), and heavy hydrogenated pyridine (C₅D₅N) are available.

Heavy water for nuclear magnetic resonance spectroscopy D₂O Prepared for nuclear magnetic resonance spectroscopy.

Helium He Not less than 99.995 vol%.

Hematoxylin C₁₆H₁₄O₆.nH₂O White or light yellow to brownish crystals or crystalline powder. It is soluble in hot water and in ethanol (95), and sparingly soluble in cold water.

Residue on ignition <2.44>: not more than 0.1% (1 g).

Hematoxylin TS Dissolve 1 g of hematoxylin in 12 mL of ethanol (99.5). Dissolve 20 g of aluminum potassium sulfate 12-water in 200 mL of warm water, cool, and filter. After 24 hours, mix these two prepared solutions. Allow to stand for 8 hours in a wide-mouthed bottle without using a stopper, and filter.

Heparin sodium [Same as the namesake monograph]

HEPES buffer solution, pH 7.5 Dissolve 2.38 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 90 mL of water, adjust to pH 7.5 with 5 mol/L sodium hydroxide TS, and add water to make 100 mL.

Heptane CH₃(CH₂)₅CH₃ [K 9701, Special class]

Hesperidin for component determination Hesperidin for thin-layer chromatography. It meets the following requirements.

Optical rotation <2.49> [α]_D²⁰: -100 - -120° (5 mg dried with silica gel for 24 hours, methanol, 50 mL, 100 mm).

Purity Related substances—Dissolve 2 mg in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integra-

tion method: the total area of the peaks other than the peak of hesperidin and the solvent is not larger than the peak area of hesperidin obtained with the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Hochuekkito Extract.

Time span of measurement: About 6 times as long as the retention time of hesperidin.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of hesperidin obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Hochuekkito Extract.

Hesperidin for thin-layer chromatography C₂₈H₃₄O₁₅ A white to light brown-yellow, crystalline powder or powder. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 245°C (with decomposition).

Absorbance <2.24> *E*₁^{1%}_{cm} (284 nm): 310 - 340 (8 mg dried in a desiccator (silica gel) for 24 hours, methanol, 500 mL).

Purity Related substances—Dissolve 1 mg in 2 mL of methanol. Proceed the test with 20 μL of this solution as directed in the Identification (6) under Hochuekkito Extract: no spot other than the principle spot of around *R*_f 0.3 appears.

Hexaammonium heptamolybdate-cerium (IV) sulfate TS Dissolve 2.5 g of hexaammonium heptamolybdate tetrahydrate and 1.0 g of cerium (IV) sulfate tetrahydrate in diluted sulfuric acid (3 in 50) to make 100 mL. Prepare before use.

Hexaammonium heptamolybdate-sulfuric acid TS Dissolve 1.0 g of hexaammonium heptamolybdate tetrahydrate in diluted sulfuric acid (3 in 20) to make 40 mL. Prepare before use.

Hexaammonium heptamolybdate tetrahydrate (NH₄)₆Mo₇O₂₄·4H₂O [K 8905, Special class]

Hexaammonium heptamolybdate TS dissolve 21.2 g of hexaammonium heptamolybdate tetrahydrate in water to make 200 mL (10%). Prepare before use.

Hexamethylenetetramine (CH₂)₆N₄ [K 8847, Special class]

Hexamethylenetetramine TS See Test Methods for Plastic Containers <7.02>.

Hexamine See hexamethylenetetramine.

Hexane C₆H₁₄ [K 8848, Special class]

Hexane for liquid chromatography CH₃(CH₂)₄CH₃ Colorless, clear liquid. Miscible with ethanol (95), with diethyl ether, with chloroform and with benzene.

Boiling point <2.57>: about 69°C

Purity (1) Ultraviolet absorptive substances—Read the absorbances of hexane for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: not more than 0.3 at the wavelength of 210 nm, and not more than 0.01 between 250 nm and 400

nm.

(2) Peroxide—To a mixture of 100 mL of water and 25 mL of dilute sulfuric acid add 25 mL of a solution of potassium iodide (1 in 10) and 20 g of hexane for liquid chromatography. Stopper tightly, shake, and allow to stand in a dark place for 15 minutes. Titrate <2.50> this solution, while shaking well, with 0.01 mol/L sodium thiosulfate (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

***n*-Hexane for liquid chromatography** See hexane for liquid chromatography.

Hexane for purity of crude drug [K 8848, Special class] Use hexane meeting the following additional specification. Evaporate 300.0 mL of hexane for purity of crude drug in vacuum at a temperature not higher than 40°C, add the hexane to make exactly 1 mL, and use this solution as the sample solution. Separately, dissolve 2.0 mg of γ -BHC in hexane to make exactly 100 mL. Pipet 1 mL of this solution, and add hexane to make exactly 100 mL. Further pipet 2 mL of this solution, add hexane to make exactly 100 mL, and use this solution as the standard solution I. Perform the test with exactly 1 μ L each of the sample solution and standard solution I as directed under Gas Chromatography <2.02> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of peak other than the solvent peak from the sample solution is not larger than the peak area of γ -BHC from the standard solution I.

Operating conditions

Proceed the operating conditions in the Purity (2) under Crude Drugs Test <5.01> except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution I, add hexane to make exactly 20 mL, and use this solution as the standard solution II. Adjust the detection sensitivity so that the peak area of γ -BHC obtained from 1 μ L of the standard solution II can be measured by the automatic integration method, and the peak height of γ -BHC from 1 μ L of the standard solution I is about 20% of the full scale.

Time span of measurement: About three times as long as the retention time of γ -BHC beginning after the solvent peak.

Hexane for ultraviolet-visible spectrophotometry [K 8848, Special class]. When determining the absorbance of hexane for ultraviolet-visible spectrophotometry as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution, its value is not more than 0.10 at 220 nm and not more than 0.02 at 260 nm, and it has no characteristic absorption between 260 nm and 350 nm.

***n*-Hexane for ultraviolet-visible spectrophotometry** See hexane for ultraviolet-visible spectrophotometry.

Hirsutine C₂₂H₂₈N₂O₃ White to light yellow, crystals or crystalline powder. Melting point: about 100°C.

Optical rotation <2.49> [α]_D²⁰: about +70° (10 mg, methanol, 1 mL, 50 mm).

Absorbance <2.24> E_{1cm}^{1%} (245 nm): 354 – 379 (5 mg calculated on the anhydrous basis, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances – Dissolve 5 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), proceed with 20 μ L of this solution as directed in the Compo-

nent determination under Uncaria Thorn, and perform the Liquid Chromatography <2.01>: the sum of the peak areas except the areas of hirsutine and the solvent is not more than 1/10 of the sum of the peak areas except the solvent.

L-Histidine hydrochloride See L-histidine hydrochloride monohydrate.

L-Histidine hydrochloride monohydrate C₆H₉N₃O₂·HCl·H₂O [K 9050, Special class]

Homatropine hydrobromide C₁₆H₂₁NO₃·HBr [Same as the namesake monograph]

Honokiol C₁₈H₁₈O₂·xH₂O Odorless white, crystals or crystalline powder.

Purity—Dissolve 1 mg of honokiol in the mobile phase to make 10 mL, and use this solution as the sample solution. Perform the Liquid Chromatography <2.01> with 10 μ L of the sample solution as directed in the Component determination under Magnolia Bark: when measure the peak areas 2 times as long as the retention time of magnorole, the total area of peaks other than honokiol is not larger than 1/10 times the total area of the peaks other than the solvent peak.

Horseradish peroxidase An oxidase (Molecular weight: about 40,000) derived from horseradish

Horse serum Collect the blood from horse in a flask, coagulate, and allow to stand at room temperature until the serum is separated. Transfer the separated serum in glass containers, and preserve at –20°C.

Human antithrombin III Serine protease inhibition factor obtained from normal plasma of health human. It is a protein, which inhibits the activities of thrombin and activated blood coagulation factor X. It contains not less than 300 Units per mg protein. One unit indicates an amount of the antithrombin III which inhibits 1 unit of thrombin at 25°C under the existence of heparin.

Human chorionic gonadotrophin TS Weigh accurately a suitable amount of Human Chorionic Gonadotrophin according to the labeled amount, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2 so that each mL contains 80 human chorionic gonadotrophin Units.

Human insulin desamide substance-containing TS Dissolve 1.5 mg of Insulin Human (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS, allow to stand at 25°C for 3 days, and when the procedure is run with this solution according to the conditions as directed in the Purity (1) under Insulin Human (Genetical Recombination), the solution contains about 5% of the desamide substance.

Human insulin dimer-containing TS Allow to stand Insulin Human (Genetical Recombination) at 25°C for 10 days or more, and dissolve 4 mg of this in 1 mL of 0.01 mol/L hydrochloric acid TS.

Human normal plasma Dissolve an amount of dried human normal plasma powder, equivalent to 1 mL of the normal plasma of human, in 1 mL of water. Store between 2 and 10°C, and use within one week.

Human serum albumin for assay White to pale-yellow powder. Albumin content is at least 99%. Convert to the dehydrate using the following water determination method.

Water content <2.48>: (0.20 g, direct titration). However, in a dehydration solvent, use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1).

Hydralazine hydrochloride $C_8H_8N_4.HCl$ [Same as the namesake monograph]

Hydralazine hydrochloride for assay $C_8H_8N_4.HCl$
[Same as the monograph Hydralazine Hydrochloride. When dried, it contains not less than 99.0% of hydralazine hydrochloride ($C_8H_8N_4.HCl$).]

Hydrazine hydrate See hydrazine monohydrate.

Hydrazine monohydrate $NH_2NH_2.H_2O$ Colorless liquid, having a characteristic odor.

Hydrazine sulfate See hydrazinum sulfate.

Hydrazinum sulfate $N_4H_6SO_4$ [K 8992, Special class]

Hydrazinum sulfate TS Dissolve 1.0 g of hydrazinum sulfate in water to make 100 mL.

Hydrobromic acid HBr [K 8509, Special class]

Hydrochloric acid HCl [K 8180, Special class]

Hydrochloric acid-ammonium acetate buffer solution, pH 3.5 Dissolve 25 g of ammonium acetate in 45 mL of 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

Hydrochloric acid, dilute Dilute 23.6 mL of hydrochloric acid with water to make 100 mL (10%).

Hydrochloric acid-ethanol TS See hydrochloric acid-ethanol (95) TS.

Hydrochloric acid-ethanol (95) TS Dilute 23.6 mL of hydrochloric acid with ethanol to make 100 mL.

0.01 mol/L Hydrochloric acid-methanol TS To 20 mL of 0.5 mol/L hydrochloric acid TS add methanol to make 1000 mL.

0.05 mol/L Hydrochloric acid-methanol TS To 100 mL of 0.5 mol/L hydrochloric acid add methanol to make 1000 mL.

Hydrochloric acid-2-propanol TS Add 0.33 mL of hydrochloric acid to 100 mL of 2-propanol, mix, and store in a dark and cool place.

Hydrochloric acid-potassium chloride buffer solution, pH 2.0 To 10.0 mL of 0.2 mol/L hydrochloric acid VS add 88.0 mL of 0.2 mol/L potassium chloride TS, adjust the pH to 2.0 ± 0.1 with 0.2 mol/L hydrochloric acid VS or 0.2 mol/L potassium chloride TS, then add water to make 200 mL.

Hydrochloric acid, purified Add 0.3 g of potassium permanganate to 1000 mL of diluted hydrochloric acid (1 in 2), distil, discard the first 250 mL of the distillate, and collect the following 500 mL of the distillate.

0.001 mol/L Hydrochloric acid TS Dilute 10 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.01 mol/L Hydrochloric acid TS Dilute 100 mL of 0.1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.02 mol/L Hydrochloric acid TS Dilute 100 mL of 0.2 mol/L hydrochloric acid TS with water to make 1000 mL.

0.05 mol/L Hydrochloric acid TS Dilute 100 mL of 0.5 mol/L hydrochloric acid TS with water to make 1000 mL.

0.1 mol/L Hydrochloric acid TS Dilute 100 mL of 1 mol/L hydrochloric acid TS with water to make 1000 mL.

0.2 mol/L Hydrochloric acid TS Dilute 18 mL of hydrochloric acid with water to make 1000 mL.

0.5 mol/L Hydrochloric acid TS Dilute 45 mL of hydrochloric acid with water to make 1000 mL.

1 mol/L Hydrochloric acid TS Dilute 90 mL of hydrochloric acid with water to make 1000 mL.

2 mol/L Hydrochloric acid TS Dilute 180 mL of hydrochloric acid with water to make 1000 mL.

3 mol/L Hydrochloric acid TS Dilute 270 mL of hydrochloric acid with water to make 1000 mL.

5 mol/L Hydrochloric acid TS Dilute 450 mL of hydrochloric acid with water to make 1000 mL.

6 mol/L Hydrochloric acid TS Dilute 540 mL of hydrochloric acid with water to make 1000 mL.

7.5 mol/L Hydrochloric acid TS Dilute 675 mL of hydrochloric acid with water to make 1000 mL.

10 mol/L Hydrochloric acid TS Dilute 900 mL of hydrochloric acid with water to make 1000 mL.

Hydrocortisone $C_{21}H_{30}O_5$ [Same as the namesake monograph]

Hydrocortisone acetate $C_{23}H_{32}O_6$ [Same as the namesake monograph]

Hydrocotarnine hydrochloride for assay
 $C_{12}H_{15}NO_3.HCl.H_2O$ [Same as the monograph Hydrocotarnine Hydrochloride Hydrate. When dried, it contains not less than 99.0% of hydrocotarnine hydrochloride ($C_{12}H_{15}NO_3.HCl$).]

Hydrofluoric acid HF [K 8819, Special class] It contains not less than 46.0% of HF .

Hydrogen H_2 [K 0512, Standard substance, Third class] It contains not less than 99.99% of H_2 .

Hydrogen chloride-ethanol TS See hydrogen chloride-ethanol (99.5) TS.

Hydrogen chloride-ethanol (99.5) TS Pass dry hydrogen chloride, which is generated by slowly adding 100 mL of sulfuric acid dropwise to 100 mL of hydrochloric acid and dried by washing with sulfuric acid, through 75 g of ethanol (99.5) cooled in an ice bath until the increase in mass has reached 25 g. Prepare before use.

Hydrogen hexachloroplatinate (IV) hexahydrate
 $H_2PtCl_6.6H_2O$ [K 8153, Special class]

Hydrogen hexachloroplatinate (IV)-potassium iodide TS To 3 mL of hydrogen hexachloroplatinate (IV) TS add 97 mL of water and 100 mL of a solution of potassium iodide (3 in 50). Prepare before use.

Hydrogen hexachloroplatinate (IV) TS Dissolve 2.6 g of chloroplatinic acid in water to make 20 mL (0.25 mol/L).

Hydrogen peroxide-sodium hydroxide TS To a mixture

of water and hydrogen peroxide (30) (9:1) add 3 drops of bromophenol blue TS, and then add 0.01 mol/L sodium hydroxide TS until a purple-blue color develops. Prepare before use.

Hydrogen peroxide TS Dilute 1 volume of hydrogen peroxide (30) with 9 volumes of water. Prepare before use (3%).

Hydrogen peroxide TS, dilute Dilute 1 mL of hydrogen peroxide (30) with 500 mL of water, and dilute 5 mL of this solution with water to make 100 mL. Prepare before use.

Hydrogen peroxide water, strong See hydrogen peroxide (30).

Hydrogen peroxide (30) H_2O_2 [K 8230, Hydrogen peroxide, Special class, Concentration: 30.0 – 35.5%].

Hydrogen sulfide H_2S Colorless, poisonous gas, heavier than air. It dissolves in water. Prepare by treating iron (II) sulfide heptahydrate with dilute sulfuric acid or dilute hydrochloric acid. Other sulfides yielding hydrogen sulfide with dilute acids may be used.

Hydrogen sulfide TS A saturated solution of hydrogen sulfide. Prepare by passing hydrogen sulfide into cold water. Preserve in well-filled, light-resistant bottles, in a dark, cold place.

Hydrogen tetrachloroaurate (III) tetrahydrate $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ [K 8127, Special class]

Hydrogen tetrachloroaurate (III) tetrahydrate TS Dissolve 1 g of hydrogen tetrachloroaurate (III) tetrahydrate in 35 mL of water (0.2 mol/L).

Hydroiodic acid HI [K 8917, Special class]

Hydroquinone $\text{C}_6\text{H}_4(\text{OH})_2$ [K 8738, Special class]

Hydroxocobalamin acetate $\text{C}_{62}\text{H}_{89}\text{CoN}_{13}\text{O}_{15}\text{P} \cdot \text{C}_2\text{H}_4\text{O}_2$ Dark red crystals or powder.

Loss on drying <2.41>: not more than 12% (50 mg, in vacuum not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 6 hours).

Content: not less than 98.0%. Assay—Proceed as directed in the Assay under Hydroxocobalamin Acetate.

***m*-Hydroxyacetophenone** $\text{C}_8\text{H}_8\text{O}_2$ White to light yellowish white crystalline powder.

Melting point <2.60>: about 96°C

Purity Related substances—Perform the test with 10 μL of a solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 15,000) as directed in the Assay under Cefalexin: Any obstructive peaks for determination of cefalexin are not observed.

***p*-Hydroxyacetophenone** $\text{C}_8\text{H}_8\text{O}_2$ White to pale yellow crystals or crystalline powder. It is freely soluble in methanol.

Melting point <2.60>: 107 – 111°C

Purity—Weigh 1 mg of *p*-hydroxyacetophenone, add methanol and dissolve to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the Assay under Peony Root: the total area of the peaks other than the peak of *p*-hydroxyacetophenone from the sample solution is not larger than 3/100 of the total area of the peaks other than the solvent peak.

3-Hydroxybenzoic acid $\text{HOC}_6\text{H}_4\text{COOH}$ White, crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum according to the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3300 cm^{-1} , 1690 cm^{-1} , 1600 cm^{-1} , 1307 cm^{-1} , 1232 cm^{-1} and 760 cm^{-1} .

Melting point <2.60>: 203 – 206°C

Purity Clarity—Dissolve 1 g of 3-hydroxybenzoic acid in 20 mL of methanol: the solution is clear.

Content: not less than 99.0%. Assay—Weigh accurately about 0.2 g of 3-hydroxybenzoic acid, dissolve in 20 mL of diluted ethanol (95) (1 in 2), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of cresol red TS) until the color of the solution changes from yellow to dark orange-red. Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 13.81 mg of $\text{C}_7\text{H}_6\text{O}_3$

***p*-Hydroxybenzoic acid** See parahydroxybenzoic acid.

***N*-(2-Hydroxyethyl)isonicotinamide nitric ester** $\text{C}_8\text{H}_9\text{N}_3\text{O}_4$ A white crystalline powder.

Identification—Determine the infrared absorption spectrum of *N*-(2-hydroxyethyl)isonicotinamide nitric ester as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3270 cm^{-1} , 1653 cm^{-1} , 1546 cm^{-1} and 1283 cm^{-1} .

***N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid** $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_4\text{S}$ White crystalline powder.

Purity Clarity and color of solution—Dissolve 11.9 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid in 50 mL of water: the solution is clear and colorless.

Content: not less than 99.0%. Assay—Weigh accurately about 1 g of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, dissolve in 60 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (Potentiometric titration).

Each mL of 0.5 mol/L sodium hydroxide VS
= 119.2 mg of $\text{C}_8\text{H}_{18}\text{N}_2\text{O}_4\text{S}$

1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol $\text{C}_3\text{H}_6\text{N}_4\text{OS}$ White, crystals or powder.

Melting point <2.60>: 136 – 141°C

Purity Related substances—Dissolve 0.10 g of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol in 1 mL of water, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, water, methanol and formic acid (60:10:7:6) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5*H*)-one hydrochloride** $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_3\text{S} \cdot \text{HCl}$ To 9 g of diltiazem hy-

drochloride add 50 mL of ethanol (99.5), and dissolve by heating at 80°C. To this solution add slowly 50 mL of a solution of potassium hydroxide in ethanol (99.5) (33 in 500) dropwise, and heat for 4 hours with stirring. Cool in an ice bath, filter, and evaporate the filtrate to dryness. Dissolve the residue in ethanol (99.5), add slowly a solution of hydrochloric acid in ethanol (99.5) (59 in 250) to make acidic, and filter. Add diethyl ether slowly to the filtrate, and filter the crystals produced. To the crystals add ethanol (99.5), heat to dissolve, add 0.5 g of activated charcoal, allow to stand, and filter. After cooling the filtrate in an ice-methanol bath, filter the crystals formed, and wash with diethyl ether. Further, add ethanol (99.5) to the crystals, and heat to dissolve. After cooling, filter the crystals produced, and dry under reduced pressure. White crystals or crystalline powder, having a slight, characteristic odor.

Purity—Dissolve 50 mg of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4-(5*H*)-one hydrochloride in chloroform to make exactly 10 mL, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), chloroform, water and acetic acid (100) (12:10:3:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly iodine TS on the plate: any spot other than the principal spot does not appear.

Water <2.48>: not more than 1.0% (0.5 g).

Content: not less than 99.0%, calculated on the anhydrous basis. **Assay**—Weigh accurately about 0.5 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4-(5*H*)-one hydrochloride, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 40.89 mg of C₂₀H₂₄N₂O₃S.HCl

***d*-3-Hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino) ethyl]-2-(*p*-methoxyphenyl)-1,5-benzothiazepine-4 (5*H*)-one hydrochloride** See *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino) ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4 (5*H*)-one hydrochloride.

2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid C₂₁H₁₄N₂O₇S [K 8776, Special class]

Hydroxylamine hydrochloride See hydroxylammonium chloride.

Hydroxylamine hydrochloride-ferric chloride TS See hydroxylammonium chloride-iron (III) chloride TS.

Hydroxylamine hydrochloride TS See hydroxylammonium chloride TS.

Hydroxylamine perchlorate NH₂OH.HClO₄ Hygroscopic, white crystals. Dissolves in water and in ethanol (95). **Melting point** <2.60>: 87.5 – 90°C

Hydroxylamine perchlorate-dehydrated ethanol TS See hydroxylamine perchlorate-ethanol (99.5) TS.

Hydroxylamine perchlorate-ethanol (99.5) TS Dilute

2.99 mL of hydroxylamine perchlorate TS with ethanol (99.5) to make 100 mL.

Storage—Preserve in tight containers, in a cold place.

Hydroxylamine perchlorate TS An ethanol (95) solution which contains 13.4% of hydroxylamine perchlorate.

Storage—Preserve in tight containers, in a cold place.

Hydroxylamine TS Dissolve 10 g of hydroxylammonium chloride in 20 mL of water, and add ethanol (95) to make 200 mL. To this solution add, with stirring, 150 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and filter. Prepare before use.

Hydroxylamine TS, alkaline Mix equal volumes of a solution of hydroxylammonium chloride in methanol (7 in 100) and a solution of sodium hydroxide in methanol (3 in 25), and filter. Prepare before use.

Hydroxylamine hydrochloride TS, pH 3.1 See hydroxylammonium chloride TS, pH 3.1.

Hydroxylammonium chloride NH₂OH.HCl [K 8201, Special class]

Hydroxylammonium chloride-iron (III) chloride TS Acidify 100 mL of a solution of iron (III) chloride hexahydrate in ethanol (95) (1 in 200) with hydrochloric acid, and dissolve 1 g of hydroxylammonium chloride in the solution.

Hydroxylammonium chloride TS Dissolve 20 g of hydroxylammonium chloride in water to make 65 mL, transfer it to a separator, add 2 to 3 drops of thymol blue TS, then add ammonia solution (28) until the solution exhibits a yellow color. Shake well after adding 10 mL of a solution of sodium *N,N*-diethyldithiocarbamate trihydrate (1 in 25), allow to stand for 5 minutes, and extract this solution with 10 to 15 mL portions of chloroform. Repeat the extraction until 5 mL of the extract does not exhibit a yellow color, upon adding 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) and shaking it. Add 1 to 2 drops of thymol blue TS, add dropwise dilute hydrochloric acid to this aqueous solution until it exhibits a red color, then add water to make 100 mL.

Hydroxylammonium chloride TS, pH 3.1 Dissolve 6.9 g of hydroxylammonium chloride in 80 mL of water, adjust the pH to 3.1 by adding dilute sodium hydroxide TS, and add water to make 100 mL.

Hydroxylammonium chloride-ethanol TS Dissolve 34.8 g of hydroxylammonium chloride in water to make 100 mL, and use this solution as Solution A. Dissolve 10.3 g of sodium acetate trihydrate and 86.5 g of sodium hydroxide in water to make 1000 mL, and use this solution as Solution B. Mix 1 volume of Solution A, 1 volume of Solution B and 4 volumes of ethanol (95).

4-Hydroxy-3-methoxybenzyl nonylic acid amide C₁₇H₂₇NO₃ A white crystalline powder, having a faint, characteristic odor.

Purity Related substances—Dissolve 10 mg in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed in the Component determination under Capsicum: when measure the peak areas 2 times as long as the retention time of capsaicin, the total area of the

peaks other than 4-hydroxy-3-methoxybenzyl nonylic acid amide is not larger than the peak area of 4-hydroxy-3-methoxybenzyl nonylic acid amide from the standard solution.

***N*-(3-Hydroxyphenyl)acetamide** $C_8H_9NO_2$ White to pale yellowish white crystals. It is freely soluble in ethanol (95), and sparingly soluble in water.

Melting point <2.60>: 146 – 149°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of *N*-(3-hydroxyphenyl)acetamide in 50 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.1 g of *N*-(3-hydroxyphenyl)acetamide in 1000 mL of water. Pipet 10 mL of this solution, add 6.5 mL of acetonitrile and water to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed in the Assay under Aspoxicillin Hydrate: any peak other than those of *N*-(3-hydroxyphenyl)acetamide and the solvent does not appear.

3-(3-Hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid

$C_{10}H_{10}O_4$ White to light yellow, crystals or crystalline powder. Sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 230°C (with decomposition).

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, between 238 nm and 242 nm, between 290 nm and 294 nm, and between 319 nm and 323 nm.

Purity Related substances—Dissolve 1 mg in 1 mL of methanol. Proceed the test with 2 μ L of this solution as directed in the Identification (11) under Hochuekkito Extract: no spot other than the principle spot of around *R_f* 0.6 appears.

3-(3-Hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography Dissolve 1 mg of 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid and 1 mg of (*E*)-ferulic acid in 2 mL of methanol.

2-[4-(2-Hydroxymethyl)-1-piperazinyl] propanesulfonic acid $C_8H_{18}N_2O_4S$ A white crystalline powder.

Residue on ignition <2.44>: not more than 0.1%.

Content: not less than 99%.

3-(*p*-Hydroxyphenyl)propionic acid $C_9H_{10}O_3$

Description—White to light yellow-brown crystals or crystalline powder, having a faint, characteristic odor.

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.2 g of 3-(*p*-hydroxyphenyl)propionic acid, previously dried (in vacuum, 60°C, 4 hours), dissolve in 5 mL of methanol, add 45 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 16.62 mg of $C_9H_{10}O_3$

Hypaconitine for purity $C_{33}H_{45}NO_{10}$ White, crystals or crystalline powder. Soluble in acetonitrile, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water. Melting point: about 175°C (with decomposition).

Identification—Determine the infrared absorption spectrum of hypaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500 cm^{-1} , 1728 cm^{-1} , 1712 cm^{-1} , 1278 cm^{-1} , 1118 cm^{-1} , 1099 cm^{-1} and 714 cm^{-1} .

Absorbance <2.24> $E_{1\%}^{1\text{cm}}$ (230 nm): 217 – 252 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

Purity Related substances—(1) Dissolve 5.0 mg of hypaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of hypaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of hypaconitine and the solvent obtained with the sample solution is not larger than the peak area of hypaconitine with the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of hypaconitine is about 23 minutes.

Time span of measurement: About 3 times as long as the retention time of hypaconitine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of hypaconitine obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μ L of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hypaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μ L of this solution under the above operating conditions, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hypaconitine is not more than 1.5%.

Water <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding

0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Hypophosphorus acid See phosphinic acid.

Hypoxanthine $C_5H_4N_4O$ White crystals or crystalline powder. Freely soluble in ammonia TS, sparingly soluble in dilute hydrochloric acid and in hot water, very slightly soluble in water, and practically insoluble in methanol.

Purity Related substances—Dissolve 5.0 mg of hypoxanthine in 100 mL of a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL. Proceed with this solution as directed in the Purity (4) under Mercaptopurine Hydrate: any spot other than the principal spot at the *R_f* value of about 0.2 does not appear.

Content: not less than 97.0% and not more than 103.0%. Assay—Weigh accurately about 0.15 g of hypoxanthine, previously dried at 105°C for 3 hours, and dissolve in phosphate buffer solution, pH 7.0, to make exactly 1000 mL. Pipet 10 mL of this solution, and dilute with phosphate buffer solution, pH 7.0, to make exactly 250 mL. Read the absorbance *A* of this solution at the wavelength of 250 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using phosphate buffer solution, pH 7.0, as the blank solution.

$$\text{Amount (mg) of } C_5H_4N_4O = \frac{A}{779} \times 250,000$$

Ibuprofen $C_{13}H_{18}O_2$ [Same as the namesake monograph]

Icariin for thin-layer chromatography $C_{33}H_{40}O_{15}$ Light yellow crystals. Very slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 234°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of icariin for thin-layer chromatography in 1 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification under Epimedium Herb: no spot other than the principal spot having *R_f* value about 0.4 appears.

Imidazole $C_3H_4N_2$ White crystalline powder. Very soluble in water and in methanol.

Melting point <2.60>: 89 – 92°C

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (313 nm): not more than 0.031 (8 g, water, 100 mL).

Imidazole for Karl Fischer method See the Water Determination <2.48>.

Imidazole for thin-layer chromatography $C_3H_4N_2$ White, crystalline powder. Very soluble in water and in methanol, and freely soluble in ethyl acetate and in dichloromethane.

Melting point <2.60>: 89 – 92°C

Purity Related substances—Dissolve 10 mg of imidazole for thin-layer chromatography in exactly 20 mL of dichloromethane, and proceed with this solution as directed in the Purity (6) under Clotrimazole: any spot other than the principal spot does not appear.

Imidazole TS Dissolve 8.25 g of imidazole in 65 mL of water, adjust the pH to 6.8 with 5 mol/L hydrochloric acid TS, and add water to make 100 mL.

Iminodibenzyl $C_{14}H_{13}N$ White to light brown crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 104 – 110°C

Purity (1) Clarity of solution—Dissolve 1.0 g of iminodibenzyl in 20 mL of methanol by heating on a water bath: the solution is clear.

(2) Related substances—Proceed as directed in the Purity (6) under Carbamazepine: any spot other than the principal spot at the *R_f* value of about 0.9 does not appear.

Nitrogen <1.08>: 6.8 – 7.3%.

Imipramine hydrochloride $C_{19}H_{24}N_2 \cdot HCl$ [Same as the namesake monograph]

Indigo carmine $C_{16}H_8N_2Na_2O_8S_2$ [K 8092, Special class]

Indigo carmine TS Dissolve 0.20 g of indigo carmine in water to make 100 mL. Use within 60 days.

2,3-Indolinedione $C_8H_5NO_2$ [K 8089, Special class]

Indometacin $C_{19}H_{16}ClNO_4$ [Same as the namesake monograph]

Interleukin-2 dependent mouse natural killer cell (NKC3) Fractionate using discontinuous concentration gradient method cells obtained by removing adhesive cells and phagocytic cells from C3H/He mouse spleen cells. Then, cultivate in soft agar containing interleukin-2 the cell fraction with potent NK activity and obtain the colonies. From among the cell lines obtained, one of the cell lines dependent on interleukin-2 that grows in liquid medium and serially subcultured in liquid medium containing interleukin-2 is identified as NKC3.

Iodine I [K 8920, Special class]

Iodine for assay I [Same as the monograph Iodine]

Iodine monobromide IBr Blackish brown crystals or masses.

It dissolves in water, in ethanol (95), in diethyl ether, in carbon disulfide and in acetic acid (100).

Melting point <2.60>: 40°C

Storage—Preserve in light-resistant glass containers, in a cold place.

Iodine-starch TS To 100 mL of starch TS add 3 mL of dilute iodine TS.

Iodine trichloride ICl_3 [K 8403, Special class]

Iodine TS Dissolve 14 g of iodine in 100 mL of a solution of potassium iodide (2 in 5), add 1 mL of dilute hydrochloric acid, and dilute with water to make 1000 mL (0.05 mol/L).

Storage—Preserve in light-resistant containers.

Iodine TS, dilute To 1 volume of iodine TS add 4 volumes of water.

0.0002 mol/L Iodine TS Measure exactly 1 mL of 0.5 mol/L iodine TS, add water to make exactly 250 mL, pipet 10 mL of the solution, and add water to make exactly 100 mL. Prepare before use.

0.5 mol/L Iodine TS To 12.7 g of iodine and 25 g of potassium iodide add 10 mL of water, triturate, and add water to make 100 mL.

Iodoethane C_2H_5I A colorless or a dark-brown, clear liquid, having diethyl ether-like odor.

Distilling range <2.57>: 71.0 – 72.5°C, not less than 94 vol %.

Iodomethane CH_3I [K 8919, Special class]

5-Iodouracil for liquid chromatography $\text{C}_4\text{H}_3\text{IN}_2\text{O}_2$
White, crystalline powder. Melting point: about 275°C (with decomposition).

Purity—Dissolve 3 mg of 5-iodouracil for liquid chromatography in a mixture of diluted methanol (1 in 25) to make 10 mL. Perform the test with $10\ \mu\text{L}$ of this solution as directed under Liquid Chromatography <2.01>, according to the operating conditions in the Purity under Idoxuridine Eye Drops. Measure each peak area by the automatic integration method over a time span of twice as long as the retention time of the principal peak, and calculate the amount of 5-iodouracil by the area percentage method: It shows the purity of not less than 98.5%.

Content: not less than 98.5%. **Assay**—Weigh accurately about 5 mg of 5-iodouracil for liquid chromatography, previously dried at 60°C for 3 hours under reduced pressure, dissolve in water to make exactly 250 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbance A at the wavelength of maximum absorption at about 282 nm.

$$\begin{aligned} \text{Amount (mg) of 5-iodouracil (C}_4\text{H}_3\text{IN}_2\text{O}_2\text{)} \\ = \frac{A}{265} \times 2500 \end{aligned}$$

Iotalamic acid for assay $\text{C}_{11}\text{H}_9\text{I}_3\text{N}_2\text{O}_4$ [Same as the monograph Iotalamic Acid]

Iron Fe Iron in the forms of strips, sheets, granules or wires. Fe: not less than 97.7%. It is attracted by a magnet.

Iron (III) chloride-acetic acid TS Dissolve 0.1 g of iron (III) chloride hexahydrate in diluted acetic acid (3 in 100) to make 100 mL.

Iron (III) chloride hexahydrate $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ [K 8142, Special class]

Iron (III) chloride-iodine TS Dissolve 5 g of iron (III) chloride hexahydrate and 2 g of iodine in a mixture of 50 mL of acetone and 50 mL of a solution of tartaric acid (1 in 5).

Iron (III) chloride-methanol TS Dissolve 1 g of iron (III) chloride hexahydrate in methanol to make 100 mL.

Iron (III) chloride-potassium hexacyanoferrate (III) TS Dissolve 0.1 g of potassium hexacyanoferrate (III) in 20 mL of iron (III) chloride TS. Prepare before use.

Iron (III) chloride-pyridine TS, anhydrous Heat gradually 1.7 g of iron (III) chloride hexahydrate by direct application of flame, melt, and solidify. After cooling, dissolve the residue in 100 mL of chloroform, add 8 mL of pyridine, and filter.

Iron (III) chloride TS Dissolve 9 g of iron (III) chloride hexahydrate in water to make 100 mL (1/3 mol/L).

Iron (III) chloride TS, acidic To 60 mL of acetic acid (100) add 5 mL of sulfuric acid and 1 mL of iron (III) chloride hexahydrate TS.

Iron (III) chloride TS, dilute Dilute 2 mL of iron (III) chloride hexahydrate TS with water to make 100 mL. Prepare before use.

Iron (III) nitrate enneahydrate $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$

[K 8559, Special class]

Iron (III) nitrate TS Dissolve 1 g of iron (III) nitrate enneahydrate in hydrochloric acid-potassium chloride buffer solution (pH 2.0) to make 300 mL.

Iron (III) perchlorate-ethanol TS Dissolve 0.8 g of iron (III) perchlorate hexahydrate in perchloric acid-ethanol TS to make 100 mL.

Storage—Preserve in tight containers, in a cold place.

Iron (III) perchlorate hexahydrate $\text{Fe}(\text{ClO}_4)_3 \cdot 6\text{H}_2\text{O}$
Hygroscopic, light purple crystals, and a solution in ethanol (99.5) (1 in 125) is clear and orange in color.

Iron (III) sulfate *n*-hydrate $\text{Fe}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$ [K 8981, Special class]

Iron (III) sulfate TS Dissolve 50 g of iron (III) sulfate *n*-hydrate in an excess of water, and add 200 mL of sulfuric acid and water to make 1000 mL.

Iron (II) sulfate heptahydrate $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ [K 8978, Special class]

Iron (II) sulfate TS Dissolve 8 g of iron (II) sulfate heptahydrate in 100 mL of freshly boiled and cooled water. Prepare before use.

Iron (II) sulfide FeS [K 8948, for hydrogen sulfide development]

Iron (II) tartrate TS Dissolve 1 g of iron (II) sulfate heptahydrate, 2 g of potassium sodium tartrate tetrahydrate and 0.1 g of sodium hydrogen sulfite in water to make 100 mL.

Iron (II) thiocyanate TS Add 3 mL of dilute sulfuric acid to 35 mL of water, and remove the dissolved oxygen by boiling the solution. Dissolve 1 g of iron (II) sulfate heptahydrate in this hot solution, cool, and then dissolve 0.5 g of potassium thiocyanate. When the solution is pale red in color, decolorize by adding reduced iron, separate the excess of reduced iron by decanting, and preserve the solution with protection from oxygen. Do not use a solution showing a pale red color.

Iron (II) trisodium pentacyanoamine TS To 1.0 g of sodium pentacyanonitrosylferrate (III) dihydrate add 3.2 mL of ammonia water, shake, stopper closely, and allow to stand in a refrigerator overnight. Add this solution to 10 mL of ethanol (99.5), filter a yellow colored precipitate by suction, wash with ether (99.5), dry, and preserve in a desiccator. Before using, dissolve in water to make a solution of 1.0 mg/mL, and store in a refrigerator. Use within 7 days after preparation.

Iron-phenol TS Dissolve 1.054 g of ammonium iron (II) sulfate hexahydrate in 20 mL of water, add 1 mL of sulfuric acid and 1 mL of hydrogen peroxide (30), heat until effervescence ceases, and dilute with water to make 50 mL. To 3 volumes of this solution contained in a volumetric flask add sulfuric acid, with cooling, to make 100 volumes, yielding the iron-sulfuric acid solution. Purify phenol by distillation, discarding the first 10% and the last 5%, and collect the distillate, with exclusion of moisture, in a dry, tared, glass-stoppered flask of about twice the volume of the phenol. Stopper the flask, solidify the phenol in an ice bath, breaking the top crust with a glass rod to ensure complete crystallization, and after drying, weigh the flask. To the glass-stoppered flask add 1.13 times the mass of phenol of the iron sulfuric acid solu-

tion, insert the stopper in the flask, and allow to stand, without cooling but with occasional shaking, until the phenol is liquefied, then shake the mixture vigorously. Allow to stand in a dark place for 16 to 24 hours. To the mixture add diluted sulfuric acid (10 in 21) equivalent to 23.5% of its mass, mix well, transfer to dry glass-stoppered bottles, and preserve in a dark place, with protection from atmospheric moisture. Use within 6 months.

Iron-phenol TS, dilute To 10 mL of iron-phenol TS add 4.5 mL of water. Prepare before use.

Iron powder Fe A lusterless, gray to grayish black powder, being attracted by a magnet.

Identification—To 1 mL of a solution in hydrochloric acid (1 in 50) add water to make 15 mL, and add 0.1 mL of potassium hexacyanoferrate (III) TS: a blue color appears.

Iron salicylate TS Dissolve 0.1 g of ammonium iron (III) sulfate dodecahydrate in 50 mL of diluted sulfuric acid (1 in 250), and add water to make 100 mL. Measure 20 mL of this solution, and add 10 mL of a solution of sodium salicylate (23 in 2000), 4 mL of dilute acetic acid, 16 mL of sodium acetate TS and water to make 100 mL. Prepare before use.

Isatin See 2,3-indolinedione.

Isoamyl acetate See 3-methylbutyl acetate.

Isoamyl alcohol See 3-methyl-1-butanol.

Isoamyl benzoate $C_{12}H_{16}O_2$

Specific gravity <2.56> d_4^{15} : 0.993

Boiling point <2.57>: 260 – 262°C

Isoamyl parahydroxybenzoate $C_{12}H_{16}O_3$ White crystalline powder, having a faint characteristic odor.

It is very soluble in acetonitrile, in ethanol (95), in acetone and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 62 – 64°C

Isobutanol See 2-methyl-1-propanol.

Isobutyl parahydroxybenzoate $C_{11}H_{14}O_3$ Colorless crystals or white crystalline powder. Odorless. Freely soluble in ethanol (95), in acetone and in diethyl ether, and practically insoluble in water.

Melting point <2.60>: 75 – 77°C

Residue on ignition <2.44>: not more than 0.1%.

Content: not less than 98.0%. *Assay*—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS
= 194.2 mg of $C_{11}H_{14}O_3$

Isobutyl salicylate $C_{11}H_{14}O_3$ Colorless, clear liquid, having a characteristic odor.

Refractive index <2.45> n_D^{20} : 1.506 – 1.511

Specific gravity <2.56> d_4^{20} : 1.068 – 1.073

Boiling point <2.57>: 260 – 262°C

Purity—Perform the test with 1 μ L of isobutyl salicylate as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of isobutyl salicylate by the area percentage method: It shows the purity of not less than 97.0%.

Operating conditions

Detector: A thermal conductivity detector.

Column: A column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μ m in particle diameter, coated with polyethylene glycol 20 M for gas chromatography at the ratio of 10%.

Column temperature: A constant temperature of about 220 °C.

Carrier gas: Helium

Flow rate: About 20 mL per minute.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of isobutyl salicylate obtained from 1 μ L of the sample solution is about 60% to 80% of the full scale.

Time span of measurement: About 3 times as long as the retention time of isobutyl salicylate beginning after the solvent peak.

Isoelectric point markers for tecceleukin Dissolve 0.02 to 0.05 mg of cytochrome C, trypsinogen, lentil-lectin basic band, lentil-lectin middle band, lentil-lectin acidic band, horse myoglobin basic band, horse myoglobin acidic band, human carbonic anhydrase B, bovine carbonic anhydrase B, and β -lactoglobulin A, in 0.1 mL of saccharose solution (3 in 10).

L-Isoleucine $C_6H_{13}NO_2$ [Same as the namesake monograph]

Isoniazid for assay $C_6H_7N_3O$ [Same as the monograph Isoniazid. When dried, it contains not less than 99.0% of isoniazid ($C_6H_7N_3O$).]

Isoniazid $C_6H_7N_3O$ [Same as the namesake monograph]

Isoniazid TS Dissolve 0.1 g of isoniazid for assay in a mixture of 50 mL of methanol and 0.12 mL of hydrochloric acid, and add methanol to make 200 mL.

Isonicotinic acid White, crystals or powder. *Melting point*: about 315°C (decomposition).

Isonicotinic acid amide $C_6H_6N_2O$ White, crystals or crystalline powder.

Melting point <2.60>: 155 – 158°C

Purity Clarity of solution—Dissolve 1.0 g of the substance to be tested in 20 mL of methanol: the solution is clear.

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.3 g of isonicotinic acid amide, previously dried, and dissolve in 20 mL of acetic acid (100) by heating. After cooling, add 100 mL of benzene, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.21 mg of $C_6H_6N_2O$

Isooctane See octane, iso.

Isopromethazine hydrochloride for thin-layer chromatography $C_{17}H_{20}N_2S.HCl$ White, odorless, crystalline powder. Freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

Melting point <2.60>: 193 – 197°C

Purity Related substances—Dissolve 5.0 mg of iso-

promethazine hydrochloride for thin-layer chromatography in exactly 25 mL of ethanol, and perform the test with this solution as directed in the Purity (3) under Promethazine Hydrochloride: any spot other than the principal spot at the R_f value of about 0.65 does not appear.

Isopropanol See 2-propanol.

Isopropanol for liquid chromatography See 2-propanol for liquid chromatography.

Isopropyl benzoate $C_6H_5COOCH(CH_3)_2$ A clear, colorless liquid, having a characteristic odor.

Refractive index <2.45> n_D^{20} : 1.490 – 1.498

Specific gravity <2.56> d_{20}^{20} : 1.008 – 1.016

Isopropylamine See propylamine, iso.

Isopropylamine-ethanol TS To 20 mL of isopropylamine add ethanol (99.5) to make 100 mL. Prepare before use.

Isopropylether See propylether, iso.

Isopropyl iodide for assay C_3H_7I Colorless, clear liquid. On exposure to light it liberates iodine and becomes brown. Miscible with ethanol (95), with diethyl ether and with petroleum benzin, and not miscible with water. Use the distillate obtained between 89.0°C and 89.5°C.

Specific gravity <2.56> d_4^{20} : 1.700 – 1.710

Purity—Perform the test with 1 μ L of isopropyl iodide for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of isopropyl iodide by the area percentage method: It shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of isopropyl iodide from 1 μ L of isopropyl iodide for assay is about 80% of the full scale.

Content: not less than 98.0%. **Assay**—Transfer 10 mL of ethanol (95) into a brown volumetric flask, weigh accurately, add 1 mL of isopropyl iodide for assay, and weigh accurately again. Add ethanol (95) to make exactly 100 mL, pipet 20 mL of this solution into the second brown volumetric flask, add exactly 50 mL of 0.1 mol/L silver nitrate VS and then 2 mL of nitric acid, stopper, shake occasionally for 2 hours in a dark place, and allow to stand overnight in a dark place. Shake occasionally for 2 hours, add water to make exactly 100 mL, and filter through dry filter paper. Discard the first 20 mL of the filtrate, pipet the next 50 mL, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L silver nitrate VS
= 17.00 mg of C_3H_7I

Isopropyl myristate $C_{17}H_{34}O_2$ Colorless, clear, oily liquid, and odorless. Congeals at about 5°C. Soluble in 90% alcohol, miscible with many organic solvents and with solid oils, and insoluble in water, in glycerin and in propylene glycol.

Refractive index <2.45> n_D^{20} : 1.432 – 1.436

Specific gravity <2.56> d_{20}^{20} : 0.846 – 0.854

Saponification value <1.13>: 202 – 212

Acid value <1.13>: not more than 1.

Iodine value <1.13>: not more than 1.

Residue on ignition <2.44>: not more than 0.1% (1 g).

Isopropyl myristate for sterility test $C_{17}H_{34}O_2$ Transfer 100 mL of isopropyl myristate into a centrifuge tube, add 100 mL of twice-distilled water, and shake vigorously for 10 minutes. Then centrifuge at a rate of 1800 revolutions per minute for 20 minutes, separate the supernatant liquid (isopropyl myristate layer), and determine the pH of the residual water layer: not less than 5.5.

Treat isopropyl myristate which meets the requirements of pH determination as follows: 500 mL of isopropyl myristate, which has met the requirements of pH determination, is percolated through a 15-cm high layer of activated alumina filled in a glass column 20 mm in diameter and 20 cm in length with a slightly positive pressure in order to facilitate adequate flow, and then sterilized by filtration.

Isopropyl *p*-aminobenzoate See isopropyl 4-aminobenzoate.

Isopropyl 4-aminobenzoate $NH_2C_6H_4COOCH(CH_3)_2$
Pale brown crystals.

Melting point <2.60>: 83 – 86°C

Isopropyl *p*-hydroxybenzoate See isopropyl parahydroxybenzoate.

Isopropyl parahydroxybenzoate $C_{10}H_{12}O_3$ Odorless and colorless fine crystals, or white, crystalline powder. Freely soluble in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

Melting point <2.60>: 84 – 86°C

Residue on ignition <2.44>: not more than 0.1%.

Content: not less than 99.0%. **Assay**—Proceed as directed in the Assay under Ethyl Parahydroxybenzoate.

Each mL of 1 mol/L sodium hydroxide VS
= 180.2 mg of $C_{10}H_{12}O_3$

Isotonic sodium chloride solution [Same as the namesake monograph]

Japanese acid clay Natural hydrous aluminum silicate, grayish white powder, having a particle size of about 74 μ m.

Loss on drying <2.41>: not more than 10% (1 g, 105°C, 4 hours).

Water adsorbing capacity: not less than 2.5%. Weigh accurately about 10 g of Japanese acid clay in weighing bottle, allow to stand for 24 hours with cover in a chamber, in which humidity is maintained to 80% by means of sulfuric acid (specific gravity 1.19), reweigh, and determine the increase of mass of the sample.

Jesaconitine for purity $C_{35}H_{49}NO_{12}$ A white powder. Freely soluble in acetonitrile, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification—Determine the infrared absorption spectrum of jesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3500 cm^{-1} , 1715 cm^{-1} , 1607 cm^{-1} , 1281 cm^{-1} , 1259 cm^{-1} , 1099 cm^{-1} and 772 cm^{-1} .

Absorbance <2.24> $E_{1\%}^{1\text{cm}}$ (258 nm): 270 – 291 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

Purity Related substances—(1) Dissolve 5.0 mg of

jesaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification in Processed Aconite Root: the spot other than the principal spot is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of jesaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of jesaconitine and the solvent is not larger than the peak area of jesaconitine with the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of jesaconitine is about 36 minutes.

Time span of measurement: About 3 times as long as the retention time of jesaconitine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of jesaconitine obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μ L of the standard solution.

System performance: Dissolve 5 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 1 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μ L of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of jesaconitine is not more than 1.5%.

Water <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Josamycin $C_{42}H_{69}NO_{15}$ [Same as the namesake monograph]

Josamycin propionate $C_{45}H_{73}NO_{16}$ [Same as the namesake monograph]

Kainic acid $C_{10}H_{15}NO_4 \cdot H_2O$ [Same as the monograph Kainic Acid Hydrate]

Kainic acid for assay [Same as the monograph Kainic Acid Hydrate]

Kanamycin sulfate $C_{18}H_{36}N_4O_{11} \cdot xH_2SO_4$ [Same as the namesake monograph]

Karl Fischer TS See Water Determination <2.48>.

Kerosene It is mainly a mixture of hydrocarbons in the methane series, and a colorless, clear liquid, having not a disagreeable, characteristic odor.

Specific gravity <2.56>: about 0.80

Distilling range <2.57>: 180 – 300°C

Kininogen Produced by purifying from bovine plasma. Dissolve an appropriate amount of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 so that 10 mL of the solution contains 1 mg of kininogen, and use this solution as the sample solution. Perform the following tests with the sample solution: it meets the requirement of each test.

(i) Immediately after the sample solution is prepared, add 0.1 mL of a solution of trichloroacetic acid (1 in 5) to 0.5 mL of the sample solution, shake, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution. Proceed with 0.1 mL of this solution as directed in the Purity (2) under Kallidinogenase, and determine the amount of kinin: kinin is not detected.

(ii) Warm 0.5 mL of the sample solution at $30 \pm 0.5^\circ\text{C}$ for 20 minutes, and proceed as directed in (i): kinin is not detected.

(iii) Perform the test with 0.5 mL of the sample solution as directed in the Purity (2) under Kallidinogenase: the decomposition of bradykinin is not observed.

(iv) To 0.5 mL of the sample solution add 0.5 mL of 0.02 mol/L phosphate buffer solution, pH 8.0 containing 500 μ g of crystal trypsin, previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, warm this solution at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, add 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Then boil for 3 minutes, cool in ice immediately, and centrifuge. To 0.5 mL of the supernatant liquid add 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. To 0.1 mL of this solution add 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution. To 0.1 mL of this solution add trichloroacetic acid-gelatin-tris buffer solution to make 20 mL, then proceed as directed in (i), and determine the amount, B_K , of kinin per well. Calculate the kinin-releasing activity per mg by the following equation: not less than 10 μ g bradykinin equivalent per mg.

Kinin-releasing activity per mg (μ g bradykinin equivalent/mg) = $B_K \times 0.0096$

Kininogen TS Dissolve a sufficient quantity of kininogen in 0.02 mol/L phosphate buffer solution, pH 8.0 to prepare a solution having an ability in each mL to release kinin corresponding to not less than 1 μ g of bradykinin.

Lactic acid $CH_3CH(OH)COOH$ [K 8726, Special class]

Lactic acid TS Dissolve 12.0 g of lactic acid in water to make 100 mL.

α -Lactoalbumin White powder. Derived from milk. Molecular weight of about 14,200.

Lactobionic acid $C_{12}H_{22}O_{12}$ Colorless crystals or white crystalline powder, having no odor.

Melting point <2.60>: 113 – 118°C

Purity—Dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and perform the test with 10 μ L of this solution as directed in the Identification (2) under Erythromycin Lactobionate: the spot other than the principal spot is not found.

β -Lactoglobulin Prepare from milk. White to light yellow powder.

Nitrogen content <1.08>: not less than 14% (calculated on the dried basis).

Lactose See lactose monohydrate.

α -Lactose and β -lactose mixture (1:1) Use a mixture of lactose monohydrate and anhydrous lactose (3:5).

Lactose broth After adding lactose monohydrate to ordinary broth in the ratio of 0.5%, add about 12 mL of bromothymol blue-sodium hydroxide TS to 1000 mL of the medium. Then dispense portions of about 10 mL into tubes for fermentation, and sterilize fractionally on each of three successive days for 15 to 30 minutes at 100°C by using an autoclave, or sterilize by autoclaving for not more than 20 minutes at 121°C, and cool quickly by immersing in cold water.

Lactose broth, three times concentrated Add lactose monohydrate to ordinary broth prepared by using 330 mL in place of 1000 mL of water in the ratio of 1.5%, and prepare according to the method of preparation under lactose broth, with 25 mL portions in tubes for fermentation.

Lactose broth, twice concentrated Add lactose monohydrate to ordinary broth prepared by using 500 mL in place of 1000 mL of water in the ratio of 1.0% and prepare according to the method of preparation under lactose broth.

Lactose monohydrate $C_{12}H_{22}O_{11} \cdot H_2O$ [Same as the monograph Lactose].

Lactose substrate TS Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

Lactose substrate TS for β -galactosidase (penicillium) Dissolve 6.0 g of lactose monohydrate in diluted disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 (1 in 10) to make 100 mL.

Lanthanum-alizarin complexone TS To 1 mL of ammonia water (28) add 10 mL of water. To 4 mL of this solution add 4 mL of a solution of ammonium acetate (1 in 5) and 192 mg of alizarin complexone, and label this solution as alizarin complexone stock solution. Dissolve 41 g of sodium acetate trihydrate in 400 mL of water, and add 24 mL of acetic acid (100). To this solution add the total volume of the alizarin complexone stock solution, add 400 mL of acetone, and label this solution as alizarin complexone solution. To 10 mL of diluted hydrochloric acid (1 in 6) add 163 mg of lanthanum (III) oxide, heat to dissolve, and label this solution as lanthanum solution. To the alizarin complexone solution add the lanthanum solution, and mix. After cooling, adjust to pH 4.7 with acetic acid (100) or ammonia water (28), and add water to make 1000 mL. Prepare before use.

Lanthanum (III) oxide La_2O_3 White crystals.

Loss on ignition <2.43>: not more than 0.5% (1 g, 1000°C, 1 hour)

Lauromacrogol [Same as the namesake monograph]

Lead acetate See lead (II) acetate trihydrate.

Lead acetate TS See lead (II) acetate TS.

Lead (II) acetate TS To 9.5 g of lead (II) acetate trihydrate add freshly boiled and cooled water to make 100 mL. Preserve in tightly stoppered bottles (0.25 mol/L).

Lead dioxide See lead (IV) oxide.

Lead (II) acetate trihydrate $Pb(CH_3COO)_2 \cdot 3H_2O$ [K 8374, Special class]

Lead (II) nitrate $Pb(NO_3)_2$ [K 8563, Special class]

Lead (II) oxide PbO [K 8090, Special class]

Lead (IV) oxide PbO_2 A dark brown to black-brown, powder or granules.

Identification—A supernatant liquid of a solution in dilute acetic acid (1 in 100) responds to Quality Tests <1.09> (3) for lead salt.

Lead monoxide See lead (II) oxide.

Lead nitrate See lead (II) nitrate.

Lead subacetate TS Place the yellowish mixture, obtained by triturating 3 g of lead (II) acetate trihydrate and 1 g of lead (II) oxide with 0.5 mL of water, in a beaker, and heat on a water bath, covering with a watch glass, until it shows a homogeneous, white to reddish white color. Then add 9.5 mL of hot water in small portions, cover it again with a watch glass, and set it aside. Decant the supernatant liquid, and adjust the specific gravity to 1.23 to 1.24 (15°C) by adding water. Preserve in tightly stoppered bottles.

Lead subacetate TS, dilute To 2 mL of lead subacetate TS add freshly boiled and cooled water to make 100 mL. Prepare before use.

L-Leucine $C_6H_{13}NO_2$ [Same as the namesake monograph]

Levallorphan tartrate for assay $C_{19}H_{25}NO \cdot C_6H_6O_6$ [Same as the monograph Levallorphan Tartrate. When dried, it contains not less than 99.0% of $C_{19}H_{25}NO \cdot C_6H_6O_6$.]

Levothyroxine sodium $C_{15}H_{11}I_4NNaO_4 \cdot xH_2O$ [Same as the monograph Levothyroxine Sodium Hydrate]

Levothyroxine sodium for thin-layer chromatography [Same as the monograph Levothyroxine Sodium Hydrate. Proceed the test as directed in the Identification (3) under Levothyroxine Sodium Hydrate: any spot other than the principal spot at the R_f value of about 0.26 does not appear.

Lidocaine for assay ($C_{14}H_{22}N_2O$) [same as the monograph Lidocaine]

Limonene $C_{10}H_{16}$ Clear and colorless liquid, having a specific perfume and a bitter taste.

Refractive index <2.45> n_D^{20} : 1.427 – 1.474

Specific gravity <2.56> d_{20}^{20} : 0.841 – 0.846

Melting point <2.60>: 176 – 177°C

Purity Related substances—Dissolve 0.1 g of limonene in 25 mL of hexane and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed

under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of limonene: it is not less than 97.0%.

Operating conditions

Proceed the operating conditions in the Assay under Eucalyptus Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Measure 1 mL of limonene, add hexane to make 100 mL, and adjust the detection sensitivity so that the peak height of limonene obtained from 2 μ L of this solution is 40% to 60% of the full scale.

Time span of measurement: About 3 times as long as the retention time of limonene beginning after the solvent peak.

(Z)-Ligustilide for thin-layer chromatography $C_{12}H_{14}O_2$
A clear, yellow-grown liquid, having a characteristic odor. Miscible with methanol and with ethanol (99.5), and practically insoluble in water.

Identification—Determine the absorption spectrum of a solution in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 320 nm and 324 nm.

Purity Related substances—Dissolve 1 mg in 10 mL of methanol. Proceed the test with 1 μ L of this solution as directed in the Identification (5) under Hochuekkito Extract: no spot other than the principle spot of around *Rf* 0.6 appears.

Liothyronine sodium $C_{15}H_{11}I_3NNaO_4$ [Same as the namesake monograph]

Liothyronine sodium for thin-layer chromatography [Same as the monograph Liothyronine Sodium. Proceed as directed for the Identification (1) under Liothyronine Sodium Tablets: any spot other than the principal spot at the *Rf* value of 0.3 to 0.4 does not appear.]

Liquid paraffin See paraffin, liquid.

Liquiritin for thin-layer chromatography $C_{21}H_{22}O_9 \cdot xH_2O$
White crystals or crystalline powder. Sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water. Melting point: about 210°C (with decomposition).

Identification—Determine the absorption spectrum of a solution in diluted methanol (1 in 2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 215 nm and 219 nm, and between 275 nm and 279 nm.

Purity Related substances—Dissolve 1.0 mg in 1 mL of methanol, and perform the test with 1 μ L of this solution as directed in the Identification (5) under Kakkonto Extract: no spot other than the principal spot (*Rf* value is about 0.4) appears.

Lisinopril $C_{21}H_{31}N_3O_5 \cdot 2H_2O$ [Same as the monograph Lisinopril Hydrate]

Lisinopril for assay $C_{21}H_{31}N_3O_5 \cdot 2H_2O$ [Same as the monograph Lisinopril Hydrate. It contains not less than 99.5% of lisinopril ($C_{21}H_{31}N_3O_5$; 405.49), calculated on the anhydrous basis.]

Lithium acetate dihydrate $CH_3COOLi \cdot 2H_2O$ Colorless crystals.

Dilute acetic acid insoluble substances—To 40.0 g of lithi-

um acetate dihydrate add 45 mL of water, heat in a water bath to dissolve, cool, then dissolve in dilute acetic acid, and filter by suction. Wash the filter with water, dry the filter at $105 \pm 2^\circ C$ for 1 hour, and weigh the mass of the residue after cooling: not more than 0.0025%.

Content: not less than 97.0%. **Assay**—Weigh accurately 0.3 g of lithium acetate dihydrate, add exactly 50 mL of acetic acid (100) and exactly 5 mL of acetic anhydride, dissolve by heating in a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS after cooling (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.20 mg of $CH_3COOLi \cdot 2H_2O$

Lithium bromide LiBr White crystals or crystalline powder. It is hygroscopic.

Purity (1) Chloride <1.03>: not more than 0.1%.

(2) Sulfate <1.14>: not more than 0.01%.

Lithium chloride LiCl White crystals or masses.

Identification—Perform the test as directed under Flame Coloration Test (1) <1.04>: a persistent red color appears.

Lithium sulfate See lithium sulfate monohydrate.

Lithium sulfate monohydrate $Li_2SO_4 \cdot H_2O$ [K 8994, Special class]

Lithocholic acid for thin-layer chromatography

$C_{24}H_{40}O_3$ White crystals or crystalline powder. Soluble in ethanol (95), in acetic acid (100) and in acetone, slightly soluble in chloroform, and practically insoluble in water. Melting point: about 186°C.

Purity Related substances—Dissolve 25 mg of lithocholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 25 mL. Dilute 1.0 mL of this solution with a mixture of chloroform and ethanol (95) (9:1) to make exactly 100 mL. Perform the test with 10 μ L of this solution as directed in the Purity (7) under Ursodeoxycholic Acid: any spot other than the principal spot with the *Rf* value of about 0.7 does not appear.

Content: 98.0%. **Assay**—Weigh accurately about 0.5 g of lithocholic acid for thin-layer chromatography, previously dried at 80°C for 4 hours under reduced pressure (phosphorus (V) oxide), dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, titrate <2.50> with 0.1 mol/L sodium hydroxide VS, add 100 mL of freshly boiled and cooled water near the end point, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 37.66 mg of $C_{24}H_{40}H_3$

Locke-Ringer's TS

Sodium chloride	9.0 g
Potassium chloride	0.42 g
Calcium chloride dihydrate	0.24 g
Magnesium chloride hexahydrate	0.2 g
Sodium hydrogen carbonate	0.5 g
Dextrose	0.5 g
Water, freshly distilled with a hard-glass apparatus	a sufficient quantity

To make 1000 mL

Prepare before use. The constituents except dextrose and

sodium hydrogen carbonate can be made up in concentrated stock solutions, stored in a dark place, and diluted before use.

Loganin for thin-layer chromatography $C_{17}H_{26}O_{10}$
White, crystals or crystalline powder. Soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5). Melting point: 221 – 227°C.

Purity Related substances—Dissolve 1.0 mg of loganin for thin-layer chromatography in 2 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification under Cornus Fruit: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Low-molecular weight heparin for calculation of molecular mass.

It is a low-molecular weight heparin with a disaccharide unit prepared, and display the molecular mass distribution between 600 and more than 10,000. When the average of molecular mass of Low-molecular weight heparin international standard is determined as a reference with this, the difference compared as a reference with the Low-molecular weight heparin international standard is not less than 5%.

Luteolin for thin-layer chromatography $C_{15}H_{10}O_6$
Light yellow to yellow-brown crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 310°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of luteolin for thin-layer chromatography in 1 mL of methanol. Proceed the test with 10 μ L of this solution as directed in the Identification under Chrysanthemum Flower: any spot other than the principal spot of *R_f* about 0.7 does not appear.

Lysate reagent A lyophilized product obtained from amebocyte lysate of horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*). Amebocyte lysate preparations which do not react to β -glucans are available: they are prepared by removing the G factor reacting to β -glucans from amebocyte lysate or by inhibiting the G factor reacting system of amebocyte lysate.

Lysate TS Dissolve a lysate reagent in water for bacterial endotoxins test, or in a suitable buffer, by gentle stirring.

Lysil endopeptidase White powder or masses, An exotoxin produced by *Achromobacter*. Molecular weight: 27,500.

L-Lysine hydrochloride $C_6H_{14}N_2O_2 \cdot HCl$ [Same as the namesake monograph]

Macrogol 600 $HOCH_2(CH_2OCH_2)_nCH_2OH$, $n = 11 - 13$
Clear, colorless, viscous liquid or a white, petrolatum-like solid, having a faint, characteristic odor. Very soluble in water, in ethanol (95), in acetone and in macrogol 400, soluble in diethyl ether, and practically insoluble in petroleum benzene. Congealing point: 18 – 23°C

Average molecular weight: When perform the test as directed in the Average molecular weight test under Macrogol 400, it is between 570 and 630.

4-(N-Maleimidylmethyl)-cyclohexane-1-carboxylate-N-hydroxysuccinimide ester $C_{16}H_{18}N_2O_6$ Colorless crystals. Being decomposed by acid and alkaline treatment.

Magnesia TS Dissolve 5.5 g of magnesium chloride hexahydrate and 7 g of ammonium chloride in 65 mL of water,

add 35 mL of ammonia TS, allow the mixture to stand for a few days in tightly stoppered bottles, and filter. If the solution is not clear, filter before use.

Magnesium Mg [K 8875, Special class]

Magnesium chloride See magnesium chloride hexahydrate.

Magnesium chloride hexahydrate $MgCl_2 \cdot 6H_2O$
[K 8159, Special class]

Magnesium nitrate See magnesium nitrate hexahydrate.

Magnesium nitrate hexahydrate $Mg(NO_3)_2 \cdot 6H_2O$
[K 8567, Special class]

Magnesium oxide MgO [K 8432, Special class]

Magnesium powder Mg [K 8876, Special class]

Magnesium sulfate See magnesium sulfate heptahydrate.

Magnesium sulfate heptahydrate $MgSO_4 \cdot 7H_2O$
[K 8995, Special class]

Magnesium sulfate TS Dissolve 12 g of magnesium sulfate hexahydrate in water to make 100 mL (0.5 mol/L).

Magneson [K 8879, Special class]

Magneson TS Dissolve 0.1 g of magneson in 100 mL of *N,N*-dimethylformamide.

Magnolol for component determination $C_{18}H_{18}O_2$
Odorless white, crystals or crystalline powder. Freely soluble in methanol and in diethyl ether, and practically insoluble in water. Melting point: about 102°C.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (290 nm): 270 – 293 [0.01 g dried for 1 hour or more a desiccator (silica gel), methanol, 500 mL].

Purity Related substances—(1) Dissolve 1.0 mg of magnolol for component determination in exactly 1 mL of methanol, and perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, then develop the plate with a mixture of hexane, acetone and acetic acid (100) (20:15:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot at the *R_f* value of about 0.5 does not appear.

(2) Dissolve 5.0 mg of magnolol for component determination in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak from these solutions by the automatic integration method: the total area of peaks other than the peak of magnolol from the sample solution is not larger than the peak area of magnolol from the standard solution.

Operating conditions

Proceed the operating conditions under Magnolia Bark except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution,

add the mobile phase to make exactly 20 mL. Adjust the sensitivity so that the peak area of magnolol obtained with 10 μ L of this solution can be measured, and is about 20% of full-scale by the automatic integration method.

Time span of measurement: About 3 times as long as the retention time of magnolol beginning after the solvent peak.

Malachite green See malachite green oxalate.

Malachite green oxalate $C_{52}H_{54}N_2O_{12}$ [K 8878, Malachite green (oxalate), Special class]

Maleic acid $C_4H_4O_4$ [K 8884, Special class]

Maltose See maltose monohydrate.

Maltose monohydrate $C_{12}H_{22}O_{11} \cdot H_2O$ [Same as the namesake monograph].

Manganese dioxide MnO_2 Black to black-brown, mass- or powder.

Identification—To 0.5 g add 20 mL of water and 3 mL of hydrochloric acid, and 3 mL of hydrogen peroxide (30). Alkalinize the solution with ammonia solution (28) while cooling, and add 25 mL of hydrogen sulfide TS: pale red precipitates appear.

D-Mannitol $C_6H_{14}O_6$ [Same as the monograph D-Mannitol]

D-Mannose $C_6H_{12}O_6$ White crystal or crystalline powder. It is very soluble in water. Melting point: about 132°C (with decomposition).

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: +13.7 – +14.7° (4 g, diluted ammonia TS (1 in 200), 20 mL, 100 mm).

Marker protein for celmoleukin molecular weight determination Add 10 μ L of cytochrome C prepared to a concentration of 2 mg per mL to 10 μ L of a commercially available marker protein with a known molecular weight (6 ingredients: phosphorylase b, bovine serum albumin, ovalbumin, carbonic dehydratase, soy trypsin inhibitor, and lysozyme) and then dilute 10-fold with buffer solution for celmoleukin.

Identification: Use the solution to be examined as the sample solution. Separately, to an amount of cytochrome C add distilled water for injection so that each mL contains 100 mg of protein, and use this as the standard solution. When 20 μ L each of the sample solution and standard solution are tested using SDS polyacrylamide gel electrophoresis under the operating conditions outlined in the Identification (3) of Celmoleukin (Genetical Recombination), the sample solution exhibits 7 major electrophoretic bands. Furthermore, the degree of mobility of the sample solution cytochrome C is consistent with that of the band obtained from the standard solution.

Meat extract Concentrated extract of fresh meat of bovine, equine or other animals. A yellow-brown to dark brown paste-like mass, having a meat-like odor.

Medium for float culture Dissolve 6.000 g of sodium chloride, 0.400 g of potassium chloride, 0.677 g of anhydrous sodium dihydrogen phosphate (NaH_2PO_4), 0.100 g of calcium nitrite tetrahydrate, 0.100 g of magnesium sulfate hydrate, 2.000 g of glucose, 0.164 g of sodium succinate hexahydrate, 46 mg of succinic acid, 0.240 g of L-arginine hydrochloride, 56.8 mg of L-asparagine monohydrate, 20 mg

of L-aspartic acid, 72.9 mg of L-cysteine hydrochloride monohydrate, 20 mg of L-glutamic acid, 1 mg of glutathione, 10 mg of glycine, 20.3 mg of L-histidine hydrochloride monohydrate, 20 mg of L-hydroxyproline, 50 mg of L-isoleucine, 40 mg of L-lysine hydrochloride, 15 mg of methionine, 20 mg of L-threonine, 5 mg of L-tryptophan, 20 mg of L-valine, 50 mg of L-leucine, 15 mg of L-phenylalanine, 20 mg of L-proline, 30 mg of L-serine, 20 mg of L-tyrosine, 0.2 mg of D-biotin (crystals), 0.25 mg of calcium pantothenate, 3 mg of choline chloride, 35 mg of *i*-inositol, 1 mg of 4-aminobenzoic acid, 5 μ g of cyanocobalamin, 1 mg of folic acid, 1 mg of nicotinamide, 0.2 mg of riboflavin, 1 mg of thiamine hydrochloride, 1 mg of pyridoxine hydrochloride, and 5 mg of phenol red in a suitable amount of water, add 1 mL of kanamycin sulfate solution (3 in 50), add water to make 1000 mL, and then sterilize by autoclaving for 15 minutes at 121°C. After cooling, add 10 mL of L-glutamine solution (3 in 100) and 20 mL of 7% sodium bicarbonate injection, and then mix. Store at 4°C.

Mefruside for assay $C_{13}H_{19}ClN_2O_5S_2$ [Same as the monograph Mefruside. When dried, it contains not less than 99.0% of mefruside ($C_{13}H_{19}ClN_2O_5S_2$).]

Meglumine $C_7H_{17}NO_5$ [same as the namesake monograph]

Mentha oil [Same as the namesake monograph]

Menthol $C_{10}H_{20}O$ [Same as the monograph *dl*-Menthol or *l*-Menthol]

***l*-Menthol for assay** [Same as the monograph *l*-Menthol. It contains not less than 99.0% of $C_{10}H_{20}O$ and meets the following additional specifications.]

Optical rotation $\langle 2.49 \rangle$ $[\alpha]_D^{20}$: –48.0 – –51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of *l*-menthol for assay in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 5 μ L each of the sample solution and standard solution (1) as directed under Gas Chromatography $\langle 2.02 \rangle$ according to the following conditions, measure each peak area of these solutions by the automatic integration method: the total peak area other than the peak area of *l*-menthol from the sample solution is not larger than the peak area of *l*-menthol from the standard solution (1).

Operatin conditions

Proceed the operating conditions in the Assay under Mentha Oil except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add dichloromethane to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of *l*-menthol obtained from 5 μ L of the standard solution (2) can be measured, and the peak height of *l*-menthol from 5 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of *l*-menthol beginning after the solvent peak.

Mepivacaine hydrochloride for assay $C_{15}H_{22}N_2O \cdot HCl$ [Same as the monograph Mepivacaine Hydrochloride. When dried, it contains not less than 99.0%

of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$.)]

Mercapto acetic acid $HSCH_2COOH$ [K 8630, Special class] Place in an ampule, and preserve in a dark, cold place. Do not use after storing for a long period.

2-Mercaptoethanol $HSCH_2CH_2OH$ Clear and colorless liquid.

Specific gravity <2.56> d_4^{20} : 1.112 – 1.117

Content: not less than 97.0%. *Assay*—Perform the test with 0.6 μ L of the substance to be examined as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of each component by the automatic integration method.

Content (%) = (the peak area of 2-mercaptoethanol/the total of the peak areas of each component) \times 100

Operating conditions

Detector: A hydrogen flame-ionization detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (177–250 μ m in particle diameter) coated in 20% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 120 °C

Carrier gas: Helium

Flow rate: Adjust the flow rate of about 50 mL/min and so that the retention time of 2-mercaptoethanol is 3–4 minutes.

Time span of measurement: About 7 times as long as the retention time of 2-mercaptoethanol.

Mercaptopurine $C_5H_4N_4S.H_2O$ [Same as the monograph Mercaptopurine Hydrate]

Mercuric acetate See mercury (II) acetate.

Mercuric acetate TS for nonaqueous titration See mercury (II) acetate TS for nonaqueous titration.

Mercuric chloride See mercury (II) chloride.

Mercury Hg [K 8572, Special class]

Mercury (II) acetate $Hg(CH_3COO)_2$ [K 8369, Special class]

Mercury (II) acetate TS for nonaqueous titration Dissolve 5 g of mercury (II) acetate in acetic acid (100) for nonaqueous titration to make 100 mL.

Mercury (II) chloride $HgCl_2$ [K 8139, Special class]

Mercury (II) chloride TS Dissolve 5.4 g of mercury (II) chloride in water to make 100 mL.

Mesaconitine for purity $C_{33}H_{45}NO_{11}$ White, crystals or crystalline powder. Slightly soluble in acetonitrile and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water. Melting point: about 190°C (with decomposition).

Identification—Determine the infrared absorption spectrum of mesaconitine for purity as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3510 cm^{-1} , 1713 cm^{-1} , 1277 cm^{-1} , 1116 cm^{-1} , 1098 cm^{-1} and 717 cm^{-1} .

Absorbance <2.24> $E_{1\%}^{1\text{cm}}$ (230 nm): 211 – 247 [5 mg dried for not less than 12 hours in a desiccator (reduced pressure

not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), ethanol (99.5), 200 mL].

Purity Related substances—(1) Dissolve 5.0 mg of mesaconitine for purity in 2 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and proceed the test as directed in the Identification under Processed Aconite Root: the spot other than the principal spot is not more intense than the spot with the standard solution.

(2) Dissolve 5.0 mg of mesaconitine for purity in 5 mL of acetonitrile, and use as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 50 mL, and use as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of mesaconitine and the solvent is not larger than the peak area of mesaconitine with the standard solution.

Operating conditions

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity under Processed Aconite Root.

Mobile phase: A mixture of phosphate buffer solution for processed aconite root and tetrahydrofuran (9:1).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of mesaconitine.

System suitability

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of mesaconitine obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μ L of the standard solution.

System performance: Dissolve 1 mg each of aconitine for purity, hyaconitine for purity and mesaconitine for purity, and 8 mg of jesaconitine for purity in 200 mL of acetonitrile. When the procedure is run with 10 μ L of this solution under the above operating conditions, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between these peaks is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mesaconitine is not more than 1.5%.

Water <2.48>: not more than 1.0% [5 mg dried for not less than 12 hours in a desiccator (reduced pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 40°C), coulometric titration].

Mesityl oxide $CH_3COCH=C(CH_3)_2$ A colorless or pale yellow, clear liquid, having a characteristic odor.

Specific gravity <2.56> d_4^{20} : 0.850 – 0.860

Metacycline hydrochloride $C_{22}H_{22}N_2O_8.HCl$ Yellow to dark yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of metacy-

cline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μ L of the sample solution as directed in the Purity (2) under Doxycycline Hydrochloride Hydrate, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total area of peaks other than metacycline is not more than 10%.

Metallic sodium See sodium.

Metanil yellow $C_{18}H_{14}N_3NaO_3S$ Yellow-brown powder. Sparingly soluble in water, and very slightly soluble in ethanol (95) and in *N,N*-dimethylformamide.

Metanil yellow TS Dissolve 0.1 g of metanil yellow in 200 mL of *N,N*-dimethylformamide.

Metaphosphoric acid HPO_3 [K 8890, Special class]

Metaphosphoric acid-acetic acid TS Dissolve 15 g of metaphosphoric acid and 40 mL of acetic acid (100) in water to make 500 mL. Preserve in a cold place, and use within 2 days.

Metenolone enanthate $C_{27}H_{42}O_3$ [Same as the name-sake monograph]

Metenolone enanthate for assay To 1 g of metenolone enanthate add 30 mL of water, and add slowly 70 mL of methanol with warming to dissolve. Filter while hot, and allow the filtrate to stand on a water bath for 30 minutes. Allow to stand overnight in a cold place, collect the crystals thus formed, and wash with a small amount of diluted methanol (1 in 3). Recrystallize in the same manner, and dry the crystals in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours. It is white, odorless crystals.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (242 nm): 321 – 328 (1 mg, methanol, 100 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: +40 – +42° (0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60>: 69 – 72°C

Purity Related substances—Dissolve 50 mg of metenolone enanthate for assay in chloroform to make exactly 10 mL, and use this solution as the sample solution. Proceed with 10 μ L of this solution as directed in the Purity (3) under Metenolone Enanthate: any spot other than the principal spot does not appear.

Metformin hydrochloride for assay $C_4H_{11}N_5.HCl$ [Same as the monograph Metformin Hydrochloride. When dried, it contains not less than 99.0% of metformin hydrochloride ($C_4H_{11}N_5.HCl$)]

Methanesulfonic acid CH_3SO_3H Clear, colorless liquid or colorless or white, crystalline mass, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether.

Congealing point <2.42>: 15 – 20°C

Specific gravity <2.56> d_{20}^{20} : 1.483 – 1.488

Content: not less than 99.0%. Assay—Weigh accurately about 2 g of methanesulfonic acid, dissolve in 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS
= 96.11 mg of CH_3SO_3H

Methanesulfonic acid TS To 35 mL of methanesulfonic acid add 20 mL of acetic acid (100) and water to make 500 mL.

0.1 mol/L Methanesulfonic acid TS To 4.8 g of methanesulfonic acid add water to make 500 mL.

Methanol CH_3OH [K 8891, Special class]

Methanol, anhydrous CH_4O To 1000 mL of methanol add 5 g of magnesium powder. If necessary, add 0.1 mL of mercury (II) chloride TS to start the reaction. After the evolving of a gas is stopped, distillate the solution, and preserve the distillate protecting from moisture. Water content per mL is not more than 0.3 mg.

Methanol for Karl Fischer method See Water Determination <2.48>.

Methanol for liquid chromatography CH_3OH A clear, colorless liquid. Mixable with water.

Purity Ultraviolet-absorbing substances—Perform the test as directed in Ultraviolet-visible Spectrophotometry <2.24> using water as the blank: the absorbances at 210 nm, at 220 nm, at 230 nm, at 240 nm and at 254 nm are not more than 0.70, 0.30, 0.15, 0.07 and 0.02, respectively.

Methanol-free ethanol See ethanol (95), methanol-free.

Methanol-free ethanol (95) See ethanol (95), methanol-free.

Methanol, purified Distil methanol before use.

Methionine $C_5H_{11}NO_2S$ [Same as the monograph L-Methionine]

2-Methoxyethanol $CH_3OCH_2CH_2OH$ [K 8895, Special class]

4-Methoxybenzaldehyde $C_8H_8O_2$ Clear, colorless to light yellow liquid. Miscible with ethanol (95) and with diethyl ether, and practically insoluble in water.

Specific gravity <2.56> d_4^{20} : 1.123 – 1.129

Content: not less than 97.0%. Assay—Weigh accurately about 0.8 g of 4-methoxybenzaldehyde, add exactly 7.5 mL of hydroxylamine TS, shake well, allow to stand for 30 minutes, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS) until the color of the solution changes from blue through green to yellow-green. Perform a blank determination.

Each mL of 0.5 mol/L hydrochloric acid VS
= 68.08 mg of $C_8H_8O_2$

4-Methoxybenzaldehyde-acetic acid TS To 0.5 mL of 4-methoxybenzaldehyde add acetic acid (100) to make 100 mL.

4-Methoxybenzaldehyde-sulfuric acid TS To 9 mL of ethanol (95) add 0.5 mL of 4-methoxybenzaldehyde and 0.5 mL of sulfuric acid, and mix thoroughly.

1-Methoxy-2-propanol $C_4H_{10}O_2$ A colorless, clear liquid.

Clarity of solution—To 5 mL of 1-methoxy-2-propanol add 20 mL of water, and mix: the solution is clear.

Specific gravity <2.56> d_4^{20} : 0.920 – 0.925

Refractive index <2.45> n_D^{20} : 1.402 – 1.405

Water <2.48>: not more than 0.5% (5 g).

Content: not less than 98.0%.

Assay—Proceed as directed under Gas Chromatography <2.02> using the area percentage method according to the following conditions:

Operating conditions

Detector: Thermal conductivity detector

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm) coated with polyethylene glycol 20 M for gas chromatography in 20%.

Column temperature: A constant temperature of about 90°C.

Carrier gas: Helium

Flow rate: A constant flow rate of 20 mL per minute.

p-Methyl aminophenol sulfate See 4-methyl aminophenol sulfate.

4-Methyl aminophenol sulfate ($\text{HOC}_6\text{H}_4\text{NHCH}_3$) $\cdot\text{H}_2\text{SO}_4$ White to pale yellow or very pale grayish white, crystals or crystalline powder. Melting point: about 260°C (with decomposition).

p-Methyl aminophenol sulfate TS See 4-methyl aminophenol sulfate TS.

4-Methyl aminophenol sulfate TS Dissolve 0.35 g of 4-methyl aminophenol sulfate and 20 g of sodium hydrogen sulfite in water to make 100 mL. Prepare before use.

2-Methylaminopyridine $\text{C}_6\text{H}_8\text{N}_2$ A pale yellow liquid.

Specific gravity <2.56> d_{20}^{20} : 1.050 – 1.065

Boiling point <2.57>: 200 – 202°C

Water <2.48>: less than 0.1%.

2-Methylamino pyridine for Karl Fischer method See Water Determination <2.48>.

Methyl behenate $\text{C}_{23}\text{H}_{46}\text{O}_2$ White, odorless and tasteless, scaly crystals or powder. Dissolves in acetone, in diethyl ether and in chloroform.

Melting point <2.60>: 54°C

Saponification value <1.13>: 155.5 – 158.5

Methyl benzoate $\text{C}_6\text{H}_5\text{COOCH}_3$ Clear, colorless liquid.

Refractive index <2.45> n_D^{20} : 1.515 – 1.520

Specific gravity <2.56> d_{20}^{20} : 1.087 – 1.095

Purity—Dissolve 0.1 mL of methyl benzoate in the mobile phase in Assay under Thiamine Hydrochloride to make 50 mL. Perform the test as directed under Liquid Chromatography <2.01> with 10 μL of this solution according to the Assay under Thiamine Hydrochloride. Measure each peak area by the automatic integration method in a range about twice the retention time of methyl benzoate, and calculate the amount of methyl benzoate by the area percentage method: it shows the purity of not less than 99.0%.

Methyl benzoate for estriol test $\text{C}_8\text{H}_8\text{O}_2$ Clear, colorless liquid, having a characteristic odor.

Refractive index <2.45> n_D^{20} : 1.515 – 1.520

Specific gravity <2.56> d_{20}^{20} : 1.087 – 1.095

Acid value <1.13>: not more than 0.5.

D-(+)- α -Methylbenzylamine $\text{C}_6\text{H}_5\text{CH}(\text{CH}_3)\text{NH}_2$ Colorless or pale yellow clear liquid, having an amine like odor. Miscible with ethanol (95) and with acetone, and slightly

soluble in water.

Refractive index <2.45> n_D^{20} : 1.524–1.529

Optical rotation <2.49> $[\alpha]_D^{20}$: +37 – +41° (50 mm)

Content: not less than 98.0%. **Assay**—Perform the test with exact 0.6 μL of D-(+)- α -methylbenzylamine as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of D-(+)- α -methylbenzylamine.

Operating conditions

Detector: Hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (180 to 250 μm in particle diameter) coated with polyethylene glycol 20 M for gas chromatography and potassium hydroxide at the ratio of 10% and 5%, respectively.

Column temperature: A constant temperature of about 140°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of D-(+)- α -methylbenzylamine is about 5 minutes.

Selection of column: To 5 mL of D-(+)- α -methylbenzylamine add 1 mL of pyridine. Proceed with 0.6 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pyridine and D-(+)- α -methylbenzylamine in this order with the resolution between these peaks being not less than 3.

Time span of measurement: About 3 times as long as the retention time of D-(+)- α -methylbenzylamine beginning after the solvent peak.

3-Methyl-1-butanol $\text{C}_5\text{H}_{12}\text{O}$ [K 8051, Special class]

3-Methylbutyl acetate $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$ [K 8358, Special class]

Methyl cellosolve See 2-methoxyethanol.

Methyl docosanate $\text{C}_{23}\text{H}_{46}\text{O}_2$ White, tabular crystals or crystalline powder.

Melting point <2.60>: 51.0 – 56.0°C

Methyldopa $\text{C}_{10}\text{H}_{13}\text{NO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$ [Same as the monograph Methyldopa Hydrate]

Methyldopa for assay $\text{C}_{10}\text{H}_{13}\text{NO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$ [Same as the monograph Methyldopa Hydrate. When dried, it contains not less than 99.0% of methyldopa ($\text{C}_{10}\text{H}_{13}\text{NO}_4$).]

N,N'-Methylenebisacrylamide $\text{CH}_2(\text{NHCOCHCH}_2)_2$ White crystalline powder.

Content: not less than 97.0%.

Methylene blue See methylene blue trihydrate.

Methylene blue-potassium perchlorate TS To 500 mL of a solution of potassium perchlorate (1 in 1000) add dropwise, with shaking, a solution of methylene blue (1 in 100) until a slight, permanent turbidity results. Allow the solution to stand, and filter the supernatant liquid.

Methylene blue-sulfuric acid-monobasic sodium phosphate TS See methylene blue-sulfuric acid-sodium dihydrogenphosphate TS.

Methylene blue-sulfuric acid-sodium dihydrogenphosphate TS To 30 mL of a solution of methylene blue (1 in 1000)

add 500 mL of water, 6.8 mL of sulfuric acid and 50 g of sodium dihydrogenphosphate dihydrate, dissolve, and add water to make 1000 mL.

Methylene blue trihydrate $C_{16}H_{18}ClN_3S_3 \cdot 3H_2O$
[K 8897, Special class]

Methylene blue TS Dissolve 0.1 g of methylene blue trihydrate in water to make 100 mL. Filter if necessary.

***dl*-Methylephedrine hydrochloride** $C_{11}H_{17}NO \cdot HCl$
[Same as the namesake monograph]

***dl*-Methylephedrine hydrochloride for assay** [Same as the monograph *dl*-Methylephedrine Hydrochloride]

Methylergometrine maleate for assay
 $C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$ [Same as the monograph Methylergometrine Maleate. When dried, it contains not less than 99.0% of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$).

Methyl ethyl ketone See 2-butanone.

Methyl iodide See iodomethane.

Methyl iodide for assay CH_3I Clear, colorless liquid. On exposure to light, it liberates iodine and becomes brown. Miscible with ethanol (95) and with diethyl ether, and sparingly soluble in water. Use the distillate obtained between 42.2°C and 42.6°C.

Specific gravity <2.56> d_{25}^{25} : 2.27 – 2.28

Purity—Perform the test with 1 μ L of methyl iodide for assay as directed under Gas Chromatography <2.02> according to the operating conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of methyl iodide by the area percentage method: it shows the purity of not less than 99.8%. Adjust the detection sensitivity so that the peak height of methyl iodide from 1 μ L of methyl iodide for assay is about 80% of the full scale.

Content: not less than 98.0%. *Assay*—Proceed as directed in the Assay under Isopropyl iodide for assay.

Each mL of 0.1 mol/L silver nitrate VS
= 14.19 mg of CH_3I

Methyl isobutyl ketone See 4-methyl-2-pentanone.

3-O-Methylmethyldopa for thin-layer chromatography
 $C_{11}H_{15}NO_4$

Purity Related substances—Dissolve 5 mg of 3-O-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL. Perform the test with 20 μ L of this solution as directed in the Purity (5) under Methyl-dopa: any spot other than the principal spot at the *R_f* value of about 0.7 does not appear.

2-Methyl-5-nitroimidazole for thin-layer chromatography $C_4H_5N_3O_2$ White crystalline powder. Slightly soluble in water and in acetone. Melting point: about 253°C (with decomposition).

Purity Related substances—Dissolve 40 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in 8 mL of acetone, and use as the sample solution. Pipet 2.5 mL of the sample solution, add acetone to make exactly 100 mL, and use as the standard solution. Perform the test as directed in the Purity (3) under Metronidazole: the spots other than the principal spot from the sample solution are not more intense

than the spot from the standard solution.

Methyl orange $C_{14}H_{14}N_3NaO_3S$ [K 8893, Special class]

Methyl orange-boric acid TS Add 0.5 g of methyl orange and 5.2 g of boric acid in 500 mL of water, and dissolve by warming on a water bath. After cooling, wash this solution with three 50-mL portions of chloroform.

Methyl orange TS Dissolve 0.1 g of methyl orange in 100 mL of water, and filter if necessary.

Methyl orange-xylene cyanol FF TS Dissolve 1 g of methyl orange and 1.4 g of xylene cyanol FF in 500 mL of dilute ethanol.

Methyl parahydroxybenzoate $HOC_6H_4COOCH_3$
[Same as the namesake monograph]

4-Methylpentan-2-ol $C_6H_{14}O$ A clear and colorless, volatile liquid.

Refractive index <2.45> n_D^{20} : about 1.411

Specific gravity <2.56> d_{20}^{20} : about 0.802

Boiling point <2.57>: about 132°C

4-Methyl-2-pentanone $CH_3COCH_2CH(CH_3)_2$
[K 8903, Special class]

3-Methyl-1-phenyl-5-pyrazolone $C_{10}H_{10}N_2$ [K 9548, Special class]

Methyl prednisolone $C_{22}H_{30}O_3$ [Same as the namesake monograph]

2-Methyl-1-propanol $(CH_3)_2CHCH_2OH$ [K 8811, Special class]

***N*-Methylpyrrolidine** $C_5H_{11}N$ Colorless, clear liquid, having a characteristic order.

Identification—Determine the spectrum of *N*-methylpyrrolidine in a solution of deuterated chloroform for nuclear magnetic resonance spectroscopy (4 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits a big signal, at around δ 2.3 ppm.

Content: not less than 95%. *Assay*—Put 30 mL of water in a beaker, weigh accurately the beaker, add dropwise about 0.15 g of *N*-methylpyrrolidine, weigh accurately the beaker again, and titrate <2.50> with 0.05 mol/L sulfuric acid VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid
= 8.515 mg of $C_5H_{11}N$

Methyl red $C_{15}H_{15}N_3O_2$ [K 8896, Special class]

Methyl red-methylene blue TS Dissolve 0.1 g of methyl red and 0.1 g of methylene blue in ethanol (95) to make 100 mL, and filter if necessary. Preserve in light-resistant containers.

Methyl red TS Dissolve 0.1 g of methyl red in 100 mL of ethanol (95), and filter if necessary.

Methyl red TS, dilute Dissolve 25 mg of methyl red in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

Methyl red TS for acid or alkali test To 0.1 g of methyl red add 7.4 mL of 0.05 mol/L sodium hydroxide VS or 3.7

mL of 0.1 mol/L sodium hydroxide VS, triturate to dissolve in a mortar, and add freshly boiled and cooled water to make 200 mL. Preserve in light-resistant, glass-stoppered bottles.

Methyrosaniline chloride See crystal violet.

Methyrosaniline chloride TS See crystal violet TS.

Methyl salicylate $C_8H_8O_3$ [Same as the namesake monograph]

Methylsilicone polymer for gas chromatography Prepared for gas chromatography.

Methyltestosterone $C_{20}H_{30}O_2$ [Same as the namesake monograph]

1-Methyl-1H-tetrazole-5-thiol for liquid chromatography $C_2H_4N_4S$ White, crystals or crystalline powder. Very soluble in methanol, and freely soluble in water.

Melting point <2.60>: 123 – 127°C

Loss on drying <2.41>: not more than 1.0% (1 g, in vacuum, phosphorous (V) oxide, 2 hours).

Content: not less than 99.0%. *Assay*—Weigh accurately about 0.2 g of 1-methyl-1H-tetrazole-5-thiol, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-*N,N*-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 11.61 mg of $C_2H_4N_4S$

Methylthymol blue $C_{37}H_{43}N_2NaO_{13}S$ [K 9552]

Methylthymol blue-potassium nitrate indicator Mix 0.1 g of methylthymol blue with 9.9 g of potassium nitrate, and triturate until the mixture becomes homogeneous.

Sensitivity—When 0.02 g of methylthymol blue-potassium nitrate indicator is dissolved in 100 mL of 0.02 mol/L sodium hydroxide VS, the solution is slightly blue in color. On adding 0.05 mL of 0.01 mol/L barium chloride VS to this solution, the solution shows a blue color, then on the subsequent addition of 0.1 mL of 0.01 mol/L disodium ethylenediaminetetraacetate VS, it becomes colorless.

Methylthymol blue-sodium chloride indicator Mix 0.25 g of methylthymol blue and 10 g of sodium chloride, and grind to homogenize.

1-Methyl-1H-tetrazole-5-thiol $C_2H_4N_4S$ White, crystals or crystalline powder.

Melting point <2.60>: 125 – 129°C

Identification—(1) Determine the ultraviolet-visible absorption spectrum of a solution of 1-methyl-1H-tetrazole-5-thiol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 222 nm and 226 nm.

(2) Determine the infrared absorption spectrum of 1-methyl-1H-tetrazole-5-thiol according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3060 cm^{-1} , 2920 cm^{-1} , 2780 cm^{-1} , 1500 cm^{-1} , 1430 cm^{-1} and 1410 cm^{-1} .

Purity Related substances—Dissolve 0.10 g of 1-methyl-1H-tetrazole-5-thiol in exactly 100 mL of water. Perform the test with 1 μL of this solution as directed in the Purity (4) un-

der Cefmetazole Sodium: any spot other than the principal spot at the *R_f* value of about 0.77 does not appear.

Methyl yellow $C_{14}H_{15}N_3$ [K 8494, Special class]

Methyl yellow TS Dissolve 0.1 g of methyl yellow in 200 mL of ethanol (95).

Metoclopramide for assay $C_{14}H_{22}ClN_3O_2$ [Same as the monograph Metoclopramide. When dried, it contains not less than 99.0% of metoclopramide ($C_{14}H_{22}ClN_3O_2$).]

Metoprolol tartrate for assay $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ [Same as the monograph Metoprolol Tartrate. When dried, it contains not less than 99.5% of metoprolol tartrate ($(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$).]

Metronidazole $C_6H_9N_3O_3$ [Same as the namesake monograph]

Metronidazole for assay $C_6H_9N_3O_3$ [Same as the monograph Metronidazole. It meets the following additional requirement.]

Related substances—Weigh accurately about 25 mg of metronidazole for assay, dissolve in a mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mixture of water and methanol (4:1) to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mixture of water and methanol (4:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than metronidazole is not more than the peak area of metronidazole with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay under Metronidazole Tablets.

Time span of measurement: About 4 times as long as the retention time of metronidazole.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, add a mixture of water and methanol (4:1) to make exactly 20 mL. Confirm that the peak area of metronidazole obtained with 10 μL of this solution is equivalent to 7 to 13% of that with the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 2.0%.

Microplates Polystyrene plates with an inside diameter of 7 (upper edge) to 6.4 (lower edge) mm, and 11.3 mm thickness. Have 96 flat-bottomed truncated cone-shaped wells.

Milk casein See casein, milk.

Milk of lime Place 10 g of calcium oxide in a mortar, and add gradually 40 mL of water under grinding.

Mixture of petroleum hexamethyl tetracosane branching hydrocarbons (L) for gas chromatography Prepared for gas chromatography.

Molecular weight markers for teceleukin Dissolve 0.4 mg each of lysozyme, soy trypsin inhibitor, carbonic anhydrase, egg white albumin, bovine serum albumin, and phosphorylase b in 200 μ L of diluted glycerin (1 in 2).

Molybdenum (III) oxide MoO_3 A white to yellowish green powder.

Identification—Dissolve 0.5 g in 5 mL of ammonia solution (28), acidify 1 mL of this solution with a suitable amount of nitric acid, add 5 mL of sodium phosphate TS, and warm: yellow precipitates appear.

Molybdenum (III) oxide-citric acid TS To 54 g of molybdenum (III) oxide and 11 g of sodium hydroxide add 200 mL of water, and dissolve by heating while stirring. Separately, dissolve 60 g of citric acid monohydrate in 250 mL of water, and add 140 mL of hydrochloric acid. Mix these solutions, filter if necessary, add water to make 1000 mL, and add a solution of potassium bromate (1 in 100) until a yellow-green color appears.

Storage—Preserve in tightly stoppered containers, protected from light.

Molybdenum trioxide See molybdenum (III) oxide.

Molybdenum trioxide-citric acid TS See molybdenum (III) oxide-citric acid TS.

Monobasic ammonium phosphate See ammonium dihydrogenphosphate.

Monobasic potassium phosphate See potassium dihydrogenphosphate.

Monobasic potassium phosphate for pH determination See potassium dihydrogenphosphate for pH determination.

0.05 mol/L Monobasic potassium phosphate, pH 3.0 See 0.05 mol/L potassium dihydrogenphosphate, pH 3.0.

0.02 mol/L Monobasic potassium phosphate TS See 0.02 mol/L potassium dihydrogenphosphate TS.

0.05 mol/L Monobasic potassium phosphate TS See 0.05 mol/L potassium dihydrogenphosphate TS.

0.2 mol/L Monobasic potassium phosphate TS See 0.2 mol/L potassium dihydrogenphosphate TS.

0.2 mol/L Monobasic potassium phosphate TS for buffer solution See 0.2 mol/L potassium dihydrogenphosphate TS for buffer solution.

0.05 mol/L Monobasic potassium phosphate TS, pH 4.7 See 0.05 mol/L potassium dihydrogenphosphate TS, pH 4.7.

Monobasic sodium phosphate See sodium dihydrogenphosphate dihydrate.

0.05 mol/L Monobasic sodium phosphate TS See 0.05 mol/L sodium dihydrogenphosphate TS.

0.1 mol/L Monobasic sodium phosphate TS See 0.1 mol/L sodium dihydrogenphosphate TS.

2 mol/L Monobasic sodium phosphate TS See 2 mol/L sodium dihydrogenphosphate TS.

0.05 mol/L Monobasic sodium phosphate TS, pH 2.6 See 0.05 mol/L sodium dihydrogenphosphate TS, pH 2.6.

0.05 mol/L Monobasic sodium phosphate TS, pH 3.0 See 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0.

0.1 mol/L Monobasic sodium phosphate TS, pH 3.0 See 0.1 mol/L sodium dihydrogenphosphate TS, pH 3.0.

0.02 mol/L Monobasic ammonium phosphate TS See 0.02 mol/L ammonium dihydrogenphosphate TS.

Monoethanolamine See 2-Aminoethanol.

Morphine hydrochloride [Same as the monograph Morphine Hydrochloride Hydrate]

Morphine hydrochloride for assay $\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$ [Same as the monograph Morphine Hydrochloride Hydrate. It contains not less than 99.0% of morphine hydrochloride ($\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl}$), calculated on the anhydrous basis.]

3-(N-Morpholino)propanesulfonic acid $\text{C}_7\text{H}_{15}\text{NO}_4\text{S}$ White crystalline powder, freely soluble in water, and practically insoluble in ethanol (99.5).

Melting point <2.60>: 275 – 280°C

0.02 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0 Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

0.02 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 8.0 Dissolve 4.2 g of 3-(N-morpholino)propanesulfonic acid in 700 mL of water, adjust the pH to 8.0 with dilute sodium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L 3-(N-Morpholino)propanesulfonic acid buffer solution, pH 7.0 Dissolve 20.92 g of 3-(N-morpholino)propanesulfonic acid in 900 mL of water, adjust the pH to 7.0 with sodium hydroxide TS, and add water to make 1000 mL.

MTT TS Dissolve 8 g of sodium chloride, 0.2 g of calcium chloride, 1.15 g of anhydrous sodium dihydrogen phosphate (NaH_2PO_4) and 0.2 g of potassium dihydrogen phosphate (KH_2PO_4) in water to make 1000 mL, and sterilize in an autoclave for 15 minutes at 121°C to make the PBS(–) solution. Dissolve 0.3 g of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide in this PBS(–) solution to make 100 mL. Sterilize by membrane filtration (pore size, 0.45 μm), and store in a cool place shielded from light.

Murexide $\text{C}_8\text{H}_8\text{N}_6\text{O}_6$ Red-purple powder. Practically insoluble in water, in ethanol (95) and in diethyl ether.

Purity Clarity of solution—Dissolve 10 mg of murexide in 100 mL of water: the solution is clear.

Residue on ignition <2.44>: not more than 0.1% (1 g).

Sensitivity—Dissolve 10 mg of murexide in 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, and add water to make 100 mL, and use this solution as the sample solution. Separately, add 2 mL of ammonia-ammonium chloride buffer solution, pH 10.0, to 5 mL of diluted Standard Calcium Solution (1 in 10), add water to make 25 mL, and render the solution to pH 11.3 with sodium hydroxide TS.

Add 2 mL of the sample solution and water to this solution to make 50 mL: a red-purple color develops.

Murexide-sodium chloride indicator Prepared by mixing 0.1 g of murexide and 10 g of sodium chloride and grinding to get homogeneous.

Storage—Preserve in light-resistant containers.

Myoglobin A hemoprotein obtained from horse heart muscle. White crystalline powder. It contains not less than 95 % of myoglobin in the total protein.

Nalidixic acid $C_{12}H_{12}N_2O_3$ [Same as the namesake monograph]

Naphazoline nitrate $C_{14}H_{14}N_2 \cdot HNO_3$ [Same as the namesake monograph]

Naphazoline nitrate for assay [Same as the monograph Naphazoline Nitrate. When dried, it contains not less than 99.0% of naphazoline nitrate ($C_{14}H_{14}N_2 \cdot NHO_3$).]

Naphthalene $C_{10}H_8$ Colorless flake-like or lustrous stick-like crystals, having a characteristic odor.

Melting point <2.60>: 78 – 82°C

1,3-Naphthalenediol $C_{10}H_8O_2$ Red-brown crystals or gray-brown powder. Freely soluble in water, in methanol and in ethanol (99.5). Melting point: about 124°C.

1,3-Naphthalenediol TS Dissolve 50 mg of 1,3-naphthalenediol in 25 mL of ethanol (99.5), and add 2.5 mL of phosphoric acid.

2-Naphthalenesulfonic acid $C_{10}H_8O_3S \cdot H_2O$ White to pale yellowish white powder. Very soluble in water, in methanol and in ethanol (95), and sparingly soluble in diethyl ether and in chloroform.

Water <2.48>: 7.0 – 11.5% (0.5 g, volumetric titration, direct titration).

Content: not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.5 g of 2-naphthalenesulfonic acid, dissolve in 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromothymol blue TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.82 mg of $C_{10}H_8O_3S$

1-Naphthol $C_{10}H_7OH$ [K 8698, Special class] Preserve in light-resistant containers.

2-Naphthol $C_{10}H_7OH$ [K 8699, Special class] Preserve in light-resistant containers.

α -Naphthol See 1-naphthol.

β -Naphthol See 2-naphthol.

***p*-Naphtholbenzein** $C_{27}H_{20}O_3$ [K 8693, Special class]

α -Naphtholbenzein See *p*-naphtholbenzein.

***p*-Naphtholbenzein TS** Dissolve 0.2 g of *p*-naphtholbenzein in acetic acid (100) to make 100 mL.

Purity Clarity and color of solution—Dissolve 0.1 g of *p*-naphtholbenzein in 100 mL of ethanol (95): the solution is red in color and clear.

Sensitivity—Add 100 mL of freshly boiled and cooled water to 0.2 mL of a solution of *p*-naphtholbenzein in etha-

nol (95) (1 in 1000), and add 0.1 mL of 0.1 mol/L sodium hydroxide VS: a green color develops. Add subsequently 0.2 mL of 0.1 mol/L hydrochloric acid VS: the color of the solution changes to yellow-red.

α -Naphtholbenzein TS See *p*-naphtholbenzein TS.

1-Naphthol-sulfuric acid TS Dissolve 1.5 g of 1-naphthol in 50 mL of ethanol (95), add 3 mL of water and 7 mL of sulfuric acid, and mix well. Prepare before use.

1-Naphthol TS Dissolve 6 g of sodium hydroxide and 16 g of anhydrous sodium carbonate in water to make 100 mL. In this solution dissolve 1 g of 1-naphthol. Prepare before use.

2-Naphthol TS Dissolve 1 g of 2-naphthol in sodium carbonate TS to make 100 mL. Prepare before use.

α -Naphthol TS See 1-naphthol TS.

β -Naphthol TS See 2-naphthol TS.

1-Naphthylamine $C_{10}H_7NH_2$ [K 8692, Special class] Preserve in light-resistant containers.

α -Naphthylamine See 1-naphthylamine.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate** See *N*, *N*-diethyl-*N'*-1-naphthylethylenediamine oxalate.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS** See *N*, *N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS.

***N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS** See *N*, *N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS.

Naphthylethylenediamine TS Dissolve 0.1 g of *N*-1-naphthylethylenediamine dihydrochloride in water to make 100 mL. Prepare before use.

***N*-1-Naphthylethylenediamine dihydrochloride** $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$ [K8197, Special class]

Naringin for thin-layer chromatography $C_{27}H_{32}N_{14} \cdot 2H_2O$ White crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and slightly soluble in water. Melting point: about 170°C (with decomposition). *Optical rotation* <2.49> $[\alpha]_D^{20}$: – 87 – – 93° (0.1 g, ethanol (95), 10 mL, 100 mm).

Purity Related substances—Proceed with 10 μ L of a solution, prepared by dissolving 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), as directed in the Identification under Bitter Orange Peel: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Neutral alumina containing 4% of water Take 50 g of neutral alumina for column chromatography, previously dried at 105°C for 2 hours, in a tight container, add 2.0 mL of water, shake well to make homogeneous, and allow to stand for more than 2 hours.

Neutral detergent Synthetic detergent containing anionic or non-ionic surfactant, and pH of its 0.25% solution is between 6.0 and 8.0. Dilute to a suitable concentration before use.

Neutralized ethanol See ethanol, neutralized.

Neutral red $C_{15}H_{17}N_4Cl$ Slightly metallic, dark green

powder or masses.

Identification—Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3310 cm^{-1} , 3160 cm^{-1} , 1621 cm^{-1} , 1503 cm^{-1} , 1323 cm^{-1} , 1199 cm^{-1} and 732 cm^{-1} .

Neutral red TS Dissolve 0.1 g of neutral red in acetic acid (100) to make 100 mL.

Nicardipine hydrochloride for assay $\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_6\cdot\text{HCl}$ [Same as the monograph Nicardipine Hydrochloride. When dried, it contains not less than 99.0% of nicardipine hydrochloride ($\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_6\cdot\text{HCl}$).]

Nicergoline for assay $\text{C}_{24}\text{H}_{26}\text{BrN}_3\text{O}_3$ [Same as the monograph Nicergoline, or Nicergoline purified according to the method below. When dried, it contains not less than 99.0% of nicergoline ($\text{C}_{24}\text{H}_{26}\text{BrN}_3\text{O}_3$), and when perform the test of the Purity (2) under Nicergoline, the total area of the peaks other than nicergoline from the sample solution is not more than 2.5 times the peak area of nicergoline from the standard solution.

Method of purification: Dissolve 1 g of Nicergoline in 20 mL of acetonitrile, allow to stand in a dark place for about 36 hours, filter, and dry the crystals so obtained at 60°C for 2 hours in vacuum.]

Nicomol for assay $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_9$ [Same as the monograph Nicomol. When dried, it contains not less than 99.0% of $\text{C}_{34}\text{H}_{32}\text{N}_4\text{O}_9$.]

Nicotinamide $\text{C}_6\text{H}_6\text{N}_2\text{O}$ [Same as the namesake monograph]

β -Nicotinamide-adenine dinucleotide (β -NAD)
 $\text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_{14}\text{P}_2$ [K 9802]

Content: not less than 94.5%. **Assay**—Weigh accurately about 25 mg of β -nicotinamide-adenine dinucleotide, oxidized form, and dissolve in water to make exactly 25 mL. Pipet 0.2 mL of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0, to make exactly 10 mL, and use this solution as the sample solution. Determine the absorbances, A_T and A_B , of the sample solution and 0.1 mol/L phosphate buffer solution, pH 7.0, at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\begin{aligned} &\text{Amount (mg) of } \text{C}_{21}\text{H}_{27}\text{N}_7\text{O}_{14}\text{P}_2 \\ &= \frac{0.6634 \times 10}{17.6 \times 0.20} \times (A_T - A_B) \times 25 \end{aligned}$$

Nicotinamide adenine dinucleotide TS See β -nicotinamide-adenine dinucleotide TS.

β -Nicotinamide adenine dinucleotide (β -NAD) TS Dissolve 40 mg of β -nicotinamide adenine dinucleotide, oxidized form in 10 mL of water. Prepare before use.

Nifedipine $\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_6$ [Same as the namesake monograph]

Ninhydrin $\text{C}_9\text{H}_6\text{O}_4$ [K 8870, Special class]

Ninhydrin TS Dissolve 0.2 g of ninhydrin in water to make 10 mL. Prepare before use.

Ninhydrin-acetic acid TS Dissolve 1.0 g of ninhydrin in

50 mL of ethanol (95), and add 10 mL of acetic acid (100).

Ninhydrin-L-ascorbic acid TS Dissolve 0.25 g of ninhydrin and 0.01 g of L-ascorbic acid in water to make 50 mL. Prepare before use.

Ninhydrin-butanol TS Dissolve 0.3 g of ninhydrin in 100 mL of 1-butanol, and add 3 mL of acetic acid (100).

Ninhydrin-citric acid-acetic acid TS Dissolve 70 g of citric acid monohydrate in 500 mL of water, add 58 mL of acetic acid (100), 70 mL of a solution of sodium hydroxide (21 in 50) and water to make 1000 mL. In 100 mL of this solution dissolve 0.2 g of ninhydrin.

Ninhydrin-stannous chlorid TS See ninhydrin-tin (II) chloride TS.

Ninhydrin-sulfuric acid TS Dissolve 0.1 g of ninhydrin in 100 mL of sulfuric acid. Prepare before use.

Ninhydrin-tin (II) chloride TS Dissolve 21.0 g of citric acid in water to make 200 mL, adjust the pH to 5.6 ± 0.2 by adding sodium hydroxide TS, add water to make 500 mL, and dissolve 1.3 g of tin (II) chloride. To 50 mL of the solution, add 50 mL of a 2-methoxyethanol solution of ninhydrin (2 in 50). Prepare before use.

0.2% Ninhydrin-water saturated 1-butanol TS Dissolve 2 g of ninhydrin in 1-butanol saturated with water to make 1000 mL.

Nitrendipine for assay [Same as the monograph Nitrendipine. It, when dried, contains not less than 99.0% of nitrendipine ($\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_6$), and meets the following requirement. When perform the test as directed in the Purity (2) under Nitrendipine, the area of the peak of dimethyl ester, having the relative retention time of about 0.8 with respect to nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution, the area of the peak other than nitrendipine and the dimethyl ester is not larger than 1/5 times the peak area of nitrendipine from the standard solution, and the total area of the peak other than nitrendipine is not larger than 1/2 times the peak area of nitrendipine from the standard solution.]

Nitric acid HNO_3 [K 8541, Special class, Concentration: 69 - 70%, Density: about 1.42 g/mL]

Nitric acid, dilute Dilute 10.5 mL of nitric acid with water to make 100 mL (10%).

Nitric acid, fuming [K 8739, Special class, Concentration: not less than 97%, Density: 1.52 g/mL]

Nitric acid TS, 2 mol/L Dilute 12.9 mL of nitric acid with water to make 100 mL.

2,2',2''-Nitrilotrisethanol $(\text{CH}_2\text{CH}_2\text{OH})_3\text{N}$ [K 8663, Special class]

2,2',2''-Nitrilotrisethanol buffer solution, pH 7.8
Dissolve 149.2 g of 2,2',2''-nitrilotrisethanol in about 4500 mL of water, adjust to pH 7.8 with 4 mol/L hydrochloric acid, and add water to make 5000 mL.

p-Nitroaniline See 4-nitroaniline.

4-Nitroaniline $\text{O}_2\text{NC}_6\text{H}_4\text{NH}_2$ [K 8708, p-Nitroaniline, Special class]

p-Nitroaniline-sodium nitrite TS See 4-nitroaniline-sodi-

um nitrite TS.

4-Nitroaniline-sodium nitrite TS To 90 mL of a solution of 0.3 g of 4-nitroaniline in 100 mL of 10 mol/L hydrochloric acid TS add 10 mL of a solution of sodium nitrite (1 in 20), and mix well. Prepare before use.

***o*-Nitrobenzaldehyde** See 2-nitrobenzaldehyde.

2-Nitrobenzaldehyde $O_2NC_6H_4CHO$ Pale yellow crystals or crystalline powder.

Melting point <2.60>: 42 – 44°C

Nitrobenzene $C_6H_5NO_2$ [K 8723, Special class]

***p*-Nitrobenzenediazonium chloride TS** See 4-nitrobenzenediazonium chloride TS.

4-Nitrobenzenediazonium chloride TS Dissolve 1.1 g of 4-nitroaniline in 1.5 mL of hydrochloric acid, add 1.5 mL of water, and then add a solution prepared by dissolving 0.5 g of sodium nitrite in 5 mL of water, while cooling in an ice bath. Prepare before use.

***p*-Nitrobenzenediazonium chloride TS for spraying** See 4-nitrobenzenediazonium chloride TS for spraying.

4-Nitrobenzenediazonium chloride TS for spraying Dissolve 0.4 g of 4-nitroaniline in 60 mL of 1 mol/L hydrochloric acid TS, and add, while cooling in an ice bath, sodium nitrite TS until the mixture turns potassium iodide-starch paper to blue in color. Prepare before use.

***p*-Nitrobenzenediazonium fluoroborate** See 4-nitrobenzenediazonium fluoroborate.

4-Nitrobenzenediazonium fluoroborate $O_2NC_6H_4N_2BF_4$ Pale yellowish white, almost odorless powder. Freely soluble in dilute hydrochloric acid, slightly soluble in water, and very slightly soluble in ethanol (95) and in chloroform. *Melting point*: about 148°C (with decomposition).

Identification—Add 1 mL each of a solution of phenol (1 in 1000) and sodium hydroxide TS to 10 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 1000); a red color develops.

Loss on drying <2.41>: not more than 1.0% (1 g, silica gel, 2 hours).

***p*-Nitrobenzoyl chloride** See 4-nitrobenzoyl chloride.

4-Nitrobenzoyl chloride $O_2NC_6H_4COCl$ Light yellow crystals.

Melting point <2.60>: 70 – 74°C

Content: not less than 98.0%. *Assay*—Weigh accurately about 0.5 g of 4-nitrobenzoyl chloride, add an excess of silver nitrate-ethanol TS, and boil under a reflux condenser for 1 hour. After cooling, filter the precipitate, wash with water, dry at 105°C to constant mass, and weigh. The mass of 4-nitrobenzoyl chloride, multiplied by 1.107, represents the mass of 4-nitrobenzoyl chloride ($C_7H_4ClNO_3$).

***p*-Nitrobenzyl chloride** See 4-nitrobenzyl chloride.

4-Nitrobenzyl chloride $O_2NC_6H_4CH_2Cl$ Light yellow crystals or crystalline powder. Soluble in ethanol (95).

Melting point <2.60>: 71 – 73°C

Content: not less than 98.0%. *Assay*—Weigh accurately about 0.5 g of 4-nitrobenzyl chloride, add 15 mL of a solution prepared by dissolving 4 g of silver nitrate in 10 mL of

water and adding ethanol (95) to make 100 mL, and heat on a water bath under a reflux condenser for 1 hour. After cooling, filter the precipitate with a glass filter, wash with water, dry at 105°C to constant mass, and weigh. The mass of the precipitate represents the amount of silver chloride (AgCl: 143.32).

Amount (mg) of 4-nitrobenzyl chloride

$$= \text{amount (mg) of silver chloride (AgCl: 143.32)} \times 1.197$$

4-(4-Nitrobenzyl)pyridine $C_{12}H_{10}N_2O_2$ Pale yellow, crystalline powder. Freely soluble in acetone, and soluble in ethanol (95).

Melting point <2.60>: 69 – 71°C

Nitrogen [Same as the namesake monograph]

Nitrogen monoxide NO A colorless gas. Prepare by adding sodium nitrite TS to a solution of iron (II) sulfate heptahydrate in dilute sulfuric acid. Nitrogen monoxide from a metal cylinder may be used.

Nitromethane CH_3NO_2 [K 9523, Special class]

3-Nitrophenol $C_6H_5NO_3$ A light yellow crystalline powder.

Melting point <2.60>: 96 – 99°C

4-Nitrophenol $C_6H_5NO_3$ [K 8721, Special class]

***o*-Nitrophenol** $C_6H_5NO_3$ [K 8719, Special class]

***o*-Nitrophenyl- β -D-galactopyranoside** See 2-nitrophenyl- β -D-galactopyranoside.

2-Nitrophenyl- β -D-galactopyranoside $C_{12}H_{15}NO_8$ White crystalline powder. Odorless. It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point <2.60>: 193 – 194°C

Purity Clarity and color of solution—A solution of 2-nitrophenyl- β -D-galactopyranoside (1 in 100) is clear and colorless.

Loss on drying <2.41>: not more than 0.1% (0.5 g, 105°C, 2 hours).

Content: not less than 98.0%. *Assay*—Weigh accurately about 0.05 g of 2-nitrophenyl- β -D-galactopyranoside, previously dried, dissolve in water to make exactly 100 mL. Pipet 20 mL of this solution, and add water to make exactly 50 mL. Determine the absorbance, *A*, of this solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of 2-nitrophenyl- β -D-galactopyranoside

$$= \frac{A}{133} \times 25,000$$

1-Nitroso-2-naphthol $C_{10}H_7NO_2$ [K 8713, Special class]

1-Nitroso-2-naphthol TS Dissolve 0.06 g of 1-nitroso-2-naphthol in 80 mL of acetic acid (100), and add water to make 100 mL.

α -Nitroso- β -naphthol See 1-nitroso-2-naphthol.

α -Nitroso- β -naphthol TS See 1-nitroso-2-naphthol TS.

Nitrous oxide N_2O Colorless and odorless gas. Use nitrous oxide from a metal cylinder.

NK-7 cells Cells derived from mouse NK cells

NN Indicator Mix 0.5 g of 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid with 50 g of anhydrous sodium sulfate, and triturate until the mixture becomes homogeneous.

Nonylphenoxypoly(ethyleneoxy)ethanol for gas chromatography Prepared for gas chromatography.

Normal agar media for teceleukin Dissolve 5.0 g of meat extract, 10.0 g of peptone, 5.0 g of sodium chloride, and 15.0 to 20.0 g of agar in water to make 1000 mL, and sterilize. Adjust the pH to 6.9 to 7.1.

Normal human plasma Dissolve the lyophilized normal human plasma derived from 1 mL of normal human plasma with 1 mL of water.

***n*-Octadecane** C₁₈H₃₈ Colorless or white solid at ordinary temperature.

Purity Clarity of solution—A solution of *n*-octadecane in chloroform (1 in 25) is clear.

Octadecylsilanized silica gel for pretreatment Prepared for pretreatment.

***n*-Octane** C₈H₁₈

Specific gravity <2.56> d_4^{20} : 0.700 – 0.705

Purity—Perform the test with 2 μ L of *n*-octane as directed under Gas Chromatography <2.02> according to the conditions in the Assay under Hypromellose. Measure each peak area by the automatic integration method, and calculate the amount of *n*-octane by the area percentage method: not less than 99.0%.

Octane, iso A colorless liquid. Practically insoluble in water. Miscible with diethyl ether and with chloroform.

Purity—Determine the absorbances of isooctane at 230 nm, 250 nm and 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank solution: these values are not more than 0.050, 0.010 and 0.005, respectively.

1-Octanol CH₃(CH₂)₆CH₂OH [K 8213, Special class]

Octyl alcohol See 1-octanol.

***n*-Octylbenzen** C₁₄H₂₂ Clear and colorless liquid, having a characteristic odor.

Specific gravity <2.56> d_4^{20} : 0.854 – 0.863

Distillation test <2.57>: 263 – 265 °C, not less than 95 vol%.

Ofloxacin demethyl substance (\pm)-9-Fluoro-2,3-dihydro-3-methyl-7-oxo-7*H*-10-(1-piperazinyl)-pirido[1, 2, 3-*de*] [1, 4]benzoxazine-6-carboxylic acid C₁₇H₁₈FN₃O₄ White to light green-yellowish white, crystals or crystalline powder.

Identification—Determine the infrared absorption spectrum of ofloxacin demethyl substance as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3050 cm⁻¹, 2840 cm⁻¹, 1619 cm⁻¹, 1581 cm⁻¹, 1466 cm⁻¹, 1267 cm⁻¹, 1090 cm⁻¹, 1051 cm⁻¹ and 816 cm⁻¹.

Olive oil [Same as the namesake monograph]

Orcine C₇H₃O₂ White to light red-brown crystals or crystalline powder, having an unpleasant, sweet taste. It

turns to red in color when oxidized in air. Soluble in water, in ethanol (95), and in diethyl ether.

Meting point <2.60>: 107 – 111 °C

Orcine-ferric chloride TS See orcine-iron (III) chloride TS.

Orcine-iron (III) chloride TS Dissolve 10 mg of orcine in 1 mL of a solution of iron (III) chloride hexahydrate in hydrochloric acid (1 in 1000). Prepare before use.

Ordinary agar medium Dissolve 25 to 30 g of agar in 1000 mL of ordinary broth with the aid of heat, add water to make up for the loss, adjust the pH to between 6.4 and 7.0, and filter. Dispense the filtrate, and sterilize by autoclaving. When powdered agar is used, 15 to 20 g of it is dissolved.

Ordinary broth Dissolve 5 g of beef extract and 10 g of peptone in 1000 mL of water by gentle heating. Adjust the pH of the mixture between 6.4 and 7.0 after sterilization, cool, add water to make up for the loss, and filter. Sterilize the filtrate by autoclaving for 30 minutes at 121 °C.

Osthole for thin-layer chromatography C₁₅H₁₆O₃ A white crystalline powder, having no odor. Freely soluble in methanol and in ethyl acetate, soluble in ethanol (99.5), and practically insoluble in water. Melting point: 83 – 84 °C.

Purity Related substances—Dissolve 1.0 mg of osthole for thin-layer chromatography in 1 mL of methanol. Perform the test with 10 μ L of this solution as directed in the Identification under Cnidium Monnieri Fruit: on spot appears other than the principal spot at around R_f 0.3.

Oxalate pH standard solution See pH Determination <2.54>.

Oxalic acid See oxalic acid dihydrate.

Oxalic acid dihydrate H₂C₂O₄·2H₂O [K 8519, Special class]

Oxalic acid TS Dissolve 6.3 g of oxalic acid dihydrate in water to make 100 mL (0.5 mol/L).

Oxycodone hydrochloride for assay C₁₈H₂₁NO₄·HCl·3H₂O [Same as the monograph Oxycodone Hydrochloride Hydrate. It contains not less than 99.0% of oxycodone hydrochloride (C₁₈H₂₁NO₄·HCl), calculated on the anhydrous basis.]

Oxygen O₂ [K 1101]

8-Oxyquinoline See 8-quinolinol.

2-Oxy-1-(2'-oxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid See 2-hydroxy-1-(2'-hydroxy-4'-sulfo-1'-naphthylazo)-3-naphthoic acid.

Oxytocin C₄₃H₆₆N₁₂O₁₂S₂ [Same as the namesake monograph]

Paeoniflorin for thin-layer chromatography C₂₃H₂₈O₁₁·*n*H₂O Colorless, odorless powder. Freely soluble in water and in methanol, and not dissolves in diethyl ether. Melting point: 123 – 125 °C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of paeoniflorin for thin layer chromatography in exactly 1 mL of methanol. Perform the test with 20 μ L of this solution as directed in the Identification (2) under Peony Root: any spot other than the principal spot at the R_f value of about 0.3 does not

appear.

Palladium chloride See palladium (II) chloride.

Palladium chloride TS See palladium (II) chloride TS.

Palladium (II) chloride PdCl₂ [K 8154, Special class]

Palladium (II) chloride TS Dissolve 0.2 g of palladium (II) chloride in 500 mL of 0.25 mol/L sulfuric acid TS, by heating if necessary, cool, and add 0.25 mol/L sulfuric acid TS to make 1000 mL.

Palmatin chloride C₂₁H₂₂ClNO₄·xH₂O A yellow-brown crystalline powder.

Purity—Dissolve 1 mg of palmatin chloride in 10 mL of methanol, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Phellodendron Bark: when measure the peak areas 2 times as long as the retention time of palmatin, the total area of the peaks other than palmatin is not larger than 1/10 of the total area except the area of solvent peak.

Palmitic acid for gas chromatography C₁₆H₃₂O₂ [K 8756, Special class]

Papaverine hydrochloride C₂₀H₂₁NO₄·HCl [Same as the namesake monograph]

Papaverine hydrochloride for assay C₂₀H₂₁NO₄·HCl [Same as the monograph Papaverine Hydrochloride. When dried, it contains not less than 99.0% of papaverine hydrochloride (C₂₀H₂₁NO₄·HCl).]

Paraffin [Same as the namesake monograph]

Paraffin, liquid [Same as the monograph Light Liquid Paraffin]

Parahydroxybenzoic acid C₇H₆O₃ White crystals.

Melting point <2.60>: 212 – 216°C

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.7 g of parahydroxybenzoic acid, dissolve in 50 mL of acetone, add 100 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 69.06 mg of C₇H₆O₃

PBS containing bovine serum To 100 mL of bovine serum add 900 mL of phosphate-buffered sodium chloride TS containing thimerosal (0.1 g) to make 1000 mL.

Storage—Store in a cool place shielded from light.

PBS containing bovine serum albumin Add PBS to 10 g of bovine serum albumin and 0.1 g of thimerosal to make 1000 mL.

Storage—Store in a cool, dark place.

Peanut oil [Same as the namesake monograph]

Pentane CH₃(CH₂)₃CH₃ Clear and colorless liquid.

Specific gravity <2.56> *d*₂₀²⁰: 0.620 – 0.650

Distilling range <2.57>: 35.5 – 37°C, not less than 98 vol%.

Peonol for component determination Use peonol for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> *E*_{1cm}^{1%} (274 nm): 853 – 934 [5 mg after

drying in a desiccator (calcium chloride for drying) for 1 hour or more, methanol, 1000 mL].

Purity Related substances—Dissolve 5.0 mg of peonol for component determination in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than peonol from the sample solution is not larger than the peak area of peonol from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Component determination under Moutan Bark except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of peonol obtained from 10 μL of the standard solution (2) can be measured, and the peak height of peonol from 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of peonol beginning after the solvent peak.

Peonol for thin-layer chromatography C₉H₁₀O₃

White, crystals or crystalline powder, having a specific odor. Freely soluble in methanol and in diethyl ether, and slightly soluble in water. Melting point: about 50°C

Purity Related substances—Dissolve 1.0 mg of peonol for thin-layer chromatography in exactly 1 mL of methanol, and perform with 10 μL of this solution as directed in the Identification under Moutan Bark: any spot other than the principal spot at the R_f value of near 0.5 does not appear.

Peptone Prepared for microbial test.

Peptone, animal tissue Prepared for microbial test.

Peptone, casein Grayish yellow powder, having a characteristic but not putrescent odor. It dissolves in water, but not in ethanol (95) and in diethyl ether.

Loss on drying <2.41>: not more than 7% (0.5 g, 105°C, constant mass).

Residue on ignition <2.44>: not more than 15% (0.5 g).

Degree of digestion—Dissolve 1 g of casein peptone in 10 mL of water, and perform the following test using this solution as the sample solution:

(1) Overlay 1 mL of the sample solution with 0.5 mL of a mixture of 1 mL of acetic acid (100) and 10 mL of dilute ethanol: no ring or precipitate forms at the junction of the two liquids, and on shaking, no turbidity results.

(2) Mix 1 mL of the sample solution with 4 mL of a saturated solution of zinc sulfate heptahydrate: a small quantity of precipitate is produced (proteoses).

(3) Filter the mixture of (2), and to 1 mL of the filtrate add 3 mL of water and 4 drops of bromine TS: a red-purple color is produced.

Nitrogen content <1.08>: not less than 10% (105°C, constant mass, after drying).

Peptone, gelatin Prepared for microbial test.

Peptone, soybean Prepared for microbial test.

Perchloric acid HClO_4 [K 8223, Special class, Density: about 1.67 g/mL. Concentration: 70.0 – 72.0%]

Perchloric acid-dehydrated ethanol TS See perchloric acid-ethanol TS.

Perchloric acid-ethanol TS Add cautiously 25.5 mL of perchloric acid to 50 mL of ethanol (99.5), cool, and add ethanol (99.5) to make 100 mL (3 mol/L).

Performic acid Mix 9 volumes of formic acid and 1 volume of hydrogen peroxide (30), and leave at room temperature for 2 hours.

Storage—Store in a cool place.

Peroxidase Obtained from horse-radish. A red-brown powder. It is freely soluble in water. It contains about 250 units per mg. One unit indicates an amount of the enzyme which produces 1 mg of purpurogallin in 20 seconds at 20°C and pH 6.0, from pyrogallol and hydrogen peroxide (30) used as the substrate.

Peroxidase-labeled antibody stock solution 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS containing antibody fragment (Fab') bound to peroxidase.

Peroxidase-labeled bradykinin A solution of horseradish origin peroxidase-binding bradykinin in gelatin-phosphate buffer solution, pH 7.0. A colorless to light brown clear solution.

Peroxidase-labeled bradykinin TS To 0.08 mL of peroxidase-labeled bradykinin, 8 mg of sodium tetraborate decahydrate, 8 mg of bovine serum albumin and 0.8 mL of gelatin-phosphate buffer solution, pH 7.0 add water to make 8 mL, and lyophilize. Dissolve this in 8 mL of water. Prepare before use.

Peroxidase-labeled rabbit anti-ECP antibody Fab' TS Mix 1 volume of ECP standard substance (equivalent to about 1 mg of protein) and 1 volume of Freund's complete adjuvant, and then immunize rabbits subcutaneously in the back region and intramuscularly in the hind leg muscle with this solution 5 times at 2 week intervals. Harvest blood on the 10th day after completing the immunization to obtain rabbit antiserum. Rabbit anti-ECP antibody Fab' is obtained by preparing an immobilized ECP column in which ECP standard substance is bound to agarose gel and then purifying by affinity column chromatography to obtain rabbit anti-ECP antibody which undergoes pepsin digestion to yield F(ab')_2 which is reacted with 2-aminoethanethiol hydrochloride.

Horseradish peroxidase is reacted with maleimido reagent [4-(maleimidomethyl) cyclohexyl carbonic acid-*N*-hydroxysuccinimide imidoester] to yield maleimido peroxidase. Perform a coupling reaction by mixing rabbit anti-ECP antibody Fab' and maleimido peroxidase at 4°C to prepare peroxidase-labeled rabbit anti-ECP antibody Fab'. Take a specific amount of peroxidase-labeled rabbit anti-ECP antibody Fab' and dilute using PBS containing bovine serum albumin. The peroxidase-labeled rabbit anti-ECP antibody Fab' TS is a diluted solution with a concentration that gives a good calibration curve with assay characteristics.

Description: Clear and colorless solution

Identification: Pipet 100 μL of the TS to be examined into flat-bottomed microtest plates. When substrate buffer solu-

tion for celmoleukin is added to this, it immediately exhibits a dark violet color, which changes to yellowish-red with time.

Perphenazine maleate for assay [Same as the monograph Perphenazine Maleate. When dried, it contains not less than 99.0 % of perphenazine maleate ($\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{O}_5 \cdot 2\text{C}_4\text{H}_4\text{O}_4$).]

Pethidine hydrochloride for assay $\text{C}_{15}\text{H}_{21}\text{NO}_2 \cdot \text{HCl}$ [Same as the monograph Pethidine Hydrochloride. When dried, it contains not less than 99.0% of pethidine hydrochloride $\text{C}_{15}\text{H}_{21}\text{NO}_2 \cdot \text{HCl}$.]

Petrolatum [Same as the monograph Yellow Petrolatum or White Petrolatum]

Petroleum benzine [K 8594, Special class]

Petroleum ether [K 8593, Special class]

Phenacetin $\text{C}_{10}\text{H}_{13}\text{NO}_2$ White crystals or crystalline powder. Soluble in ethanol (95), and very slightly soluble in water.

Melting point <2.60>: 134 – 137°C

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

***o*-Phenanthroline** See 1,10-phenanthroline monohydrate.

***o*-Phenanthroline hydrochloride** See 1,10-phenanthroline chloride monohydrate.

1,10-Phenanthroline monohydrate $\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{H}_2\text{O}$ [K 8789, Special class]

***o*-Phenanthroline TS** See 1,10-phenanthroline TS.

1,10-Phenanthroline TS Dissolve 0.15 g of 1,10-phenanthroline monohydrate in 10 mL of a freshly prepared ferrous sulfate heptahydrate solution (37 in 2500) and 1 mL of dilute sulfuric acid. Preserve in tightly stoppered containers.

1,10-Phenanthroline chloride monohydrate $\text{C}_{12}\text{H}_8\text{N}_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$ [K 8202, Special class]

Phenethylamine hydrochloride $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_2\text{NH}_2 \cdot \text{HCl}$ White crystals or crystalline powder.

Melting point: <2.60> 220 – 225°C

Phenobarbital sodium $\text{C}_{12}\text{H}_{11}\text{N}_2\text{NaO}_3$ [Same as the namesake monograph]

Phenol $\text{C}_6\text{H}_5\text{OH}$ [K 8798, Special class]

Phenol for assay $\text{C}_6\text{H}_5\text{OH}$ [K 8798, Special class]

Phenol-hydrochloric acid TS Dissolve 0.2 g of phenol in 10 mL of 6 mol/L hydrochloric acid TS.

Phenolphthalein $\text{C}_{20}\text{H}_{14}\text{O}_4$ [K 8799, Special class]

Phenolphthalein-thymol blue TS Solution A: Dissolve 0.1 g of phenolphthalein in diluted ethanol (99.5) (4 in 5). Solution B: Dissolve 0.1 g of thymol blue in 50 mL of a mixture of ethanol and dilute sodium hydroxide TS, add water to make 100 mL. Mix 2 volumes of solution A and 3 volumes of solution B before use.

Phenolphthalein TS Dissolve 1 g of phenolphthalein in 100 mL of ethanol (95).

Phenolphthalein TS, alkaline See Alcohol Number Determination <1.01>.

Phenol red $C_{19}H_{14}O_5S$ [K 8800, Special class]

Phenol red TS Dissolve 0.1 g of phenol red in 100 mL of ethanol (95), and filter if necessary.

Phenol red TS, dilute To 235 mL of a solution of ammonium nitrate (1 in 9400) add 105 mL of 2 mol/L sodium hydroxide TS and 135 mL of a solution prepared by dissolving 24 g of acetic acid (100) in water to make 200 mL. To this solution add 25 mL of a solution prepared by dissolving 33 mg of phenol red in 1.5 mL of 2 mol/L sodium hydroxide TS and adding water to make 100 mL. If necessary, adjust the pH to 4.7.

Phenol-sodium nitroprusside TS See phenol-sodium pentacyanonitrosylferrate (III) TS.

Phenol-sodium pentacyanonitrosylferrate (III) TS Dissolve 5 g of phenol and 25 mg of sodium pentacyanonitrosylferrate (III) dihydrate in sufficient water to make 500 mL. Preserve in a dark, cold place.

Phenolsulfonphthalein for assay $C_{19}H_{14}O_5S$ [Same as the monograph Phenolsulfonphthalein. When dried, it contains not less than 99.0% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$).]

50% Phenyl-50% methylpolysiloxane for gas chromatography Prepared for gas chromatography.

Phenylalanine $C_9H_{11}NO_2$ [Same as the monograph L-Phenylalanine]

Phenyl benzoate $C_6H_5COOC_6H_5$ White crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 68 – 70°C

Purity Clarity of solution—Dissolve 1.0 g of phenyl benzoate in 20 mL of methanol: the solution is clear.

***o*-Phenylenediamine** $H_2NC_6H_4NH_2$ White to dark brown crystals or crystalline powder. Freely soluble in ethanol (95) and in acetone, and soluble in water.

Content: not less than 95.0%. Assay—Accurately weigh about 0.15 g of *o*-phenylenediamine, add 50 mL of acetic acid for nonaqueous titration to dissolve, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Correct by conducting a blank test using the same method.

Each mL of 0.1 mol/L perchloric acid VS
= 10.81 mg of $H_2NC_6H_4NH_2$

***o*-Phenylenediamine dihydrochloride**

$H_2NC_6H_4NH_2 \cdot 2HCl$ White to pale yellow or pale red crystals or crystalline powder.

Clarity: a solution (1 in 20) is clear.

Content: not less than 98.0%. Assay—Weigh accurately about 0.15 g of *o*-phenylenediamine dihydrochloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS
= 9.053 mg of $H_2NC_6H_4NH_2 \cdot 2HCl$

Phenylfluorone $C_{19}H_{12}O_5$ [K 9547]

Phenylfluorone-ethanol TS Dissolve 50 mg of phenylfluorone in 10 mL of a mixture of ethanol (95) and diluted hydrochloric acid (1 in 3), and add ethanol (95) to make exactly

500 mL.

D-Phenylglycine $C_8H_9NO_2$ White, crystals or crystalline powder. Slightly soluble in water.

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 3 hours).

Content: not less than 98.5%. Assay—Weigh accurately about 0.3 g of D-phenylglycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.12 mg of $C_8H_9NO_2$

Phenylhydrazine $C_6H_5NHNH_2$ Colorless or light yellow, clear liquid, having a faint aromatic odor.

Content: not less than 99.0%. Assay—Weigh accurately about 1 g, add 30 mL of diluted hydrochloric acid (1 in 100) and water to make exactly 100 mL. Put exactly 20 mL of this solution in a glass-stoppered conical flask, and add 40 mL of diluted hydrochloric acid (3 in 4). After cooling, add 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking vigorously until the red color of the chloroform layer disappears. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L potassium iodate VS
= 5.407 mg of $C_6H_5NHNH_2$

Phenylhydrazine hydrochloride See phenylhydrazinium chloride.

Phenylhydrazine hydrochloride TS See phenylhydrazinium chloride TS.

Phenylhydrazinium chloride $C_6H_5NHNH_2 \cdot HCl$
[K 8203, Special class]

Phenylhydrazinium chloride TS Dissolve 65 mg of phenylhydrazinium chloride recrystallized from dilute ethanol, in 100 mL of a solution previously prepared by adding cautiously 170 mL of sulfuric acid to 80 mL of water.

35% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

50% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

65% Phenyl-methyl silicone polymer for gas chromatography Prepared for gas chromatography.

25% Phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography Prepared for gas chromatography.

1-phenyl-3-methyl-5-pyrazolone See 3-methyl-1-phenyl-5-pyrazolone.

Phenylpiperazine hydrochloride $C_{10}H_{14}N_2 \cdot HCl$ A white powder. Melting point: about 247°C (with decomposition).

Phloroglucin See phloroglucinol dihydrate.

Phloroglucin dihydrate See phloroglucinol dihydrate.

Phloroglucinol dihydrate $C_6H_3(OH)_3 \cdot 2H_2O$ White to pale yellow, crystals or crystalline powder.

Melting point <2.60>: 215 – 219°C (after drying).

Loss on drying <2.41>: 18.0 – 24.0% (1 g, 105°C, 1 hour).

Phosphate buffer solution for component determination of bupleurum root To 100 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 59 mL of sodium hydrogen phosphate TS.

Phosphate buffer solution for pancreatin Dissolve 3.3 g of anhydrous disodium hydrogen phosphate, 1.4 g of potassium dihydrogen phosphate and 0.33 g of sodium chloride in water to make 100 mL.

Phosphate buffer solution, pH 3.0 Dissolve 136 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust to pH 3.0 with phosphoric acid.

0.02 mol/L Phosphate buffer solution, pH 3.0 Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.0 with diluted phosphoric acid (1 in 10).

Phosphate buffer solution, pH 3.1 Dissolve 136.1 g of potassium dihydrogen phosphate in 500 mL of water, and add 6.3 mL of phosphoric acid and water to make 1000 mL.

0.05 mol/L Phosphate buffer solution, pH 3.5 To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of a solution of phosphoric acid (49 in 10,000) to make a solution having pH 3.5.

0.02 mol/L Phosphate buffer solution, pH 3.5 Dissolve 3.1 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10).

0.1 mol/L Phosphate buffer solution, pH 4.5 Dissolve 13.61 g of potassium dihydrogen phosphate in 750 mL of water, adjust to pH 4.5 with potassium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 5.3 Dissolve 0.44 g of disodium hydrogen phosphate dodecahydrate and 13.32 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 5.3 with sodium hydroxide TS or phosphoric acid, and add water to make 1000 mL.

1/15 mol/L Phosphate buffer solution, pH 5.6 Dissolve 9.07 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 5.6 with potassium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 5.9 Dissolve 6.8 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 5.9 with diluted potassium hydroxide TS (1 in 10), and add water to make 1000 mL.

Phosphate buffer solution, pH 6.0 Dissolve 8.63 g of potassium dihydrogen phosphate and 1.37 g of anhydrous disodium hydrogen phosphate in 750 mL of water, adjust the pH to 6.0 with sodium hydroxide TS or diluted phosphoric acid (1 in 15), and add water to make 1000 mL.

0.05 mol/L Phosphate buffer solution, pH 6.0 To 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution add 5.70 mL of 0.2 mol/L sodium hydroxide TS and water to make 200 mL.

Phosphate buffer solution, pH 6.2 Dissolve 9.08 g of potassium dihydrogen phosphate in 1000 mL of water (solution A). Dissolve 9.46 g of disodium hydrogen phosphate in 1000 mL of water (solution B). Mix 800 mL of the solution A

and 200 mL of the solution B, and adjust the pH to 6.2 with the solution A or the solution B if necessary.

Phosphate buffer solution, pH 6.5 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 15.20 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

Phosphate buffer solution, pH 6.8 Dissolve 3.40 g of potassium dihydrogen phosphate and 3.55 g of disodium hydrogen phosphate in water to make 1000 mL.

0.01 mol/L Phosphate buffer solution, pH 6.8 Dissolve 1.36 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 6.8 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

0.1 mol/L Phosphate buffer solution, pH 6.8 Dissolve 6.4 g of potassium dihydrogen phosphate and 18.9 g of disodium hydrogen phosphate dodecahydrate in about 750 mL of water, adjust the pH to 6.8 with sodium hydroxide TS if necessary, and add water to make 1000 mL.

Phosphate buffer solution, pH 7.0 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 29.54 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

0.05 mol/L Phosphate buffer solution, pH 7.0 Dissolve 4.83 g of dipotassium hydrogen phosphate and 3.02 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 7.0 with phosphoric acid or potassium hydroxide TS.

0.1 mol/L Phosphate buffer solution, pH 7.0 Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL (solution A). Dissolve 6.8 g of potassium dihydrogen phosphate in water to make 500 mL (solution B). To a volume of solution A add solution B until the mixture is adjusted to pH 7.0 (about 2:1 by volume of solutions A and B).

Phosphate buffer solution, pH 7.2 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 34.7 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

Phosphate buffer solution, pH 7.4 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 39.50 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

0.03 mol/L Phosphate buffer solution, pH 7.5 Dissolve 4.083 g of potassium dihydrogen phosphate in 800 mL of water, adjust the pH to 7.5 with 0.2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 8.0 Mix 50 mL of 0.2 mol/L potassium dihydrogen phosphate TS for buffer solution and 46.1 mL of 0.2 mol/L sodium hydroxide VS, and add water to make 200 mL.

0.1 mol/L Phosphate buffer solution for antibiotics, pH 8.0 Dissolve 16.73 g of dipotassium hydrogen phosphate and 0.523 g of potassium dihydrogen phosphate in about 750 mL of water, adjust the pH to 8.0 with phosphoric acid, and add water to make 1000 mL.

0.02 mol/L Phosphate buffer solution, pH 8.0 To 50 mL

of 0.2 mol/L potassium dihydrogen phosphate TS add 300 mL of water, adjust the pH to 8.0 with sodium hydroxide TS, and add water to make 500 mL.

0.1 mol/L Phosphate buffer solution, pH 8.0 Dissolve 13.2 g of anhydrous disodium hydrogen phosphate and 0.91 g of potassium dihydrogen phosphate in about 750 mL of water, adjust to pH 8.0 with phosphoric acid, and add water to make 1000 mL.

0.2 mol/L Phosphate buffer solution, pH 10.5 Dissolve 34.8 g of dipotassium hydrogen phosphate in 750 mL of water, adjust to pH 10.5 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution, pH 12 To 5.44 g of disodium hydrogen phosphate add 36.5 mL of sodium hydroxide TS and about 40 mL of water, dissolve by shaking well, and add water to make 100 mL.

Phosphate buffer solution for antibiotics, pH 6.5 Dissolve 10.5 g of disodium hydrogen phosphate dodecahydrate and 5.8 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.5 with sodium hydroxide TS, and add water to make 1000 mL.

Phosphate buffer solution for microplate washing Dissolve 0.62 g of sodium dihydrogen phosphate dihydrate, 9.48 g of disodium hydrogen phosphate dodecahydrate, 52.6 g of sodium chloride, 3.0 g of polysorbate 80 and 1.8 g of polyoxyethylene (40) octylphenyl ether in water to make 600 mL. Dilute this solution 10 times with water before use.

Phosphate buffer solution for processed aconite root Dissolve 19.3 g of disodium hydrogen phosphate dodecahydrate in 3660 mL of water, and add 12.7 g of phosphoric acid.

Phosphate-buffered sodium chloride TS Dissolve 8.0 g of sodium chloride, 0.2 g of potassium chloride, 2.9 g of disodium hydrogen phosphate dodecahydrate, and 0.2 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.01 mol/L Phosphate buffer-sodium chloride TS, pH 7.4 Dissolve 2.93 g of disodium hydrogen phosphate dodecahydrate ($\text{NaH}_2\text{PO}_4 \cdot 12\text{H}_2\text{O}$), 0.25 g of potassium dihydrogen phosphate (KH_2PO_4), and 9.0 g of sodium chloride in water to make 1000 mL.

Phosphinic acid H_3PO_2 [K 8440, First class]

Phosphate TS Dissolve 2.0 g of dipotassium hydrogen phosphate and 8.0 g of potassium dihydrogen phosphate in water to make 1000 mL.

Phosphomolybdic acid See phosphomolybdic acid *n*-hydrate.

Phosphomolybdic acid *n*-hydrate $\text{P}_2\text{O}_5 \cdot 24\text{MoO}_3 \cdot n\text{H}_2\text{O}$ Yellow crystals or crystalline powder.

Identification—(1) To 10 mL of a solution (1 in 10) add 0.5 mL of ammonia TS: yellow precipitates appear, which disappear by the addition of 2 mL of ammonia TS, and yellow precipitates appear by further addition of 5 mL of diluted nitric acid (1 in 2).

(2) To 5 mL of a solution (1 in 10) add 1 mL of ammonia TS and 1 mL of magnesia TS: white precipitates appear.

Phosphoric acid H_3PO_4 [K 9005, Special class]

Phosphoric acid-sodium sulfate buffer solution, pH 2.3

Dissolve 28.4 g of anhydrous sodium sulfate in 1000 mL of water, and add 2.7 mL of phosphoric acid. If necessary, adjust to pH 2.3 with 2-aminoethanol.

Phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0 Dissolve 6.77 mL of phosphoric acid, 5.72 mL of acetic acid (100) and 6.18 g of boric acid in water to make 1000 mL. Adjust the pH of this solution to 2.0 with 0.5 mol/L sodium hydroxide VS.

Phosphorus pentoxide See phosphorus (V) oxide.

Phosphorus, red P An odorless dark red powder. Practically insoluble in carbon disulfide and in water.

Free phosphoric acid: Not more than 0.5%.

To 5 g add 10 mL of a solution of sodium chloride (1 in 5), mix, then add 50 mL of the solution of sodium chloride (1 in 5), allow to stand for 1 hour, and filter. Wash the residue with three 10-mL portions of the solution of sodium chloride (1 in 5), combine the filtrate and the washings, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of thymol blue TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 4.90 mg of H_3PO_4

Phosphorus (V) oxide P_2O_5 [K 8342, Special class]

Phosphotungstic acid See phosphotungstic acid *n*-hydrate.

Phosphotungstic acid *n*-hydrate $\text{P}_2\text{O}_5 \cdot 24\text{WO}_3 \cdot n\text{H}_2\text{O}$ White to yellowish green, crystals or crystalline powder.

Identification—To 5 mL of a solution (1 in 10) add 1 mL of acidic tin (II) chloride TS, and heat: blue precipitates appear.

Phosphotungstic acid TS Dissolve 1 g of phosphotungstic acid *n*-hydrate in water to make 100 mL.

***o*-Phthalaldehyde** $\text{C}_6\text{H}_4(\text{CHO})_2$ Light yellow to yellow crystals.

Content: not less than 99%. **Assay**—Dissolve 1 g of *o*-phthalaldehyde in 10 mL of ethanol (95). Proceed with 2 μL of this solution as directed in Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method.

Content (%) = peak area of *o*-phthalaldehyde/total area of all peaks \times 100

Operating conditions—

Detector: A thermal conductivity detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography treated with acid and silane (177–250 μm), coated with methyl silicon polymer for gas chromatography in 10%.

Column temperature: A constant temperature of about 180 °C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of *o*-phthalaldehyde is 3–4 minutes.

Time span of measurement: About 7 times as long as the retention time of *o*-phthalaldehyde, beginning after the solvent peak.

Phthalein purple $\text{C}_{32}\text{H}_{32}\text{N}_2\text{O}_{12} \cdot x\text{H}_2\text{O}$ Yellowish white to brown power. Soluble in ethanol (95), and practically insoluble in water.

Sensitivity test—Dissolve 10 mg of phthalein purple in 1 mL of ammonia solution (28), and add water to make 100 mL. To 5 mL of this solution add 95 mL of water, 4 mL of ammonia solution (28), 50 mL of ethanol (95) and 0.1 mL of diluted barium chloride TS (1 in 5): the solution shows a blue-purple color which disappears on the addition of 0.15 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Phthalic acid $C_8H_6O_4$ Colorless or white crystalline powder. Soluble in methanol and in ethanol (95), sparingly soluble in water, and practically insoluble in chloroform. Melting point: about 200°C (with decomposition).

Content: not less than 98%. **Assay**—Weigh accurately about 2.8 g of phthalic acid, add exactly 50 mL of 1 mol/L sodium hydroxide VS and 25 mL of water, and dissolve by heating on a hot plate. After cooling, add 5 drops of phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS. Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 83.07 mg of $C_8H_6O_4$

Phthalic anhydride $C_8H_4O_3$ [K 8887, Special class]

Phthalimide $C_8H_5NO_2$ White to pale brown crystals or powder.

Melting point <2.60>: 232 – 237°C

Clarity—1.0 g of phthalimide dissolves in 20 mL of sodium hydroxide TS as a slight turbid solution.

Content: not less than 98.0%. **Assay**—Weigh accurately about 0.3 g of the substance to be tested, dissolve in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 14.71 mg of $C_8H_5NO_2$

Phytonadione $C_{31}H_{46}O_2$ [Same as the namesake monograph]

Picric acid See 2,4,6-trinitrophenol.

Picric acid-ethanol TS See 2,4,6-trinitrophenol-ethanol TS.

Picric acid TS See 2,4,6-trinitrophenol TS.

Picric acid TS, alkaline See 2,4,6-trinitrophenol TS, alkaline.

Piperidine hydrochloride $C_5H_{11}N.HCl$ A white crystalline powder. Dissolves in water and in methanol. The pH of the aqueous solution (1 in 20) is between 3.0 and 5.0.

Melting point <2.60>: 247 – 252°C

Purity Clarity and color of solution—Dissolve 1.0 g of piperidine hydrochloride in 20 mL of water: the solution is clear and colorless.

Residue on ignition <2.44>: not more than 0.1% (1 g).

Content: not less than 99.0%. **Assay**—Dissolve about 0.25 g of piperidine hydrochloride, accurately weighed, in 50 mL of water, add 5 mL of diluted nitric acid (1 in 3), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS

= 12.16 mg of $C_5H_{11}N.HCl$

Polyalkylene glycol for gas chromatography Prepared for gas chromatography.

Polyalkylene glycol monoether for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 15000-diepoxyde for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 20 M for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 400 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 600 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 1500 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol 6000 for gas chromatography Prepared for gas chromatography.

Polyethylene glycol ester for gas chromatography Prepared for gas chromatography.

Polyoxyethylene hydrogenated castor oil 60 A nonionic surfactant prepared by addition polymerization of ethylene oxide with hydrogenated castor oil. Average molar number of ethylene oxide added is about 60. A white or pale yellow petrolatum-like or waxy substance, having a faint, characteristic odor and a slight bitter taste. Very soluble in ethyl acetate and in chloroform, freely soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

Identification—(1) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water and 5 mL of ammonium thiocyanate-cobalt (II) nitrate TS, and shake thoroughly. Add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

(2) To 0.2 g of polyoxyethylene hydrogenated castor oil 60 add 0.5 g of potassium bisulfate, and heat: an acrolein-like, irritating odor is perceptible.

(3) To 0.5 g of polyoxyethylene hydrogenated castor oil 60 add 10 mL of water, shake, and add 5 drops of bromine TS: the color of the test solution does not disappear.

Congealing point <2.42>: 30 – 34°C

pH <2.54>—To 1.0 g of polyoxyethylene hydrogenated castor oil 60 add 20 mL of water, and dissolve by heating: the pH of the solution is between 3.6 and 6.0.

Acid value <1.13>: not more than 1.0.

Saponification value <1.13>: 41 – 51

Hydroxyl value <1.13>: 39 – 49

Purity (1) Clarity and color of solution—Dissolve 1.0 g of polyoxyethylene hydrogenated castor oil 60 in 20 mL of ethanol: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of polyoxyethylene hydrogenated castor oil 60 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of polyoxyethylene hydrogenated castor oil 60 according to Method 3, and perform the test (not more than 2 ppm).

Water <2.48>: not more than 2.0% (1 g).

Residue on ignition <2.44>: not more than 0.1% (1 g).

Storage—Preserve in tight containers.

Polyoxyethylene (23) lauryl ether

$C_{12}H_{25}(OCH_2CH_2)_nOH$ White masses. Melting point: about 40°C

Polyoxyethylene (40) octylphenyl ether Obtained by the addition polymerization with ethylene oxide to octylphenol. A colorless or white to pale yellow, liquid, vaseline-like or waxy, having slightly a characteristic odor.

Clarity: a solution (1 in 20) is clear.

Specific gravity <2.56> d_4^{20} : 1.10 – 1.11

pH <2.54>: 7.0 – 9.5 (5 w/v%, 25°C).

Polysorbate 20 Chiefly consists of addition polymer of sorbitan monolaurate and ethylene oxide. Pale yellow to yellow liquid, having a faint, characteristic odor.

Identification—(1) To 0.5 g of polysorbate 20 add 10 mL of water and 10 mL of sodium hydroxide TS, boil for 5 minutes, and acidify with dilute hydrochloric acid: an oily fraction is separated.

(2) To 0.5 g of polysorbate 20 add 10 mL of water, shake, and add 5 drops of bromine TS: the red color of the test solution does not disappear.

(3) Saponify 5 g of polysorbate 20 as directed under Saponification Value, and evaporate ethanol completely. Dissolve the residue in 50 mL of water, acidity with hydrochloric acid (methyl orange), and extract with two 30 mL portions of diethyl ether. Combine the diethyl ether layer, wash with 20 mL portions of water until the washings become neutral, and evaporate the diethyl ether on a water bath: the acid value of the residue is between 275 and 285. Use 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS for saponification.

Acid value <1.13>: not more than 4.0.

Saponification value <1.13>: 43 – 55

Loss on drying <2.41>: not more than 3.0% (5 g, 105°C, 1 hour).

Residue on ignition—Weigh accurately 3 g of polysorbate 20, heat gently at first, and ignite gradually (800 in 1200°C) until the residue is completely incinerated. If any carbonized substance remains, extract with hot water, filter through a sheet of filter paper for quantitative analysis (5C), and ignite the residue with the filter paper. Add the filtrate to it, evaporate to dryness, and ignite carefully until the carbonized substance does not remain. If any carbonized substance still remains, add 15 mL of ethanol (95), crush the carbonized substance with a glass rod, burn the ethanol, and ignite carefully. Cool in a desiccator (silica gel), and weigh the residue accurately: not more than 1.0%.

Polysorbate 80 [Same as the namesake monograph].

Polyvinyl alcohol $(-CH_2CHOH-)_n$ [K 9550, Special class]

Polyvinyl alcohol I Colorless to white, or pale yellow granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) or in diethyl ether. To polyvinyl alcohol I add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

Viscosity <2.53>: 25.0 – 31.0 mm²/s. Weigh 4.000 g of polyvinyl alcohol I, previously dried, add 95 mL of water, allow to stand for 30 minutes, and heat to dissolve on a water bath under a reflux condenser for 2 hours while stirring. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH <2.54>—The pH of a solution of polyvinyl alcohol I (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution—To 1.0 g of polyvinyl alcohol I add 20 mL of water, disperse by well stirring, warm between 60°C and 80°C for 2 hours, and cool: the solution is colorless and clear.

Saponification value: 98.0 – 99.0 mol%. Weigh accurately about 3.0 g of polyvinyl alcohol I, previously dried, transfer to a glass-stoppered conical flask, add 100 mL of water, and dissolve by heating on a water bath. After cooling, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.05 mol/L sulfuric acid VS, shake well, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction. However, when the volume of 0.1 mol/L sodium hydroxide VS consumed in the test is 25 mL or more, use about 2.0 g of the sample.

Saponification value (mol%)

$$= 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{0.6005 \times (a - b)f}{\text{amount (g) of the sample}}$$

a: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the test

b: Volume (mL) of 0.1 mol/L sodium hydroxide VS consumed in the blank test

f: Molarity factor of 0.1 mol/L sodium hydroxide VS

Polyvinyl alcohol II Colorless to white or pale yellow granules or powder. It is odorless, or has a faint odor of acetic acid. It is tasteless. Practically insoluble in ethanol (95) or in diethyl ether. To polyvinyl alcohol II add water, and heat: A clear, viscous solution is obtained. It is hygroscopic.

Viscosity <2.53>: 4.6 – 5.4 mm²/s. Weigh 4.000 g of polyvinyl alcohol II, previously dried, add 95 mL of water, allow to stand for 30 minutes, and dissolve by stirring on a water bath between 60°C and 80°C for 2 hours. After cooling, add water to make 100.0 g, and mix. Allow to stand still to remove bubbles, and perform the test at 20 ± 0.1°C as directed in Method 1.

pH <2.54>—The pH of a solution of polyvinyl alcohol II (1 in 25) is between 5.0 and 8.0.

Clarity and color of solution—To 1.0 g of polyvinyl alcohol II add 20 mL of water, disperse by well stirring, heat on a water bath for 2 hours, and cool: the solution is clear and colorless.

Saponification value: 86.5 – 89.5 mol%. Weigh accurately about 2.0 g of polyvinyl alcohol II, previously dried, transfer to a glass-stoppered, conical flask, add 100 mL of water, and warm while stirring for 2 hours. After cooling, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS, stopper tightly, and allow to stand for 2 hours. Then add exactly 30 mL of 0.25 mol/L sulfuric acid VS, shake well, and titrate <2.50> with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Saponification value (mol%)

$$= 100 - \frac{44.05A}{60.05 - 0.42A}$$

$$A = \frac{3.0025 \times (a - b)f}{\text{amount (g) of the sample}}$$

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank test

f: Molarity factor of 0.5 mol/L sodium hydroxide VS

Polyvinyl alcohol TS Weigh exactly 0.50 g of polyvinyl alcohol, and add water to make exactly 100 mL.

Potassium acetate CH₃COO.K [K 8363, Special class]

Potassium acetate TS Dissolve 10 g of potassium acetate in water to make 100 mL (1 mol/L).

Potassium aluminum sulfate See aluminum potassium sulfate dodecahydrate.

Potassium bicarbonate See potassium hydrogen carbonate.

Potassium biphthalate See potassium hydrogen phthalate.

Potassium biphthalate buffer solution, pH 3.5 See potassium hydrogen phthalate buffer solution, pH 3.5.

Potassium biphthalate buffer solution, pH 4.6 See potassium hydrogen phthalate buffer solution, pH 4.6.

Potassium biphthalate buffer solution, pH 5.6 See potassium hydrogen phthalate buffer solution, pH 5.6.

Potassium biphthalate for pH determination See potassium hydrogen phthalate for pH determination.

Potassium biphthalate (standard reagent) See potassium hydrogen phthalate (standard reagent).

0.2 mol/L Potassium biphthalate TS for buffer solution See 0.2 mol/L potassium hydrogen phthalate TS for buffer solution.

Potassium bisulfate See potassium hydrogen sulfate.

Potassium bromate KBrO₃ [K 8530, Special class]

Potassium bromide KBr [K 8506, Special class]

Potassium bromide for infrared spectrophotometry Crush homocrystals of potassium bromide or potassium bromide, collect a powder passed through a No. 200 (75 μm) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum <2.25>: any abnormal absorption does not appear.

Potassium carbonate K₂CO₃ [K 8615, Special class]

Potassium carbonate, anhydrous See potassium carbonate.

Potassium carbonate-sodium carbonate TS Dissolve 1.7 g of potassium carbonate and 1.3 g of anhydrous sodium carbonate in water to make 100 mL.

Potassium chlorate KClO₃ [K 8207, Special class]

Potassium chloride KCl [K 8121, Special class]

Potassium chloride for conductivity measurement [K 8121, Potassium chloride for conductivity measurement]

Potassium chloride for infrared spectrophotometry Crush homocrystals of potassium chloride or potassium chloride (Special class), collect the powder passed through a No. 200 (75 μm) sieve, and dry at 120°C for 10 hours or at 500°C for 5 hours. Prepare tablets with this powder, and determine the infrared absorption spectrum <2.25>: any abnormal absorption does not appear.

Potassium chloride-hydrochloric acid buffer solution To 250 mL of a solution of potassium chloride (3 in 20) add 53 mL of 2 mol/L hydrochloric acid TS and water to make 1000 mL.

Potassium chloride TS, acidic Dissolve 250 g of potassium chloride in water to make 1000 mL, and add 8.5 mL of hydrochloric acid.

0.2 mol/L Potassium chloride TS Dissolve 14.9 g of potassium chloride in water to make 1000 mL. Prepare before use.

Potassium chromate K₂CrO₄ [K 8312, Special class]

Potassium chromate TS Dissolve 10 g of potassium chromate in water to make 100 mL.

Potassium cyanide KCN [K 8443, Special class]

Potassium cyanide TS Dissolve 1 g of potassium cyanide in water to make 10 mL. Prepare before use.

Potassium dichromate K₂Cr₂O₇ [K 8517, Special class]

Potassium dichromate (standard reagent) K₂Cr₂O₇ [K 8005, Standard reagent for volumetric analysis]

Potassium dichromate-sulfuric acid TS Dissolve 0.5 g of potassium dichromate in diluted sulfuric acid (1 in 5) to make 100 mL.

Potassium dichromate TS Dissolve 7.5 g of potassium dichromate in water to make 100 mL.

Potassium dihydrogen phosphate KH₂PO₄ [K 9007, Special class]

Potassium dihydrogen phosphate for pH determination KH₂PO₄ [K 9007, for pH determination]

0.02 mol/L Potassium dihydrogen phosphate TS Dissolve 2.72 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.05 mol/L Potassium dihydrogen phosphate TS Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.1 mol/L Potassium dihydrogen phosphate TS, pH 2.0 Dissolve 13.6 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 2.0 with phosphoric acid.

0.25 mol/L Potassium dihydrogen phosphate TS, pH 3.5 Dissolve 34 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 3.5 with phosphoric acid, and add water to make 1000 mL.

0.33 mol/L Potassium dihydrogen phosphate TS Dissolve 4.491 g of potassium dihydrogen phosphate in water to make 100 mL.

0.05 mol/L Potassium dihydrogen phosphate, pH 3.0 Adjust the pH of 0.05 mol/L potassium dihydrogen

phosphate TS to 3.0 with phosphoric acid.

0.05 mol/L Potassium dihydrogen phosphate TS, pH 4.7 Dissolve 6.80 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to exactly 4.7 with dilute sodium hydrochloride TS, and add water to make 1000 mL.

0.1 mol/L Potassium dihydrogen phosphate TS Dissolve 13.61 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.2 mol/L Potassium dihydrogen phosphate TS Dissolve 27.22 g of potassium dihydrogen phosphate in water to make 1000 mL.

0.2 mol/L Potassium dihydrogen phosphate TS for buffer solution Dissolve 27.218 g of potassium dihydrogen phosphate for pH determination in water to make 1000 mL.

Potassium disulfate $K_2S_2O_7$ [K 8783, Potassium Disulfate, Special class]

Potassium ferricyanide See potassium hexacyanoferrate (III).

Potassium ferricyanide TS See potassium hexacyanoferrate (III) TS.

Potassium ferricyanide TS, alkaline See potassium hexacyanoferrate (III) TS, alkaline.

Potassium ferrocyanide See potassium hexacyanoferrate (II) trihydrate.

Potassium ferrocyanide TS See potassium hexacyanoferrate (II) TS.

Potassium guaiacolsulfonate $C_7H_7KO_5S$ [Same as the namesake monograph]

Potassium hexacyanoferrate (II) trihydrate $K_4Fe(CN)_6 \cdot 3H_2O$ [K 8802, Special class]

Potassium hexacyanoferrate (II) TS Dissolve 1 g of potassium hexacyanoferrate (II) trihydrate in water to make 10 mL ($\frac{1}{4}$ mol/L). Prepare before use.

Potassium hexacyanoferrate (III) $K_3Fe(CN)_6$ [K 8801, Special class]

Potassium hexacyanoferrate (III) TS Dissolve 1 g of potassium hexacyanoferrate (III) in water to make 10 mL ($\frac{1}{3}$ mol/L). Prepare before use.

Potassium hexacyanoferrate (III) TS, alkaline Dissolve 1.65 g of potassium hexacyanoferrate (III) and 10.6 g of anhydrous sodium carbonate in water to make 100 mL. Preserve in light-resistant containers.

Potassium hexahydroxoantimonate (V) $K_2H_2Sb_2O_7 \cdot 4H_2O$ White granules or crystalline powder.

Identification—To 1 g add 100 mL of water, and dissolve by warming. To 20 mL of this solution add 0.2 mL of sodium chloride TS: white precipitates appear. Rubbing the inside wall of the vessel with a glass rod accelerates the forming of the precipitates.

Potassium hexahydroxoantimonate (V) TS To 2 g of potassium hexahydroxoantimonate (V) add 100 mL of water. Boil the solution for about 5 minutes, cool quickly, add 10 mL of a solution of potassium hydroxide (3 in 20), allow to stand for 1 day, and filter.

Potassiumhydrogencarbonate $KHCO_3$ [K 8621, Special class]

Potassium hydrogen phthalate $C_6H_4(COOK)(COOH)$ [K 8809, Special class]

Potassium hydrogen phthalate buffer solution, pH 3.5 Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 7.97 mL of 0.2 mol/L hydrochloric acid VS with water to make 200 mL.

Potassium hydrogen phthalate buffer solution, pH 4.6 Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 12.0 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

Potassium iodide TS, saturated Saturate 20 g of potassium iodide in 10 mL of freshly boiled and cooled water. Prepare before use.

0.3 mol/L Potassium hydrogen phthalate buffer solution, pH 4.6 Dissolve 61.26 g of potassium hydrogen phthalate in about 800 mL of water, adjust the pH to 4.6 with sodium hydroxide TS, and add water to make 1000 mL.

Potassium hydrogen phthalate buffer solution, pH 5.6 Dilute 50 mL of 0.2 mol/L potassium hydrogen phthalate TS for buffer solution and 39.7 mL of 0.2 mol/L sodium hydroxide VS with water to make 200 mL.

Potassium hydrogen phthalate for pH determination $C_6H_4(COOK)(COOH)$ [K 8809, For pH determination]

Potassium hydrogen phthalate (standard reagent) $C_6H_4(COOK)(COOH)$ [K 8005, Standard reagent for volumetric analysis]

0.2 mol/L Potassium hydrogen phthalate TS for buffer solution Dissolve 40.843 g of potassium hydrogen phthalate for pH determination in water to make 1000 mL.

Potassium hydrogen sulfate $KHSO_4$ [K 8972, Special class]

Potassium hydroxide KOH [K 8574, Special class]

Potassium hydroxide-ethanol TS Dissolve 10 g of potassium hydroxide in ethanol (95) to make 100 mL. Prepare before use.

0.1 mol/L Potassium hydroxide-ethanol TS To 1 mL of dilute potassium hydroxide-ethanol TS add ethanol (95) to make 5 mL. Prepare before use.

Potassium hydroxide-ethanol TS, dilute Dissolve 35 g of potassium hydroxide in 20 mL of water, and add ethanol (95) to make 1000 mL (0.5 mol/L). Preserve in tightly stoppered bottles.

Potassium hydroxide TS Dissolve 6.5 g of potassium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

0.02 mol/L Potassium hydroxide TS Dilute 2 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

0.05 mol/L Potassium hydroxide TS Dilute 5 mL of potassium hydroxide TS with water to make 100 mL. Prepare before use.

8 mol/L Potassium hydroxide TS Dissolve 52 g of

potassium hydroxide in water to make 100 mL. Preserve in polyethylene bottles.

Potassium iodate KIO_3 [K 8922, Special class]

Potassium iodate (standard reagent) KIO_3 [K 8005, Standard reagent for volumetric analysis]

Potassium iodide KI [K 8913, Special class]

Potassium iodide for assay [Same as the monograph Potassium Iodide]

Potassium iodide-starch TS Dissolve 0.5 g of potassium iodide in 100 mL of freshly prepared starch TS. Prepare before use.

Potassium iodide TS Dissolve 16.5 g of potassium iodide in water to make 100 mL. Preserve in light-resistant containers. Prepare before use (1 mol/L).

Potassium iodide TS, concentrated Dissolve 30 g of potassium iodide in 70 mL of water. Prepare before use.

Storage—Preserve in light-resistant containers.

Potassium iodide-zinc sulfate TS Dissolve 5 g of potassium iodide, 10 g of zinc sulfate, and 50 g of sodium chloride in water to make 200 mL.

Potassium methanesulfonate $\text{CH}_3\text{SO}_3\text{K}$ White crystals or crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of potassium methanesulfonate in 20 mL of water: the solution is transparent and colorless.

Content: not less than 98.0%. *Assay*—Dissolve about 0.1 g of potassium methanesulfonate, accurately weighed, in 10 mL of acetic acid (100), add 20 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 13.42 \text{ mg of } \text{CH}_3\text{SO}_3\text{K} \end{aligned}$$

Potassium naphthoquinone sulfonate See potassium 1,2-naphthoquinone-4-sulfonate.

Potassium 1,2-naphthoquinone-4-sulfonate $\text{C}_{10}\text{H}_6\text{O}_2\text{SO}_3\text{K}$ [K 8696, β -Naphthoquinone-4-sulfonic acid potassium salt, Special class]

Potassium 1,2-naphthoquinone-4-sulfonate TS Dissolve 0.5 g of potassium 1,2-naphthoquinone-4-sulfonate in water to make 100 mL. Prepare before use.

Potassium nitrate KNO_3 [K 8548, Special class]

Potassium nitrite KNO_2 [K 8017, Special class]

Potassium perchlorate KClO_4 [K 8226, Special class]

Potassium periodate KIO_4 [K 8249, Special class]

Potassium periodate TS To 2.8 g of potassium periodate add 200 mL of water, dissolve by adding dropwise 20 mL of sulfuric acid under shaking, cool, and add water to make 1000 mL.

Potassium permanganate KMnO_4 [K 8247, Special class]

Potassium permanganate TS Dissolve 3.3 g of potas-

sium permanganate in water to make 1000 mL (0.02 mol/L).

Potassium permanganate TS, acidic To 100 mL of potassium permanganate TS add 0.3 mL of sulfuric acid.

Potassium peroxodisulfate $\text{K}_2\text{S}_2\text{O}_8$ [K 8253, Special class]

Potassium persulfate See potassium peroxodisulfate.

Potassium pyroantimonate See potassium hexahydroxoantimonate (V).

Potassium pyroantimonate TS See potassium hexahydroxoantimonate (V) TS.

Potassium pyrophosphate $\text{K}_4\text{O}_7\text{P}_2$ White, crystalline powder, very soluble in water.

Melting point <2.60>: 1109°C

Potassium pyrosulfate See potassium disulfate.

Potassium sodium tartarate See potassium sodium tartarate tetrahydrate.

Potassium sodium tartarate tetrahydrate $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ [K 8536, (+)-Potassium sodium tartrate tetrahydrate, Special class]

Potassium sulfate K_2SO_4 [K 8962, Special class]

Potassium sulfate TS Dissolve 1 g of potassium sulfate in water to make 100 mL.

Potassium tartrate $2\text{C}_4\text{H}_4\text{K}_2\text{O}_6 \cdot \text{H}_2\text{O}$ [K 8535, Potassium Tartrate-Water (2/1), Special class]

Potassium tellurite K_2TeO_3 White powder or small masses obtained by melting an equimolar mixture of tellurium dioxide and potassium carbonate in a stream of carbon dioxide. Soluble in water.

Content: not less than 90.0%. *Assay*—Dissolve about 1.0 g of potassium tellurite, accurately weighed, in 100 mL of water, add 5 mL of diluted acetic acid (31) (1 in 3), and boil. After cooling, filter by suction through a crucible glass filter (1G4), previously dried at $105 \pm 2^\circ\text{C}$ for 1 hour to constant mass (b (g)). Wash the filtrate with water, dry the glass filter at 110°C for 3 hours, and measure the mass a (g).

$$\begin{aligned} \text{Content (\% of potassium tellurite (K}_2\text{TeO}_3)) \\ = \frac{(a-b) \times 1.5902}{S} \times 100 \end{aligned}$$

S: Mass (g) of potassium tellurite taken.

Potassium tetraoxalate for pH determination See potassium trihydrogen dioxalate dihydrate for pH determination.

Potassium thiocyanate KSCN [K 9001, Special class]

Potassium thiocyanate TS Dissolve 1 g of potassium thiocyanate in water to make 10 mL.

Potassium trihydrogen dioxalate dihydrate for pH determination $\text{KH}_3(\text{C}_2\text{O}_4)_2 \cdot 2\text{H}_2\text{O}$ [K 8474]

Potato extract Prepared for microbial test.

Potato starch [Same as the namesake monograph]

Potato starch TS Prepare as directed under starch TS with 1 g of potato starch.

Potato starch TS for amylolytic activity test Dry about 1 g of potato starch, accurately weighed, at 105°C for 2 hours, and measure the loss. Weigh accurately an amount of potato starch, equivalent to 1.000 g on the dried basis, place into a conical flask, add 20 mL of water, and make it pasty by gradually adding 5 mL of a solution of sodium hydroxide (2 in 25) while shaking well. Heat in a water bath for 3 minutes while shaking, add 25 mL of water, and cool. Neutralize exactly with 2 mol/L hydrochloric acid TS, add 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0, and add water to make exactly 100 mL. Prepare before use.

Powdered tragacanth [Same as the namesake monograph]

Prazepam for assay $C_{19}H_{17}ClN_2O$ [Same as the monograph Prazepam. When dried, it contains not less than 99.0% of $C_{19}H_{17}ClN_2O$.]

Prednisolone $C_{21}H_{28}O_5$ [Same as the namesake monograph]

Prednisone $C_{21}H_{26}O_5$ White, crystalline powder. Slightly soluble in methanol, in ethanol (95) and in chloroform, and very slightly soluble in water.

Optical rotation <2.49> $[\alpha]_D^{20}$: +167 – +175° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Loss on drying <2.41>: not more than 1.0% (1 g, 105°C, 3 hours).

Content: 96.0 – 104.0%. *Assay*—Weigh accurately about 20 mg of prednisone, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, dilute with methanol to make exactly 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and read the absorbance *A* at the wavelength of maximum absorption at about 238 nm.

$$\text{Amount (mg) of } C_{21}H_{26}O_5 = \frac{A}{440} \times 20,000$$

Prednisolone acetate $C_{23}H_{30}O_6$ [Same as the namesake monograph]

Probenecid $C_{13}H_{19}NO_4S$ [Same as the namesake monograph]

Procainamide hydrochloride $C_{13}H_{21}N_3O.HCl$ [Same as the namesake monograph]

Procainamide hydrochloride for assay $C_{13}H_{21}N_3O.HCl$ [Same as the monograph Procainamide Hydrochloride. When dried, it contains not less than 99.0% of procainamide hydrochloride ($C_8H_8N.HCl$).]

Procaine hydrochloride $C_{13}H_{20}N_2O_2.HCl$ [Same as the namesake monograph]

Procaine hydrochloride for assay [Same as the monograph Procaine Hydrochloride]

Procatrol hydrochloride $C_{16}H_{22}N_2O_3.HCl \cdot \frac{1}{2}H_2O$ [Same as the monograph Procain Hydrochloride Hydrate]

Progesterone $C_{21}H_{30}O_2$ [Same as the namesake monograph]

L-Proline $C_5H_9NO_2$ [K 9107, Special class]

n-Propanol See 1-propanol.

1-Propanol $CH_3CH_2CH_2OH$ [K 8838, Special class]

2-Propanol $(CH_3)_2CHOH$ [K 8839, Special class]

2-Propanol for vitamin A assay $(CH_3)_2CHOH$ [K 8839, Special class] When the absorbances at 300 nm and between 320 nm and 350 nm are determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control, they are not more than 0.05 and not more than 0.01, respectively. If necessary, purify by distillation.

2-Propanol for liquid chromatography $(CH_3)_2CHOH$ Clear, colorless and volatile liquid, having a characteristic odor. Miscible with water, with ethanol (95) and with diethyl ether. Boiling point: about 82°C.

Refractive index <2.45> n_D^{20} : 1.376 – 1.378

Specific gravity <2.56> d_{20}^{20} : 0.785 – 0.788

Purity (1) Ultraviolet absorbing substances—Perform the test with 2-propanol for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at 230 nm is not more than 0.2; at 250 nm, not more than 0.03; and between 280 nm and 400 nm, not more than 0.01.

(2) Peroxide—Mix 100 mL of water and 25 mL of dilute sulfuric acid, and add 25 mL of a solution of potassium iodide (1 in 10). Add this solution to 20 g of 2-propanol for liquid chromatography. Stopper tightly, shake, allow to stand for 15 minute in a dark place, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction (not more than 0.0005%).

Propanol, iso See 2-propanol.

Propranolol hydrochloride for assay $C_{16}H_{21}NO_2.HCl$ [Same as the monograph Propranolol Hydrochloride. When dried, it contains not less than 99.5% of propranolol hydrochloride ($C_{16}H_{21}NO_2.HCl$).]

Proprantheline bromide $C_{23}H_{30}BrNO_3$ [Same as the namesake monograph]

Propionic acid CH_3CH_2COOH Colorless liquid.

Purity—Clarity and color of solution—Dissolve 1 g of propionic acid in 20 mL of ethanol (95): the solution is clear and colorless.

Specific gravity <2.56> d_{20}^{20} : 0.998 – 1.004

Distilling range <2.57>: 139 – 143°C, not less than 95 vol%.

Propylamine, iso $(CH_3)_2CHNH_2$ Colorless liquid, having a characteristic, amine-like odor. Miscible with water, with ethanol (95) and with diethyl ether.

Refractive index <2.45> n_D^{20} : 1.374 – 1.376

Specific gravity <2.56> d_{20}^{20} : 0.685 – 0.690

Distilling range <2.57>: 31 – 33°C, not less than 95 vol%.

Propyl benzoate $C_6H_5COOC_3H_7$ Clear, colorless liquid, having a characteristic odor.

Refractive index <2.45> n_D^{20} : 1.498 – 1.503

Specific gravity <2.56> d_{20}^{20} : 1.022 – 1.027

Propylene carbonate $C_4H_6O_3$ Colorless liquid.

Boiling point <2.57>: 240 – 242°C

Water <2.48>: less than 0.1%

Propylene carbonate for water determination See Water Determination <2.48>.

Propylene glycol $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{OH}$ [K 8837, Special class]

Propylene glycol cefatrizine $\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2\cdot\text{C}_3\text{H}_8\text{O}_2$
[Same as the namesake monograph]

Propylether, iso $(\text{CH}_3)_2\text{CHOCH}(\text{CH}_3)_2$ Clear, colorless liquid, having a characteristic odor. Not miscible with water.
Refractive index $\langle 2.45 \rangle n_D^{20}$: 1.368 – 1.369
Specific gravity $\langle 2.56 \rangle d_4^{20}$: 0.723 – 0.725

Propyl parahydroxybenzoate $\text{HOC}_6\text{H}_4\text{COOCH}_2\text{CH}_2\text{CH}_3$
[Same as the namesake monograph]

Propylthiouracil for assay $\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$ [Same as the monograph Propylthiouracil. When dried, it contains not less than 99.0% of propylthiouracil ($\text{C}_7\text{H}_{10}\text{N}_2\text{OS}$).]

Prostaglandin A₁ $\text{C}_{20}\text{H}_{32}\text{O}_4$ White crystals or crystalline powder. Very soluble in ethanol (95) and in ethyl acetate, and very slightly soluble in water.

Purity Related substances—Dissolve 5 mg of prostaglandin A₁ in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography $\langle 2.01 \rangle$ according to the following operating conditions. Determine areas of all peaks of both solutions by the automatic integration method: the total area of the peaks other than the peak of prostaglandin A₁ from the sample solution is not larger than the peak area of prostaglandin A₁ from the standard solution.
Operating conditions

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed the operating conditions in the Assay of Alprostadil Alfadex.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of prostaglandin A₁ obtained from 10 μL of the standard solution is 5 to 10% of the full scale.

Time span of measurement: About twice as long as the retention time of prostaglandin A₁ beginning after the solvent peak.

Protein digestive enzyme TS A solution of lysyl endopeptidase in 0.05 mol/L tris buffer solution, pH 8.6 (1 in 5000).

Pseudoephedrine hydrochloride $\text{C}_{10}\text{H}_{15}\text{NO}\cdot\text{HCl}$ White, crystals or crystalline powder. Freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (99.5), and practically insoluble in acetic anhydride. Melting point: 182 – 186°C

Purity Related substances—Dissolve 1 mg in 10 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution for twice as long as the retention time of ephedrine as directed in the Assay (1) under Kakkonto Extract: the total area of the peaks other than pseudoephedrine and the solvent is not larger than 1/10 times the total area of the peaks other than the solvent.

Puerarin for thin-layer chromatography $\text{C}_{21}\text{H}_{20}\text{O}_9$ White crystalline powder. Freely soluble in methanol, and practically insoluble in diethyl ether. Melting point: about 188°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of puerarin

for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 2 μL of this solution as directed in the Identification under Pueraria Root: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Pullulanase An enzyme obtained from *Klebsiella pneumoniae*. White crystals. It contains not less than 30 units per mg. One unit is an enzymatic activity to produce 1 μmol of maltotriose from pullulan per minute at pH 5.0 and 30°C.

Pullulanase TS A solution of pullulanase containing 10 units per mL.

Purified hydrochloric acid See hydrochloric acid, purified.

Purified methanol See methanol, purified.

Purified sulfuric acid See sulfuric acid, purified.

Purified water [Same as the namesake monograph]

Purified water for ammonium limit test To 1500 mL of purified water add cautiously 4.5 mL of sulfuric acid, distill using a hard glass distiller, discard the first distillate, and use the remaining distillate as ammonium-free purified water.

Purity—Mix 40 mL of purified water for ammonium limit test with 6.0 mL of phenol-sodium pentacyanonitrosylferrate (III) TS. Add 4.0 mL of sodium hypochlorite-sodium hydroxide TS, mix, and allow to stand for 60 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using water as the blank: absorbance at the wavelength of 640 nm is not more than 0.010.

Pyrazole $\text{C}_3\text{H}_4\text{N}_2$ White to pale yellow crystals or crystalline powder.

Melting point $\langle 2.60 \rangle$: 67 – 71°C

Pyridine $\text{C}_5\text{H}_5\text{N}$ [K 8777, Special class]

Pyridine-acetic acid TS Dilute 20 mL of pyridine with sufficient diluted acetic acid (100) (1 in 25) to make 100 mL. Prepare before use.

Pyridine, dehydrated $\text{C}_5\text{H}_5\text{N}$ To 100 mL of pyridine add 10 g of sodium hydroxide, and allow to stand for 24 hours. Decant the supernatant liquid, and distill.

Pyridine for Karl Fischer method See Water Determination $\langle 2.48 \rangle$.

Pyridine-pyrazolone TS Dissolve, with thorough shaking, 0.1 g of 3-methyl-1-phenyl-5-pyrazolone in 100 mL of water by heating between 65°C and 70°C, and cool below 30°C. Mix this solution with a solution prepared by dissolving 0.02 g of bis-(1-phenyl-3-methyl-5-pyrazolone) in 20 mL of pyridine. Prepare before use.

Pyridoxine hydrochloride $\text{C}_8\text{H}_{11}\text{NO}_3\cdot\text{HCl}$ [Same as the namesake monograph]

1-(2-Pyridylazo)-2-naphthol $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}$ Orange-yellow or orange-red powder.

Absorbance—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in methanol to make exactly 100 mL. Pipet 2.0 mL of this solution, and add methanol to make exactly 50 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$, using methanol as the blank: absorbance at the wavelength of 470 nm is not less than 0.55.

Melting point <2.60>: 137 – 140°C

Purity Clarity and color of solution—Dissolve 25 mg of 1-(2-pyridylazo)-2-naphthol in 100 mL of methanol: the solution is clear and orange-yellow.

Residue on ignition <2.44>: not more than 1.0%.

Sensitivity—On adding 50 mL of water, 30 mL of methanol and 10 mL of acetic acid-sodium acetate buffer solution, pH 5.5, to 0.2 mL of a solution of 1-(2-pyridylazo)-2-naphthol in methanol (1 in 4000), the solution is yellow in color. Add 1 drop of a solution of copper (II) chloride dihydrate (1 in 600) to this solution: the solution is red-purple in color. Add a subsequent 1 drop of diluted 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (1 in 10): the color of the solution changes to yellow again.

1-(4-Pyridyl)pyridinium chloride hydrochloride

$C_{10}H_9ClN_2 \cdot HCl$ White to yellowish white, crystalline powder. Very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point <2.60>: 154 – 156°C

Pyrogallol $C_6H_3(OH)_3$ [K 8780, Special class]

L-Pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride $C_{19}H_{26}N_8O_6 \cdot HCl$ White to light powder. Freely soluble in water, in methanol and in acetic acid (100).

Absorbance <2.24> $E_{1\%}^{1\text{cm}}$ (316 nm): 242 – 268 (2 mg, water, 100 mL).

Optical rotation <2.49> $[\alpha]_D^{25}$: –51 – –56° [0.1 g, diluted acetic acid (100) (1 in 2), 10 mL, 100 mm].

Purity Related substances—Dissolve 0.05 g of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (15:12:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

L-Pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS Dissolve 25 mg of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride and 0.04 g of D-Mannitol in 2 to 3 mL of water, lyophilize, and add 16.7 mL of water to dissolve. To 1 volume of this solution add 9 volumes of water before use.

Pyrole C_4H_5N Clear, colorless liquid, having a characteristic odor. Soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Specific gravity <2.56> d_{20}^{20} : 0.965 – 0.975

Pyrophosphate buffer solution, pH 9.0 Dissolve 3.3 g of potassium pyrophosphate, 15 mg of dithiothreitol and 40 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 70 mL of water, adjust the pH with a solution of citric acid monohydrate (21 in 100) to exactly 9.0, and add water to make 100 mL.

0.05 mol/L Pyrophosphate buffer solution, pH 9.0

Dissolve 0.83 g of potassium pyrophosphate in 40 mL of water, adjust the pH with 1 mol/L hydrochloric acid VS to 9.0, and add water to make 50 mL. Adjust the temperature to 22 \pm 2°C before use.

Quinhydrone $C_6H_4(OH)_2 \cdot C_6H_4O_2$ Green crystals or crystalline powder.

Melting point <2.60>: 169 – 172°C

Quinidine sulfate $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ [Same as the monograph Quinidine Sulfate Hydrate]

Quinine sulfate $(C_{20}H_{21}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$ [Same as the namesake monograph]

Quinoline C_9H_7N [K 8279, Special class]

Quinoline TS Mix 50 mL of quinoline with 300 mL of diluted hydrochloric acid (1 in 6), previously heated, cool, and filter if necessary.

8-Quinolinol C_9H_7NO [K 8775, Special class]

Raney nickel catalyst Grayish black powder. An alloy containing 40 to 50% of nickel and 50 to 60% of aluminum.

Ranitidinediamine $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$ White to pale yellow crystalline powder.

Identification—Determine the infrared absorption spectrum of ranitidinediamine as directed in the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2780 cm^{-1} , 1637 cm^{-1} , 1015 cm^{-1} and 788 cm^{-1} .

Content: not less than 95%. **Assay**—Weigh accurately about 0.1 g of ranitidinediamine, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple to green through blue (indicator: crystal violet TS). Perform the blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.62 mg of $(C_{10}H_{18}N_2OS)_2 \cdot C_4H_4O_4$

Reduced iron See iron powder.

Reference anti-interleukin-2 antibody for teceleukin Monoclonal antibody obtained from a fusion cell strain from mouse spleen cells sensitized to teceleukin and mouse melanoma cells, or alternately, rabbit antiserum towards human interleukin-2, that is purified using affinity chromatography. When determining the neutralizing activity, taking 1 neutralizing unit as the titer that neutralizes one unit of activity of teceleukin, contains at least 2000 neutralizing units per 1 mL.

Reinecke salt See reinecke salt monohydrate.

Reinecke salt monohydrate
 $NH_4[Cr(NH_3)_2(SCN)_4] \cdot H_2O$ [K 8926, Special class]

Reinecke salt TS To 20 mL of water add 0.5 g of Reinecke salt monohydrate, shake frequently for 1 hour, then filter. Use within 48 hours.

Resazurin $C_{12}H_6NNaO_4$ Brownish purple powder. It dissolves in water and the solution is purple in color.

Residue on ignition <2.44>: not less than 28.5% (1 g).

Resibufogenin for component determination

$C_{24}H_{32}O_4 \cdot xH_2O$ Odorless white crystalline powder.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (300 nm): 131 – 145 (0.01 g, methanol, 250 mL), dried in a desiccator (silica gel) for 24 hours.

Purity Related substances—Weigh accurately 0.04 g of resibufogenin for component determination and proceed as directed in the Purity under bufalin for component determination.

Content: not less than 98.0%. Component determination—Weigh accurately about 10 mg of resibufogenin for component determination, previously dried in a desiccator (silica gel) for 24 hours, add methanol to make exactly 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of this solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area by the automatic integration method, and calculate the amount of resibufogenin by the area percentage method.

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column about 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Selection of column: Dissolve 0.01 g each of bufalin for component determination, cinobufagin for component determination and resibufogenin for component determination in methanol to make 200 mL. Perform the test with 20 μL of this solution according to the above operating conditions, and calculate the resolution. Use a column giving elution of bufalin, cinobufagin and resibufogenin in this order, and clearly dividing each peak.

Detection sensitivity: Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of resibufogenin obtained from 20 μL of standard solution (2) can be measured by the automatic integration method and the peak height of resibufogenin from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About twice as long as the retention time of resibufogenin beginning after the peak of solvent.

Resibufogenin for thin-layer chromatography

$\text{C}_{24}\text{H}_{32}\text{O}_4 \cdot n\text{H}_2\text{O}$ White crystalline powder having no odor. It is freely soluble in acetone and in methanol.

Purity Related substances—Dissolve 5.0 mg of the substance to be tested in exactly 5 mL of acetone. Perform the test with 5 μL of this solution as directed in the Identification under Toad Venom: no other spots than the principal spot of around R_f 0.4 appear.

Resolving gel for celmoleukin Prepare the resolving gel in tris buffer solution, pH 8.8 using ammonium persulfate and TEMED so the concentrations of acrylamide and sodium lauryl sulfate are 13.5% and 0.1%, respectively.

Resorcin See resorcinol.

Resorcinol $\text{C}_6\text{H}_4(\text{OH})_2$ [K 9032, Special class]

Resorcinol sulfuric acid TS Dissolve 0.1 g of resorcinol in 10 mL of diluted sulfuric acid (1 in 10).

Resorcinol TS Dissolve 0.1 g of resorcinol in 10 mL of hydrochloric acid. Prepare before use.

Resorcin sulfuric acid TS See resorcinol sulfuric acid TS.

Resorcin TS See resorcinol TS.

L-Rhamnose monohydrate $\text{C}_6\text{H}_{12}\text{O} \cdot \text{H}_2\text{O}$ White crystalline powder having sweet taste. Freely soluble in water, and sparingly soluble in ethanol (95).

Optical rotation <2.49> $[\alpha]_D^{20}$: +7.8 – +8.3° (1 g, 20 mL of water, 2 drops of ammonia TS, 100 mm).

Melting point <2.60>: 87 – 91°C

Purity Related substances—Dissolve 1.0 mg of L-rhamnose monohydrate in 1 mL of water, and add methanol to make exactly 10 mL. Proceed with 20 μL of this solution as directed in the Identification (2) under Acacia: any spot other than the principal spot at the R_f value of about 0.5 does not appear.

Rhein for thin-layer chromatography $\text{C}_{15}\text{H}_8\text{O}_6$ A yellow powder. Very slightly soluble in acetone, and practically insoluble in water, in methanol, and in ethanol (99.5). Melting point: about 320°C (with decomposition).

Identification—Determine the absorption spectrum of a solution in methanol (3 in 500,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 228 nm and 232 nm, between 255 nm and 259 nm, and between 429 nm and 433 nm.

Purity Related substances—Dissolve 1.0 mg in 10 mL of acetone, and perform the test with 2 μL of this solution as directed in the Identification (1) under Daiokanzoto Extract: no spot other than the principal spot (R_f value is about 0.3) appears.

Rhynchophylline for component determination

$\text{C}_{22}\text{H}_{28}\text{N}_2\text{O}_4$ White, crystals or crystalline powder. Sparingly soluble in ethanol (99.5) and in acetone, and practically insoluble in water. Melting point: 205 – 209°C

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (245 nm): 473 – 502 (5 mg dried in a desiccator (silica gel) for 24 hours, a mixture of methanol and dilute acetic acid (7:3), 500 mL).

Purity Related substances—

(1) Dissolve 1.0 mg of rhynchophylline for component determination in 1 mL of acetone, and perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot of R_f about 0.5 does not appear.

(2) Dissolve 5 mg of rhynchophylline for component determination in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each

peak area obtained from these solutions by the automatic integration method: the sum of the peak areas except the areas of rhynchophylline and the solvent obtained from the sample solution is not more than the peak area of rhynchophylline from the standard solution.

Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Component determination under *Uncaria Thorn*.

Time span of measurement: About 4 times as long as the retention time of rhynchophylline beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 20 mL. Confirm that the peak area of rhynchophylline obtained from 20 μ L of this solution is equivalent to 3.5 to 6.5% of that from 20 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the operating conditions in the Component determination under *Uncaria Thorn*.

Riboflavin $C_{17}H_{20}N_4O_6$ [Same as the namesake monograph]

Riboflavin sodium phosphate $C_{17}H_{20}N_4NaO_9P$ [Same as the namesake monograph]

Ritodrine hydrochloride $C_{17}H_{21}NO_3 \cdot HCl$ [Same as the namesake monograph]

Rose Bengal See Microbial Limit Test for Crude Drugs <5.02>.

Rose Bengal TS See Microbial Limit Test for Crude Drugs <5.02>.

RPMI-1640 powdered medium Powder medium for cell culture containing 6 g of sodium chloride, 400 mg of potassium chloride, 800 mg of sodium dihydrogen phosphate, 100 mg of anhydrous calcium nitrate, 49 mg of anhydrous magnesium sulfate, 2 g of dextrose, 200 mg of L-arginine, 1 mg of glutathione, 50 mg of L-isoleucine, 15 mg of L-phenylalanine, 5 mg of L-tryptophan, 0.2 mg of biotin, 1 mg of nicotinamide, 1 mg thiamine hydrochloride, 300 mg of L-glutamine, 56.8 mg of L-asparagine, 10 mg of glycine, 50 mg of L-leucine, 20 mg of L-proline, 20 mg of L-tyrosine, 0.25 mg of D-calcium pantothenate, 5 μ g of cyanocobalamin, 1 mg of aminobenzoic acid, 20 mg of L-aspartic acid, 15 mg of L-histidine, 40 mg of L-lysine hydrochloride, 30 mg of L-serine, 20 mg of L-valine, 1 mg of folic acid, 1 mg of pyridoxine hydrochloride, 20 mg of L-glutamic acid, 20 mg of L-hydroxyproline, 15 mg of L-methionine, 20 mg of L-threonine, 3 mg of choline chloride, 35 mg of *i*-inositol, 0.2 mg of riboflavin, 59 mg of L-cystine, and 5 mg of phenol red.

Saccharated pepsin [Same as the namesake monograph]

Saikosaponin a for component determination Use saikosaponin a for thin-layer chromatography meeting the following additional specifications.

Purity Related substances—

(1) Dissolve 2.0 mg of saikosaponin a for component determination in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solu-

tion. Proceed the test with exactly 10 μ L each of the sample solution and standard solution as directed in the Purity (2) under *Bupleurum Root*: the spot other than the principal spot around *Rf* 0.4 is not larger and not more intense than the spot obtained with the standard solution.

(2) Dissolve 10 mg of saikosaponin a for component determination in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin a and the solvent is not more than the peak area of saikosaponin a obtained with the standard solution.

Operating conditions

Detector, and column: Proceed as directed in the operating conditions in the Component determination under *Bupleurum Root*.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin a is about 16 minutes.

Time span of measurement: About 6 times as long as the retention time of saikosaponin a beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin a obtained with 20 μ L of this solution is equivalent to 3.5 to 6.5% of that with 20 μ L of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin a for component determination and saikosaponin b₂ for component determination in methanol to make 100 mL. When the procedure is run with 20 μ L of the standard solution under the above operating conditions, saikosaponin a and saikosaponin b₂ are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin a is not more than 1.0%.

Saikosaponin a for thin-layer chromatography A white, crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 225 – 232°C (with decomposition).

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (206 nm): 60–68 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

Purity Related substances—Dissolve 1.0 mg of saikosaponin a for thin-layer chromatography in exactly 1 mL of methanol, and perform the test with 10 μ L of this solution as directed in the Identification (2) under *Bupleurum Root*: any spot other than the principal spot at the *Rf* value of about 0.4 does not appear.

Salicylaldazine $C_{14}H_{12}N_2O_2$ Dissolve 0.30 g of hydrazinium sulfate in 5 mL of water. To this solution add 1 mL of acetic acid (100) and 2 mL of a freshly prepared solution of salicylaldehyde in 2-propanol (1 in 5), shake well, and allow

to stand until a yellow precipitate is produced. Extract with two 15 mL portions of dichloromethane, to the combined dichloromethane extracts add 5 g of anhydrous sodium sulfate, shake, decant or filter, and evaporate the dichloromethane in the supernatant liquid or filtrate. Dissolve the residue in a warmed mixture of toluene and methanol (3:2), and cool. Filter the crystals produced, and dry in a desiccator (in vacuum, silica gel) for 24 hours. It is a yellow, crystalline powder.

Melting point <2.60>: 213 – 219°C

Purity—Dissolve 0.09 g of salicyldiazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and perform the test with this solution as directed in the Purity (6) under Povidone: any spot other than the principal spot does not appear.

Saikosaponin b₂ for component determination Saikosaponin b₂ for thin-layer chromatography. It meets the following requirements.

Purity Related substances—Dissolve 5 mg in 5 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peaks of saikosaponin b₂ and solvent is not larger than the peak area of saikosaponin b₂ obtained with the standard solution. Operating conditions

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay (1) under Saireito Extract.

Time span of measurement: About 6 times as long as the retention time of saikosaponin b₂.

System suitability

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of saikosaponin b₂ obtained with 10 μL of this solution is equivalent to 3.5 to 6.5% of that with 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay (1) under Saireito Extract.

Saikosaponin b₂ for thin-layer chromatography

C₄₂H₆₈O₁₃ White crystals or crystalline powder. Freely soluble in ethanol (99.5), soluble in methanol, and practically insoluble in water. Melting point: about 240°C

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (252 nm): 352 – 424 (5 mg, methanol, 250 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

Purity Related substances—Dissolve 2 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Proceed the test with 10 μL each of the sample solution and the standard solution as directed in the Identification (1) under Saireito Extract: the spot other than the principle spot of around Rf 0.3 is not more intense than the spot obtained with the standard solution.

Saikosaponin d for component determination A white,

crystalline powder or powder. Freely soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: about 240°C

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (206 nm): 63 – 71 (15 mg, methanol, 200 mL). Previously dried in a desiccator (in vacuum, silica gel) for 24 hours.

Purity Related substances—

(1) Dissolve 2.0 mg in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Proceed the test with 10 μL each of the sample solution and standard solution as directed in the Identification (2) under Bupleurum Root: the spot other than the principal spot around Rf 0.4 is not larger and not more intense than the spot obtained with the standard solution.

(2) Dissolve 10 mg in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than saikosaponin d and the solvent is not more than the peak area of saikosaponin d obtained with the standard solution.

Operating conditions

Detector, and column: Proceed as directed in the operating conditions in the Component determination under Bupleurum Root.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin d is about 13 minutes.

Time span of measurement: About 4 times as long as the retention time of saikosaponin d beginning after the solvent peak.

System suitability

Test for required detectability: Measure exactly 1 mL of the standard solution, and add methanol to make exactly 20 mL. Confirm that the peak area of saikosaponin d obtained with 20 μL of this solution is equivalent to 3.5 to 6.5% of that with 20 μL of the standard solution.

System performance: Dissolve 6 mg each of saikosaponin d for component determination and saikosaponin a for component determination in methanol to make 100 mL. When the procedure is run with 20 μL of the standard solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin d is not more than 1.0%.

Salicylaldehyde HOC₆H₄CHO [K 8390, Special class]

Salicylamide C₇H₇NO₂ White crystals or crystalline powder, and it is odorless and tasteless. Very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), soluble in propylene glycol, sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform. It dissolves in sodium hydroxide TS.

Melting point <2.60>: 139 – 143°C

Purity Ammonium <1.02>—Shake 1.0 g of salicylamide with 40 mL of water, and filter through filter paper previously washed well with water. Discard the first 10 mL of the filtrate, transfer the subsequent 20 mL to a Nessler tube, and add water to make 30 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: transfer 2.5 mL of Standard Ammonium Solution to a Nessler tube, and add water to make 30 mL.

Loss on drying <2.41>: not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <2.44>: not more than 0.1% (1 g).

Content: not less than 98.5%. **Assay**—Weigh accurately about 0.2 g of salicylamide, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution of 70 mL of *N,N*-dimethylformamide in 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS = 13.71 mg of $C_7H_7NO_2$

Salicylic acid HOC_6H_4COOH [K 8392, Special class]

Salicylic acid for assay HOC_6H_4COOH [K 8392, Special class]

Salicylic acid TS Dissolve 0.1 g of salicylic acid in 10 mL of sulfuric acid. Prepare before use.

Santonin $C_{15}H_{18}O_3$ [Same as the namesake monograph]

Santonin for assay [Same as the monograph Santonin. It contains not less than 99.0% of santonin ($C_{15}H_{18}O_3$).]

Schisandrin for thin-layer chromatography $C_{24}H_{32}O_7$ White crystals for crystalline powder. Freely soluble in methanol and diethyl ether, and practically insoluble in water.

Melting point <2.60>: 130 – 135°C

Purity Related substances—Dissolve 1.0 mg of schisandrin for thin-layer chromatography in exactly 1 mL of methanol. Perform the test with 5 μ L of this solution as directed in the Identification under Schisandra Fruit: any spot other than the principal spot at the *R_f* value of about 0.4 does not appear.

Scopolamine hydrobromide $C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$ [Same as the monograph Scopolamine Hydrobromide Hydrate]

Scopolamine hydrobromide for thin-layer chromatography [Same as the monograph Scopolamine Hydrobromide Hydrate. Proceed as directed in the Identification (3) under Opium Alkaloids and Atropine Injection: any spot other than the principal spot at the *R_f* value of about 0.7 does not appear.

Sea sand [K 8222, Special class]

2nd Fluid for disintegration test To 250 mL of 0.2 mol/L potassium dihydrogen phosphate TS add 118 mL of 0.2 mol/L sodium hydroxide TS and water to make 1000 mL. It is clear and colorless, and has a pH about 6.8.

2nd Fluid for dissolution test A mixture of phosphate buffer solution, pH 6.8 and water (1:1).

Selenious acid H_2SeO_3 [K 8035, Special class]

Selenious acid-sulfuric acid TS Dissolve 0.05 g of selenious acid in 10 mL of sulfuric acid.

Selenium Se [K 8598, Special class]

Selenium dioxide SeO_2 [K 8706, Special class]

Semicarbazide acetate TS Place 2.5 g of semicarbazide hydrochloride, 2.5 g of anhydrous sodium acetate and 30 mL of methanol in a flask, heat on a water bath for 2 hours, cool to 20°C, and filter. To the filtrate add methanol to make 100 mL. Preserve in a cold place. Do not use the solution showing a yellow color.

Semicarbazide hydrochloride $H_2NNHCONH_2 \cdot HCl$ White to light yellow crystals.

Identification—(1) To 10 mL of a solution (1 in 100) add 1 mL of silver nitrate TS: white precipitates appear.

(2) Determine the infrared absorption spectrum as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3420 cm^{-1} , 3260 cm^{-1} , 2670 cm^{-1} , 1684 cm^{-1} , 1582 cm^{-1} , 1474 cm^{-1} , 1386 cm^{-1} , 1210 cm^{-1} , 1181 cm^{-1} , 770 cm^{-1} and 719 cm^{-1} .

Sennoside A for component determination Use sennoside A for thin-layer chromatography meeting the following additional specifications.

Absorbance <2.24> $E_{1\%}^{1\text{cm}}$ (270 nm): 211 – 226 [10 mg dried in a desiccator (in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide) for not less than 12 hours, diluted sodium bicarbonate solution (1 in 100), 500 mL]

Purity Related substances—Dissolve 5.0 mg of sennoside A for component determination in 50 mL of the mobile phase and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μ L each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than sennoside A obtained from the sample solution is not larger than peak area of sennoside A from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Senna Leaf except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of sennoside A obtained from 10 μ L of the standard solution (2) can be measured by the automatic integration method, and the peak height of sennoside A from 10 μ L of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of sennoside A beginning after the peak of solvent.

Sennoside A for thin-layer chromatography $C_{42}H_{38}O_{20}$ Yellow crystalline powder. Insoluble in water, in chloroform and in diethyl ether, and practically insoluble in methanol and in acetone. Melting point: 200 – 240°C (with decomposition).

Purity Related substances—Dissolve 1.0 mg of sennoside A for thin-layer chromatography in exactly 4 mL of a mixture of tetrahydrofuran and water (7:3), and perform the test with 80 μL of this solution as directed in the identification under Rhubarb: any spot other than the principal spot at the *Rf* value of about 0.3 does not appear.

Sennoside B for component determination $\text{C}_{42}\text{H}_{38}\text{O}_{20}$ Yellow crystalline powder. Insoluble in water and in diethyl ether, and practically insoluble in methanol and in acetone. Melting point: 180 – 186°C (with decomposition).

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (270 nm): 210 – 225 [10 mg dried in a desiccator (in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide) for not less than 12 hours, diluted sodium bicarbonate solution (1 in 100), 500 mL]

Purity Related substances—(1) Dissolve 1.0 mg of Sennoside B for component determination in exactly 4 mL of a mixture of tetrahydrofuran and water (7:3), and perform the test as directed under Thin-layer Chromatography <2.03> with this solution. Spot 80 μL of this solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and formic acid (7:7:4:2) to a distance of about 15 cm, and air-dry the plate. Examine under the ultraviolet light (main wavelength: 365 nm): any spot other than the principal spot as the *Rf* value of about 0.5 does not appear.

(2) Dissolve 5.0 mg of sennoside B for component determination in 50 mL of the mobile phase and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution (1). Perform the test with exactly 10 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area from these solutions by the automatic integration method: the total peak area other than sennoside B obtained from the sample solution is not larger than peak area of sennoside B from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Senna Leaf except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 20 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of sennoside B obtained from 10 μL of the standard solution (2) can be measured by the automatic integration method, and the peak height of sennoside B from 10 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 4 times as long as the retention time of sennoside B beginning after the peak of solvent.

L-Serine $\text{C}_3\text{H}_7\text{NO}_3$ [K 9105, Special class]

Sesame oil [Same as the namesake monograph]

[6]-Shogaol for thin-layer chromatography $\text{C}_{17}\text{H}_{24}\text{O}_3$ A pale yellow oil. Miscible with methanol, ethanol (99.5) and with diethyl ether, and practically insoluble in water.

Purity Related substances—Dissolve 1.0 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL on a plate of

silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: no spot other than the principal spot at around *Rf* 0.5 appears.

Silica gel An amorphous, partly hydrated silicic acid occurring in glassy granules of various sizes. When used as a desiccant, it is frequently coated with a substance that changes color when the capacity to absorb water is exhausted. Such colored products may be regenerated by being heated at 110°C until the gel assumes the original color.

Loss on ignition <2.43>: not more than 6% (2 g, 950 \pm 50°C).

Water absorption: not less than 31%. Weigh accurately about 10 g of silica gel, and allow to stand for 24 hours in a closed container in which the atmosphere is maintained at 80% relative humidity with sulfuric acid having a specific gravity of 1.19. Weigh again, and calculate the increase in mass.

Siliceous earth [K 8330, Diatomaceous earth, First class]

Silicone oil Colorless clear liquid, having no odor.

Viscosity <2.53>: 50 – 100 mm^2/s .

Silicone resin Light gray, half-clear, viscous liquid or a pasty material. It is almost odorless.

Viscosity and refractive index—Place 15 g of silicone resin in a Soxhlet extractor, then extract with 150 mL of carbon tetrachloride for 3 hours. The kinematic viscosity of the residual liquid, obtained by evaporating carbon tetrachloride from the extract on a water bath, is 100 to 1100 mm^2/s (25°C). Its refractive index is 1.400 to 1.410 (25°C).

Specific gravity <2.56> *d*: 0.98 – 1.02

Loss on drying <2.41>: 0.45 – 2.25 g with the extracted residue obtained in the Viscosity and refractive index (100°C, 1 hour).

Silicotungstic acid 26-water $\text{SiO}_2 \cdot 12\text{WO}_3 \cdot 26\text{H}_2\text{O}$

White to slightly yellowish, crystals. Deliquescent. Very soluble in water and in ethanol (95).

Loss on ignition <2.43>: 14 – 15% (2 g, dry at 110°C for 2 hours then 700 – 750°C, constant mass).

Clarity and color of solution: a solution (1 in 20) is clear and colorless.

Silver chromate-saturated potassium chromate TS Dissolve 5 g of potassium chromate in 50 mL of water, add silver nitrate TS until a pale red precipitate is produced, and filter. To the filtrate add water to make 100 mL.

Silver diethyldithiocarbamate See silver *N, N*-diethyldithiocarbamate.

Silver nitrate AgNO_3 [K 8550, Special class]

Silver nitrate-ammonia TS Dissolve 1 g of silver nitrate in 20 mL of water, and add ammonia TS dropwise with stirring until the precipitate is almost entirely dissolved.

Storage—Preserve in tight, light-resistant containers.

Silver nitrate TS Dissolve 17.5 g of silver nitrate in water to make 1000 mL (0.1 mol/L). Preserve in light-resistant containers.

Silver *N, N*-diethyldithiocarbamate $\text{C}_5\text{H}_{10}\text{AgNS}_2$

[K 9512]

Soda lime [K 8603, First class]**Sodium acetate** See sodium acetate trihydrate.**Sodium acetate-acetone TS** Dissolve 8.15 g of sodium acetate trihydrate and 42 g of sodium chloride in 100 mL of water, and add 68 mL of 0.1 mol/L hydrochloric acid VS, 150 mL of acetone and water to make 500 mL.**Sodium acetate, anhydrous** CH_3COONa [K 8372, Special class]**Sodium acetate trihydrate** $\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$ [K 8371, Special class]**Sodium acetate TS** Dissolve 13.6 g of sodium acetate trihydrate in water to make 100 mL (1 mol/L).**Sodium benzoate for assay** [Same as the monograph Sodium Benzoate]**Sodium bicarbonate** See sodium hydrogen carbonate.**Sodium bicarbonate for pH determination** See sodium hydrogen carbonate for pH determination.**Sodium bicarbonate TS** See sodium hydrogen carbonate TS.**7% Sodium bicarbonate injection** [Same as the monograph Sodium Bicarbonate Injection. However, labeled amount should be 7 w/v%].**Sodium bismuthate** See bismuth sodium trioxide.**Sodium bisulfite** See sodium hydrogen sulfite.**Sodium bisulfite TS** See sodium hydrogen sulfite TS.**Sodium bitartrate** See sodium hydrogen tartrate monohydrate.**Sodium bitartrate TS** See sodium hydrogen tartrate TS.**Sodium borate for pH determination** See sodium tetraborate for pH determination.**Sodium borate decahydrate** See sodium tetraborate decahydrate.**Sodium borohydride** NaBH_4 White to grayish white, crystals, powder or masses. Freely soluble in water.**Content:** not less than 95%. **Assay**—Weigh accurately 0.25 g of sodium borohydride, dissolve in 20 mL of diluted sodium hydroxide TS (3 in 10), and add water to make exactly 500 mL. Pipet 20 mL of this solution, put in a glass-stoppered iodine flask, and cool in ice. Add exactly 40 mL of iodine TS, allow to stand at a dark place for 10 minutes, add exactly 10 mL of diluted sulfuric acid (1 in 6), and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (back titration) (indicator: starch solution). Perform a blank determination in the same manner, and make any necessary correction.Each mL of 0.1 mol/L sodium thiosulfate VS
= 0.4729 mg of NaBH_4 **Sodium bromide** NaBr [K 8514, Special class]**Sodium carbonate** See sodium carbonate decahydrate.**Sodium carbonate, anhydrous** Na_2CO_3 [K 8625, Sodium carbonate, Special class]**Sodium carbonate decahydrate** $\text{Na}_2\text{CO}_3\cdot 10\text{H}_2\text{O}$ [K 8624, Special class]**Sodium carbonate for pH determination** Na_2CO_3 [K 8625, for pH determination]**Sodium carbonate (standard reagent)** Na_2CO_3 [K 8005, Standard reagent for volumetric analysis]**Sodium carbonate TS** Dissolve 10.5 g of anhydrous sodium carbonate in water to make 100 mL (1 mol/L).**0.55 mol/L Sodium carbonate TS** Dissolve 5.83 g of anhydrous sodium carbonate in water to make 100 mL.**Sodium chloride** NaCl [K 8150, Special class]**Sodium chloride (standard reagent)** NaCl [K 8005, Standard reagent for volumetric analysis]**Sodium chloride TS** Dissolve 10 g of sodium chloride in water to make 100 mL.**0.1 mol/L Sodium chloride TS** Dissolve 6 g of sodium chloride in water to make 1000 mL.**1 mol/L Sodium chloride TS** Dissolve 29.22 g of sodium chloride in water to make 500 mL.**Sodium citrate** See trisodium citrate dihydrate.**Sodium cobaltinitrite** See sodium hexanitrocobaltate (III).**Sodium cobaltinitrite TS** See sodium hexanitrocobaltate (III) TS.**Sodium 1-decanesulfonate** $\text{C}_{10}\text{H}_{21}\text{NaO}_3\text{S}$ A white powder.**Purity** Clarity and color of solution—Dissolve 1.0 g in 20 mL of water: the solution is clear and colorless.**Loss on drying** <2.41>: not more than 3.0% (1 g, 105°C, 3 hours).**Content:** not less than 98.0%. **Assay**—Weigh accurately about 0.45 g of sodium 1-decanesulfonate, dissolve in 50 mL of water, and pass through a column, about 1.2 cm in inside diameter and about 25 cm in length, packed with about 20 mL of strongly acidic ion-exchange resin (0.3 to 1.0 mm, H type) at a flow rate of about 4 mL per minute. Wash with 150 mL of water at a flow rate of about 4 mL per minute. Combine the washing and the elute, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $\text{C}_{10}\text{H}_{21}\text{NaO}_3\text{S}$ **0.0375 mol/L Sodium 1-decanesulfonate TS** Dissolve 3.665 g of sodium 1-decanesulfonate in 400 mL of water.**Sodium desoxycholate** $\text{C}_{24}\text{H}_{39}\text{NaO}_4$ White, odorless, crystalline powder.**Identification**—Determine the infrared absorption spectrum of sodium desoxycholate, previously dried, according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3400 cm^{-1} , 2940 cm^{-1} , 1562 cm^{-1} and 1408 cm^{-1} .**Purity** Related substances—Dissolve 0.10 g of sodium

desoxycholate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol and acetic acid (100) (80:40:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly concentrated sulfuric acid on the plate, and heat at 105°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Sodium diethyldithiocarbamate See sodium *N,N*-diethyldithiocarbamate trihydrate.

Sodium *N,N*-diethyldithiocarbamate trihydrate (C₂H₅)₂NCS₂Na·3H₂O [K 8454, Special class]

Sodium dihydrogen phosphate NaH₂PO₄ A white, powder or crystalline powder. Freely soluble in water, and very slightly soluble in ethanol (99.5). It has a hygroscopic property.

A solution is acidic.

Sodium dihydrogen phosphate anhydrous See sodium dihydrogen phosphate.

2 mol/L Sodium dihydrogen phosphate TS Dissolve 312.02 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium dihydrogen phosphate TS, pH 2.5 Dissolve 2.7 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid.

Sodium dihydrogen phosphate dihydrate NaH₂PO₄·2H₂O [K 9009, Special class]

0.05 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

0.1 mol/L Sodium dihydrogen phosphate TS Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, adjust to a pH of 5.8 exactly with sodium hydroxide TS, and add water to make 500 mL.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 2.6 Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to exactly 2.6 and add water to make 1000 mL.

0.05 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 3.45 g of sodium dihydrogen phosphate dihydrate in 500 mL of water (solution A). Dilute 2.45 g of phosphoric acid with water to make 500 mL (solution B). To a volume of solution A add solution B until the mixture is adjusted to pH 3.0.

0.1 mol/L Sodium dihydrogen phosphate TS, pH 3.0 Dissolve 15.60 g of sodium dihydrogen phosphate dihydrate in 900 mL of water, adjust the pH to 3.0 with phosphoric acid, and add water to make 1000 mL.

Sodium disulfite Na₂S₂O₅ [K 8501, First class]

Sodium disulfite TS Dissolve 0.10 g of sodium disulfite in 10 mL of 1 mol/L hydrochloric acid TS, and add acetone to

make 100 mL.

Sodium dodecylbenzene sulfonate C₁₈H₂₉SO₃Na White, crystalline powder or mass.

pH <2.54>—The pH of a solution of 0.5 g of sodium dodecylbenzene sulfonate in 50 mL of freshly boiled and cooled water is between 5.0 and 7.0. Measure the pH at 25°C passing nitrogen with stirring.

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 2 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 40 mg of sodium dodecylbenzene sulfonate, previously dried, and perform the test as directed in (4) Sulfur in the Procedure of determination under Oxygen Flask Combustion Method <1.06>, using a mixture of 20 mL of water and 2 mL of strong hydrogen peroxide water as absorbing solution.

Each mL of 0.01 mol/L barium perchlorate VS
= 1.742 mg of C₁₈H₂₉SO₃Na

Sodium fluoride NaF [K 8821, Special class]

Sodium fluoride (standard reagent) NaF [K 8005, Standard reagent for volumetric analysis]

Sodium fluoride TS Dissolve 0.5 g of sodium fluoride in 100 mL of 0.1 mol/L hydrochloric acid TS. Prepare before use.

Sodium 1-heptane sulfonate C₇H₁₅NaO₃S White, crystals or crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of sodium 1-heptane sulfonate in 10 mL of water: the solution is clear and colorless.

Loss on drying <2.41>: not more than 3.0% (1 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Dissolve about 0.4 g of sodium 1-heptane sulfonate, previously dried and weighed accurately, in 50 mL of water, transfer to a chromatographic column, prepared by packing a chromatographic tube 9 mm in inside diameter and 160 mm in height with 10 mL of strongly acidic ion exchange resin for column chromatography (425 to 600 μ m in particle diameter, H type), and flow at a flow rate of about 4 mL per minute. Wash the column at the same flow rate with 150 mL of water, combine the washings with the effluent solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the color of the solution changes from yellow to blue.

Each mL of 0.1 mol/L sodium hydroxide VS
= 20.23 mg of C₇H₁₅NaO₃S

Sodium 1-hexane sulfonate C₆H₁₃NaO₃S White, crystals or crystalline powder.

Loss on drying <2.41>: not more than 3.0% (1 g, 105°C, 2 hours).

Content: not less than 98.0%. Assay—Weigh accurately about 0.4 g of sodium 1-hexane sulfonate, previously dried, and dissolve in 25 mL of water. Transfer 15–20 mL of this solution into a chromatographic column about 11 mm in diameter and about 500 mm in height of strongly acidic ion exchange resin for column chromatography (246 μ m to 833 μ m in particle diameter, type H), and elute at the rate of about 5–10 mL per minute, then wash the column with five 50-mL portions of water at the rate of about 5–10 mL per minute.

Combine the washings to the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.82 mg of $C_6H_{13}NaO_3S$

Sodium hexanitrocobaltate (III) $Na_3Co(NO_2)_6$
[K 8347, Special class]

Sodium hexanitrocobaltate (III) TS Dissolve 10 g of sodium hexanitrocobaltate (III) in water to make 50 mL, and filter if necessary. Prepare before use.

Sodium hydrogen carbonate $NaHCO_3$ [K 8622, Special class]

Sodium hydrogen carbonate for pH determination
 $NaHCO_3$ [K 8622, Sodium hydrogen carbonate, for pH determination]

Sodium hydrogen carbonate TS Dissolve 5.0 g of sodium hydrogen carbonate in water to make 100 mL.

Sodium hydrogen sulfite [K 8059, Special class]

Sodium hydrogen sulfite TS Dissolve 10 g of sodium hydrogen sulfite in water to make 30 mL. Prepare before use.

Sodium hydrogen tartrate monohydrate
 $NaHC_4H_4O_6 \cdot H_2O$ [K 8538, Sodium hydrogentartrate monohydrate, Special class]

Sodium hydrogen tartrate TS Dissolve 1 g of sodium bitartrate in water to make 10 mL (0.5 mol/L). Prepare before use.

Sodium hydrosulfite $Na_2S_2O_4$ [K 8737, Sodium dithionite, Special class]

Sodium hydroxide $NaOH$ [K 8576, Special class]

Sodium hydroxide-dioxane TS Dissolve 0.80 g of sodium hydroxide in a mixture of 1,4-dioxane and water (3:1) to make 100 mL.

Sodium hydroxide-methanol TS Dissolve by thorough shaking 4 g of sodium hydroxide in methanol to make 100 mL. To the supernatant liquid obtained by centrifugation add methanol to make 500 mL. Prepare before use.

Sodium hydroxide TS Dissolve 4.3 g of sodium hydroxide in water to make 100 mL (1 mol/L). Preserve in polyethylene bottles.

Sodium hydroxide TS, dilute Dissolve 4.3 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use (0.1 mol/L).

0.01 mol/L Sodium hydroxide TS Dilute 10 mL of sodium hydroxide TS with water to make 1000 mL. Prepare before use.

0.05 mol/L Sodium hydroxide TS To 10 mL of 0.5 mol/L sodium hydroxide TS add water to make 100 mL.

0.2 mol/L Sodium hydroxide TS Dissolve 8.0 g of sodium hydroxide in freshly boiled and cooled water to make 1000 mL. Prepare before use.

0.5 mol/L Sodium hydroxide TS Dissolve 22 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

2 mol/L Sodium hydroxide TS Dissolve 86 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

4 mol/L Sodium hydroxide TS Dissolve 168 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

6 mol/L Sodium hydroxide TS Dissolve 252 g of sodium hydroxide in water to make 1000 mL. Preserve in a polyethylene bottle.

8 mol/L Sodium hydroxide TS Dissolve 336 g of sodium hydroxide in water to make 1000 mL. Preserve in polyethylene bottles.

Sodium hypobromite TS To 8 mL of bromine TS add 25 mL of water and 25 mL of sodium carbonate TS. Prepare before use.

Sodium hypochlorite-sodium hydroxide TS To a volume of sodium hypochlorite TS for ammonium limit test, equivalent to 1.05 g of sodium hypochlorite ($NaClO$: 74.44), add 15 g of sodium hydroxide and water to make 1000 mL. Prepare before use.

Sodium hypochlorite TS Prepare the solution by passing chlorine into sodium hydroxide TS while cooling with ice, so as to contain 5% of sodium hypochlorite ($NaClO$: 74.44). Prepare before use.

Sodium hypochlorite TS for ammonium limit test Clear, colorless or pale green-yellow solution prepared by passing chlorine into sodium hydroxide or sodium carbonate solution, having the odor of chlorine.

Content: not less than 4.2 w/v% as sodium hypochlorite ($NaClO$: 74.44). Assay—Pipet 10 mL of sodium hypochlorite TS for ammonium limit test, and add water to make exactly 100 mL. Transfer exactly 10 mL of this solution to a glass-stoppered flask, add 90 mL of water, then add 2 g of potassium iodide and 6 mL of diluted acetic acid (1 in 2), stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.722 mg of $NaClO$.

Sodium iopodate for assay $C_{12}H_{12}I_3N_2NaO_2$ [Same as the monograph Sodium Iopodate. It contains not less than 99.0% of $C_{12}H_{12}I_3N_2NaO_2$, calculated on the dried basis.

Sodium lauryl sulfate [Same as the namesake monograph]

Sodium lauryl sulfate TS Dissolve 100 g of sodium lauryl sulfate in 900 mL of water, add 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.2% Sodium lauryl sulfate TS Dissolve 0.1 g of sodium lauryl sulfate in 0.1 mol/L of pH 7.0 sodium phosphate buffer to make 50 mL.

Sodium metabisulfite See sodium disulfite.

Sodium metabisulfite TS See sodium disulfite TS.

Sodium, metallic Na [K 8687, Sodium, Special class]

Sodium 1-methyl-1H-tetrazole-5-thiolate

$C_2H_3N_4NaS \cdot 2H_2O$ White, crystals or crystalline powder.

Melting point <2.60>: 90 – 94°C

Purity Related substances—Dissolve 10 mg of sodium 1-methyl-1H-tetrazole-5-thiolate in 10 mL of water, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Sodium molybdate See sodium molybdate dihydrate.

Sodium molybdate dihydrate $Na_2MoO_4 \cdot 2H_2O$

[K 8906, Special class]

Sodium Na [K 8687, special class]

Sodium 2-naphthalenesulfonate $C_{10}H_7NaO_3S$ Pale brown, crystals or powder.

Content: not less than 98.0%.

Sodium naphthoquinone sulfonate TS Dissolve 0.25 g of sodium β -naphthoquinone sulfonate in methanol to make 100 mL.

Sodium β -naphthoquinone sulfonate $C_{10}H_5NaO_5S$

Yellow to orange-yellow, crystals or crystalline powder. Soluble in water, and practically insoluble in ethanol (95).

Loss on drying <2.41>: Not more than 2.0% (1 g, in vacuum, 50°C).

Residue on ignition <2.44>: 26.5 – 28.0% (1 g, after drying).

Sodium nitrate $NaNO_3$ [K 8562, Special class]

Sodium nitrite $NaNO_2$ [K 8019, Special class]

Sodium nitrite TS Dissolve 10 g of sodium nitrite in water to make 100 mL. Prepare before use.

Sodium nitroprusside See sodium pentacyanonitrosylferrate (III) dihydrate.

Sodium nitroprusside TS See sodium pentacyanonitrosylferrate (III) TS.

Sodium 1-octane sulfonate $CH_3(CH_2)_7SO_3Na$ White crystals or powder.

Residue on ignition <2.44>: 32.2 – 33.0% (1.0 g).

Sodium oxalate (standard reagent) $C_2O_4Na_2$ [K 8005, Standard reagent for volumetric analysis]

Sodium pentacyanoammine ferroate (II) n-hydrate

$Na_3[Fe(CN)_5NH_3] \cdot nH_2O$ [K 8689, First class]

Sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS Mix an equal volume of a solution of sodium pentacyanonitrosylferrate (III) dihydrate (1 in 10), a solution of potassium hexacyanoferrate (III) (1 in 10) and a solution of sodium hydroxide (1 in 10), and allow to stand for 30 minutes. Use after the color of the solution is changed from a dark red to yellow. Prepare before use.

Sodium pentacyanonitrosylferrate (III) dihydrate

$Na_2Fe(CN)_5(NO) \cdot 2H_2O$ [K 8722, Special class]

Sodium pentacyanonitrosylferrate (III) TS Dissolve 1 g of sodium pentacyanonitrosylferrate (III) dihydrate in water to make 20 mL. Prepare before use.

Sodium 1-pentane sulfonate $C_5H_{11}NaO_3S$ White, crystals or crystalline powder. Freely soluble in water, and practically insoluble in acetonitrile.

Purity Clarity and color of solution—Dissolve 1.0 g of sodium 1-pentane sulfonate in 10 mL of water: the solution is colorless and clear.

Water <2.48>: not more than 3.0% (0.2 g).

Content: not less than 99.0%, calculated on the anhydrous basis. Assay—Dissolve about 0.3 g of sodium 1-pentane sulfonate, accurately weighed, in 50 mL of water. Transfer this solution to a chromatographic column, prepared by pouring 10 mL of strongly acidic ion-exchange resin (H type) (424 – 600 μ m in particle diameter) into a chromatographic tube, 9 mm in inside diameter and 160 mm in height, and elute at the rate of about 4 mL per minute. Wash the chromatographic column with 50 mL of water at the rate of about 4 mL per minute, and wash again with 100 mL of water in the same manner. Combine the washings with the eluate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS) until the yellow color of the solution changes to blue.

Each mL of 0.1 mol/L sodium hydroxide VS
= 17.42 mg of $C_5H_{11}NaO_3S$

Sodium perchlorate See sodium perchlorate monohydrate.

Sodium perchlorate monohydrate $NaClO_4 \cdot H_2O$

[K 8227, Special class]

Sodium periodate $NaIO_4$ [K 8256, Special class]

Sodium periodate TS Dissolve 60.0 g of sodium periodate in 120 mL of 0.05 mol/L sulfuric acid TS, and add water to make 1000 mL. If the solution is not clear, filter this through a glass-filter. Keep in a light-resistant vessel.

Sodium peroxide Na_2O_2 [K 8231, Special class]

Sodium phosphate See trisodium phosphate dodecahydrate.

Sodium phosphate TS Dissolve 5.68 g of disodium hydrogen phosphate and 6.24 g of sodium dihydrogen phosphate dihydrate in water to make 1000 mL.

Sodium p-phenol sulfonate $C_6H_5O_4NaS \cdot 2H_2O$ White to light yellow, crystals or crystalline powder, having a specific odor.

Identification—(1) To 10 mL of a solution of sodium p-phenol sulfonate (1 in 10) add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of sodium p-phenol sulfonate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 269 nm and 273 nm and between 276 nm and 280 nm.

Purity Clarity and color of solution—Dissolve 1.0 g of sodium p-phenol sulfonate in 25 mL of water: the solution is clear and colorless.

Content: not less than 90.0%. Assay—Dissolve about

0.5 g of sodium *p*-phenol sulfonate, accurately weighed, in 50 mL of water. Transfer the solution to a chromatographic column, prepared by pouring strongly acidic ion exchange resin (H type) for column chromatography (150 to 300 μm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 30 cm in height, and allow to flow. Wash the chromatographic column with water until the washing is no longer acidic, combine the washings with the above effluent solution, and titrate $\langle 2.50 \rangle$ with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of bromocresol green-methyl red TS). Separately, dissolve 0.5 g of sodium *p*-phenol sulfonate, previously weighed accurately, in 50 mL of water and titrate with 0.1 mol/L sodium hydroxide VS, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.22 mg of $\text{C}_6\text{H}_5\text{O}_4\text{NaS}\cdot 2\text{H}_2\text{O}$

0.1 mol/L Sodium phosphate buffer solution, pH 7.0
Dissolve 17.9 g of disodium hydrogen phosphate dodecahydrate ($\text{NaH}_2\text{PO}_4\cdot 12\text{H}_2\text{O}$) in water to make 500 mL. Add to this solution to a 500 mL solution prepared by dissolving 7.8 g of disodium hydrogen phosphate dihydrate in water until the pH becomes 7.0.

Sodium pyruvate Prepared for microbial test.

Sodium salicylate $\text{HOC}_6\text{H}_4\text{COONa}$ [K 8397, Special class]

Sodium salicylate-sodium hydroxide TS Dissolve 1 g of sodium salicylate in 0.01 mol/L sodium hydroxide VS to make 100 mL.

Sodium selenite Na_2SeO_3 [K 8036, Special class]

Sodium *p*-styrenesulfonate $\text{C}_8\text{H}_7\text{NaO}_3\text{S}$ White crystals or crystalline powder. Freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Recrystallize from diluted ethanol (1 in 2), and dry in vacuum.

Identification—Determine the infrared absorption spectrum of sodium *p*-styrenesulfonate according to the potassium bromide disk method under Infrared Spectrophotometry $\langle 2.25 \rangle$: it exhibits absorption at the wave numbers of about 1236 cm^{-1} , 1192 cm^{-1} , 1136 cm^{-1} , 1052 cm^{-1} , 844 cm^{-1} and 688 cm^{-1} .

Purity—Perform the test with 10 μL of a solution of sodium *p*-styrenesulfonate (1 in 1000) as directed in the Assay under Panipenem: Any obstructive peaks for determination of panipenem are not observed.

Sodium sulfate See sodium sulfate decahydrate.

Sodium sulfate, anhydrous Na_2SO_4 [K 8987, Special class]

Sodium sulfate decahydrate $\text{Na}_2\text{SO}_4\cdot 10\text{H}_2\text{O}$ [K 8986, Special class]

Sodium sulfide See sodium sulfide enneahydrate.

Sodium sulfide enneahydrate $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ [K 8949, Special class]

Sodium sulfide TS Dissolve 5 g of sodium sulfide enneahydrate in a mixture of 10 mL of water and 30 mL of glycerin. Or dissolve 5 g of sodium hydroxide in a mixture of 30 mL of water and 90 mL of glycerin, saturate a half volume of

this solution with hydrogen sulfide, while cooling, and mix with the remaining half. Preserve in well-filled, light-resistant bottles. Use within 3 months.

Sodium sulfite See sodium sulfite heptahydrate.

Sodium sulfite, anhydrous Na_2SO_3 [K 8061, Sodium sulfite, Special class]

Sodium sulfite heptahydrate $\text{Na}_2\text{SO}_3\cdot 7\text{H}_2\text{O}$ [K 8060, Special class]

1 mol/L Sodium sulfite TS Dissolve 1.26 g of anhydrous sodium sulfite in water to make 10 mL.

Sodium tartrate See sodium tartrate dihydrate.

Sodium tartrate dihydrate $\text{C}_4\text{H}_4\text{Na}_2\text{O}_6\cdot 2\text{H}_2\text{O}$
[K 8540, Special class]

Sodium tetraborate-calcium chloride buffer solution, pH 8.0 Dissolve 0.572 g of sodium tetraborate decahydrate and 2.94 g of calcium chloride dihydrate in 800 mL of freshly boiled and cooled water, adjust the pH to 8.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Sodium tetraborate decahydrate $\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$
[K 8866, Special class]

Sodium tetraborate for pH determination
[K 8866, for pH standard solution]

Sodium tetraphenylborate $(\text{C}_6\text{H}_5)_4\text{BNa}$ [K 9521]

Sodium thioglycolate $\text{HSCH}_2\text{COONa}$ A white powder, having a characteristic odor.

Identification—(1) To a solution (1 in 10) add 1 drop each of ammonia solution (28) and iron (III) chloride TS: a dark red-purple color appears.

(2) Perform the test as directed under Flame Coloration Test (1) $\langle 1.04 \rangle$: a yellow color appears.

Purity Clarity and color of solution—Dissolve 1 g in 10 mL of water: the solution is clear and colorless.

Sodium thiosulfate See sodium thiosulfate pentahydrate.

Sodium thiosulfate pentahydrate $\text{Na}_2\text{S}_2\text{O}_3\cdot 5\text{H}_2\text{O}$
[K 8637, Special class]

Sodium thiosulfate TS Dissolve 26 g of sodium thiosulfate pentahydrate and 0.2 g of anhydrous sodium carbonate in freshly boiled and cooled water to make 1000 mL (0.1 mol/L).

Sodium toluenesulfonchloramide trihydrate
 $\text{C}_7\text{H}_7\text{ClNNaO}_2\text{S}\cdot 3\text{H}_2\text{O}$ [K 8318, Sodium *p*-toluenesulfonchloramide trihydrate, Special class]

Sodium toluenesulfonchloramide TS Dissolve 1 g of sodium toluenesulfonchloramide trihydrate in water to make 100 mL. Prepare before use.

Sodium tridecanesulfonate $\text{C}_{13}\text{H}_{27}\text{SO}_3\text{Na}$ White, crystals or powder.

Purity Absorbance—Dissolve 1.43 g of sodium tridecanesulfonate in 1000 mL of water, and perform the test as directed under Ultraviolet-visible Spectrophotometry $\langle 2.24 \rangle$: the absorbances at 230 nm and 245 nm are not more than 0.05 and 0.01, respectively.

Sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy

$(\text{CH}_3)_3\text{SiCH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{Na}$ Prepared for nuclear magnetic resonance spectroscopy.

Sodium 3-trimethylsilylpropionate- d_4 for nuclear magnetic resonance spectroscopy $(\text{CH}_3)_3\text{SiCD}_2\text{CD}_2\text{COONa}$ Prepared for nuclear magnetic resonance spectroscopy.

Sodium tungstate See sodium tungstate (VI) dihydrate.

Sodium tungstate (VI) dihydrate $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ [K 8612, Special class]

Solid plates Dilute anti-E. coli protein antibody stock solution by adding 0.2 mol/L Tris hydrochloride buffer, pH 7.4, to a concentration of about 0.02 mg/mL. Add exactly 0.1 mL of this solution to each well in the microplates, cover with plate seal, and then shake gently. Centrifuge for 2 minutes if some solution sticks to the top of the microplate or elsewhere. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS (pH 7.4) to make the wash solution. After leaving the microplates for 16 to 24 hours at a constant temperature of about 25°C, remove the solution in each well by aspiration, add 0.25 mL of the wash solution, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times for each well using 0.25 mL of the wash solution. Add 0.25 mL of the block buffer solution to each well, gently shake, and let stand for 16 to 24 hours at a constant temperature of about 25°C to make solid plates. When using, remove the solution from the wells by aspiration, add 0.25 mL of the wash solution to each well, shake gently, and then remove this solution by aspiration. Repeat this procedure 2 more times using 0.25 mL of the wash solution.

Soluble starch See starch, soluble.

Soluble starch TS Triturate 1 g of soluble starch in 10 mL of cooled water, pour gradually into 90 mL of boiled water while constantly stirring, boil gently for 3 minutes, and cool. Prepare before use.

Solution of factor Xa 7 *Inkat*_{s-2222} of Factor Xa in 10 mL of water.

Sorbitan sesquioleate [Same as the namesake monograph]

D-Sorbitol [Same as the namesake monograph]

D-Sorbitol for gas chromatography Prepared for gas chromatography.

Soybean-casein digest medium See Sterility Test <4.06>.

Soybean oil [Same as the namesake monograph]

Soybean peptone See peptone, soybean.

Stacking gel for celmoleukin In 0.5 mol/L Tris buffer solution, pH 6.8, prepare stacking the gel using ammonium persulfate and TEMED so the acrylamide concentration is 5.2% and the sodium lauryl sulfate concentration is 0.1%.

Stannous chloride See tin (II) chloride dihydrate.

Stannous chloride-sulfuric acid TS See tin (II) chloride-sulfuric acid TS.

Stannous chloride TS See tin (II) chloride TS.

Stannous chloride TS, acidic See tin (II) chloride TS, acidic.

Starch [K 8658, Special class]

Starch-sodium chloride TS Saturate starch TS with sodium chloride. Use within 5 to 6 days.

Starch, soluble [K 8659, Special class]

Starch TS Triturate 1 g of starch with 10 mL of cold water, and pour the mixture slowly, with constant stirring, into 200 mL of boiling water. Boil the mixture until a thin, translucent fluid is obtained. Allow to settle, and use the supernatant liquid. Prepare before use.

Stearic acid for gas chromatography $\text{C}_{18}\text{H}_{36}\text{O}_2$ [K 8585, Special class]

Stearyl alcohol [Same as the namesake monograph]

Strong ammonia water See ammonia solution (28).

Strong cupric acetate TS See copper (II) acetate TS, strong.

Strong hydrogen peroxide water See hydrogen peroxide (30).

Strongly acidic ion exchange resin Contains strong acid ion exchange residues. Particle diameter is about 100 μm .

Strongly basic ion exchange resin Contains strong basic ion exchange residues. Particle diameter is about 100 μm .

Strontium chloride $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ [K 8132, Special class]

Strychnine nitrate for assay $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 \cdot \text{HNO}_3$ To 1 g of strychnine nitrate add 14 mL of water and about 10 mg of active carbon, heat in a water bath for 10 minutes, filter while hot, cool the filtrate quickly to form crystals, and filter the crystals. Add 8 mL of water to the crystals, dissolve by heating in a water bath, filter while hot, cool quickly, and filter the crystals formed. Repeat this procedure with 8 mL of water, and dry the crystals in a desiccator (in vacuum, silica gel) for 24 hours. Colorless or white crystals or crystalline powder. Sparingly soluble in water, in glycerin and in chloroform, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Purity Related substances—Dissolve 35 mg of strychnine nitrate for assay in 100 mL of the mobile phase and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Perform the test with exactly 20 μL each of the sample solution and standard solution (1) as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area of these solutions by the automatic integration method: the total area of the peaks other than strychnine from the sample solution is not larger than the peak area of strychnine from the standard solution (1).

Operating conditions

Proceed the operating conditions in the Assay under Nux Vomica except detection sensitivity and time span of measurement.

Detection sensitivity: Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 40 mL, and use this solution as the standard solution (2). Adjust the detection sensitivity so that the peak area of strychnine obtained from 20 μL of the standard solution (2) can be measured by the automatic integration method and the peak height of strychnine

from 20 μL of the standard solution (1) is about 20% of the full scale.

Time span of measurement: About 3 times as long as the retention time of strychnine beginning after the solvent peak.

Loss on drying <2.41>: not more than 0.5% (0.2 g, 105°C, 3 hours).

Content: not less than 99.0% calculated on the dried basis.

Assay—Dissolve about 0.5 g of strychnine nitrate for assay, accurately weighed, in 40 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), heat if necessary, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.74 mg of $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2 \cdot \text{HNO}_3$

Styrene C_8H_8 Colorless, clear liquid.

Specific gravity <2.56> *d*: 0.902 – 0.910

Purity—Perform the test with 1 μL of styrene as directed under Gas Chromatography <2.02> according to the following conditions. Measure each peak area by the automatic integration method and calculate the amount of styrene by the area percentage method: it shows the purity of not less than 99%.

Operating conditions

Detector: Thermal conductivity detector.

Column: A glass column, about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth (180 to 250 μm in particle diameter) coated with polyethylene glycol 20 M at the ratio of 10%.

Column temperature: A constant temperature of about 100°C.

Temperature of sample vaporization chamber: A constant temperature of about 150°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of styrene is about 10 minutes.

Time span of measurement: About twice as long as the retention time of styrene, beginning after the solvent peak.

Substrate buffer for celmoleukin Dissolve 32.4 g of tripotassium citrate monohydrate in water to make 1000 mL, and add 1 mol/L citric acid TS for buffer solution to adjust the pH to 5.5. To 100 mL of this solution add and dissolve 0.44 g of *o*-phenylenediamine and then 60 μL of hydrogen peroxide (30). Prepare at the time of use.

Substrate solution for lysozyme hydrochloride To a suitable amount of dried cells of *Micrococcus luteus* add a suitable amount of phosphate buffer solution, pH 6.2, gently shake to make a suspension, and add the substrate cells or the same buffer solution so that the absorbance of the suspension at 640 nm is about 0.65. Prepare before use.

Substrate solution for peroxidase determination Dissolve 0.195 mL of hydrogen peroxidase (30), 8.38 g of disodium hydrogen phosphate dodecahydrate and 1.41 g of citric acid monohydrate in water to make 300 mL. To 15 mL of this solution add 13 mg of *o*-phenylenediamine dihydrochloride before use.

Substrate TS for kallidinogenase assay (1) Dissolve an appropriate amount of H-D-valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to prepare a solution containing 1 mg of H-D-

valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride in 5 mL.

Substrate TS for kallidinogenase assay (2) Dissolve 17.7 mg of *N*- α -benzoyl-L-arginine ethyl ester hydrochloride in 0.1 mol/L tris buffer solution, pH 8.0 to make 100 mL.

Substrate TS for kallidinogenase assay (3) Suspend 0.6 g of milk casein purified by the Hammerstein's method in 80 mL of 0.05 mol/L sodium hydrogen phosphate TS, and dissolve by warming at 65°C for 20 minutes. After cooling, adjust to pH 8.0 with 1 mol/L hydrochloric acid TS or sodium hydroxide TS, and add water to make exactly 100 mL. Prepare before use.

Substrate TS for kallidinogenase assay (4) Dissolve 25 mg of H-D-valyl-L-leucyl-L-arginine-4-nitroanilide dihydrochloride in 28.8 mL of water.

Succinic acid, anhydrous $\text{C}_4\text{H}_4\text{O}_3$ White or pale yellowish white crystals or flakes. It is odorless. Soluble in water, freely soluble in hot water, and sparingly soluble in ethanol (95).

Purity (1) Chloride <1.03>: not more than 0.005%.

(2) Iron <1.10>: not more than 0.001%.

Residue on ignition <2.44>: not more than 0.1% (1 g).

Content: not less than 98.0%. **Assay**—Dissolve about 1 g of anhydrous succinic acid, accurately weighed, in 50 mL of water by warming, cool, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 50.04 mg of $\text{C}_4\text{H}_4\text{O}_3$.

Sucrose $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ [Same as the monograph Sucrose]

Sudan III $\text{C}_{22}\text{H}_{16}\text{N}_4\text{O}$ Red-brown powder. It dissolves in acetic acid (100) and in chloroform, and insoluble in water, in ethanol (95), in acetone and in ether.

Melting point <2.60>: 170 – 190°C

Sudan III TS Dissolve 0.01 g of sudan III in 5 mL of ethanol (95), filter, and add 5 mL of glycerin to the filtrate. Prepare before use.

Sulbactam sodium for sulbactam penicillamine $\text{C}_8\text{H}_{10}\text{NNaO}_5\text{S}$ White to yellowish white crystalline powder. Freely soluble in water, and slightly soluble in ethanol (95).

Identification—Determine the infrared absorption spectrum of sulbactam sodium for sulbactam penicillamine according to the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits the absorption at the wave numbers of about 1780 cm^{-1} , 1600 cm^{-1} , 1410 cm^{-1} , 1400 cm^{-1} , 1320 cm^{-1} , 1300 cm^{-1} , 1200 cm^{-1} and 1130 cm^{-1} .

Water <2.48>: not more than 1.0% (0.5 g).

Content: not less than 875 μg per mg, calculated on the anhydrous basis. **Assay**—Weigh accurately an amount of sulbactam sodium for sulbactam penicillamine and Sulbactam Reference Standard, equivalent to about 0.10 g (potency), dissolve each in a suitable volume of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak

area of sulbactam to that of the internal standard.

$$\begin{aligned} & \text{Amount } [\mu\text{g (potency)}] \text{ of sulbactam (C}_8\text{H}_{11}\text{NO}_5\text{S)} \\ & = W_S \times \frac{Q_T}{Q_S} \times 1000 \end{aligned}$$

W_S : amount [mg (potency)] of Sulbactam Reference Standard

Internal standard solution A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

Operating conditions

Detector: Ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

System suitability

System performance: When the procedure is run with 10 μL of the standard solution according to the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution according to the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 2.0%.

Sulfamic acid (standard reagent) See amido sulfuric acid (standard reagent).

Sulfanilamide $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$ [K 9066, Special class]

Sulfanilamide for titration of diazotization $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_2\text{NH}_2$ [K 9066, For titration of diazotization]

Sulfanilic acid $\text{H}_2\text{NC}_6\text{H}_4\text{SO}_3\text{H}$ [K 8586, Special class]

Sulfathiazole $\text{C}_9\text{H}_9\text{N}_3\text{O}_3\text{S}_2$ White crystalline powder.
Melting point <2.60>: 200 – 204°C

Sulfosalicylic acid See 5-sulfosalicylic acid dihydrate.

5-Sulfosalicylic acid dihydrate $\text{C}_7\text{H}_6\text{O}_6\text{S}\cdot 2\text{H}_2\text{O}$
[K 8589, Special class]

Sulfosalicylic acid TS Dissolve 5 g of 5-sulfosalicylic acid dihydrate in water to make 100 mL.

Sulfur S [K 8088, Special class]

Sulfur dioxide SO_2 Prepare by adding sulfuric acid dropwise to a concentrated solution of sodium bisulfite. Colorless gas, having a characteristic odor.

Sulfuric acid H_2SO_4 [K 8951, Special class]

Sulfuric acid, dilute Cautiously add 5.7 mL of sulfuric acid to 10 mL of water, cool, and dilute with water to make 100 mL (10%).

Sulfuric acid-ethanol TS With stirring, add slowly 3 mL

of sulfuric acid to 1000 mL of ethanol (99.5), and cool.

Sulfuric acid for readily carbonizable substances To sulfuric acid, the content of which has previously been determined by the following method, add water cautiously, and adjust the final concentration to 94.5% to 95.5% of sulfuric acid (H_2SO_4). When the concentration is changed owing to absorption of water during storage, prepare freshly.

Assay—Weigh accurately about 2 g of sulfuric acid in a glass-stoppered flask rapidly, add 30 mL of water, cool, and titrate <2.50> the solution with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of bromothymol blue TS).

Each mL of 1 mol/L sodium hydroxide VS
= 49.04 mg of H_2SO_4

Sulfuric acid, fuming $\text{H}_2\text{SO}_4\cdot n\text{SO}_3$ [K 8741, Special class]

Sulfuric acid-hexane-methanol TS To 230 mL of a mixture of hexane and methanol (1:3) add cautiously 2 mL of sulfuric acid.

Sulfuric acid-methanol TS Prepare carefully by adding 60 mL of sulfuric acid to 40 mL of methanol.

Sulfuric acid-methanol TS, 0.05 mol/L Add gradually 3 mL of sulfuric acid to 1000 mL of methanol, while stirring, and allow to cool.

Sulfuric acid-monobasic sodium phosphate TS See sulfuric acid-sodium dihydrogenphosphate TS.

Sulfuric acid, purified Place sulfuric acid in a beaker, heat until white fumes are evolved, then heat for 3 minutes cautiously and gently. Use after cooling.

Sulfuric acid-sodium dihydrogenphosphate TS Add 6.8 mL of sulfuric acid to 500 mL of water, then dissolve 50 g of sodium dihydrogenphosphate dihydrate in this solution, and add water to make 1000 mL.

Sulfuric acid-sodium hydroxide TS With stirring add slowly 120 mL of sulfuric acid to 1000 mL of water, and cool (solution A). Dissolve 88.0 g of sodium hydroxide in 1000 mL of freshly boiled and cooled water (solution B). Mix equal volumes of solution A and solution B.

Sulfuric acid TS Cautiously add 1 volume of sulfuric acid to 2 volumes of water, and while warming on a water bath add dropwise potassium permanganate TS until a pale red color of the solution remains.

0.05 mol/L Sulfuric acid TS Dilute 100 mL of 0.5 mol/L sulfuric acid TS with water to make 1000 mL.

0.25 mol/L Sulfuric acid TS With stirring, add slowly 15 mL of sulfuric acid to 1000 mL of water, then cool.

0.5 mol/L Sulfuric acid TS With stirring, add slowly 30 mL of sulfuric acid to 1000 mL of water, then cool.

2 mol/L Sulfuric acid TS To 1000 mL of water add gradually 120 mL of sulfuric acid with stirring, and cool.

Sulfurous acid See sulfurous acid solution.

Sulfurous acid solution H_2SO_3 [K 8058, Special class]

Sulpiride for assay $\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$ [Same as the monograph Sulpiride. When dried, it contains not less than 99.0% of sulpiride ($\text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$).]

Sulpyrine $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ [Same as the monograph Sulpyrine Hydrate]

Sulpyrine for assay [Same as the monograph Sulpyrine Hydrate. Calculated on the dried basis, it contains not less than 99.0% of sulpyrine ($C_{13}H_{16}N_3NaO_4S$).]

Suxamethonium chloride for thin-layer chromatography $C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$ [Same as the monograph Suxamethonium Chloride Hydrate]

Swertiamarin for thin-layer chromatography $C_{16}H_{22}O_{10}$ White, practically tasteless powder.

Melting point <2.60>: 113 – 114°C

Purity Related substances—Dissolve 2.0 mg of swertiamarin for thin-layer chromatography in exactly 1 mL of ethanol (95), and perform the test with 20 μ L of this solution as directed in the Identification under Swertia Herb: any spot other than the principal spot at the *R_f* value of about 0.5 does not appear.

Synthetic zeolite for drying A mixture of $6(Na_2O) \cdot 6(Al_2O_3) \cdot 12(SiO_2)$ and $6(K_2O) \cdot 6(Al_2O_3) \cdot 12(SiO_2)$ prepared for drying. Usually, use the spherically molded form, 2 mm in diameter, prepared by adding a binder. White to grayish white, or color transition by adsorbing water. Average fine pore diameter is about 0.3 nm, and the surface area is 500 to 700 m² per g.

Loss on ignition <2.43>: not more than 2.0% [2 g, 550 – 600°C, 4 hours, allow to stand in a desiccator (phosphorus (V) oxide)].

Talc [Same as the namesake monograph]

Tannic acid [Same as the namesake monograph]

Tannic acid TS Dissolve 1 g of tannic acid in 1 mL of ethanol (95), and add water to make 10 mL. Prepare before use.

Tartaric acid See L-tartaric acid.

L-Tartaric acid $C_4H_6O_6$ [K 8532, L(+)-Tartaric acid, Special class].

Tartrate buffer solution, pH 3.0 Dissolve 1.5 g of L-tartaric acid and 2.3 g of sodium tartrate dihydrate in water to make 1000 mL.

Taurine $H_2NCH_2CH_2SO_3H$ White crystals or crystalline powder.

Contents: not less than 95.0%. Assay—Weigh accurately about 0.2 g, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.52 mg of $C_2H_7NO_3S$

Terephthalic acid $C_6H_4(COOH)_2$ White crystals or crystalline powder. Slightly soluble in ethanol (95), and practically insoluble in water and ether.

Residue on ignition <2.44>: not more than 0.3% (1 g).

Content: not less than 95.0%. Assay—Weigh accurately about 2 g of terephthalic acid, dissolve in exactly 50 mL of 1 mol/L sodium hydroxide VS, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner.

Each mL of 1 mol/L sodium hydroxide VS
= 83.07 mg of $C_8H_6O_4$

Terephthalic acid for gas chromatography $C_6H_4(COOH)_2$ Terephthalic acid prepared for gas chromatography.

Terphenyl $C_{18}H_{14}$ White crystalline powder.

Melting point <2.60>: 208 – 213°C

Identification—Determine the absorption spectrum of a solution of terphenyl in methanol (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 276 and 280 nm.

p-Terphenyl See terphenyl.

Test bacteria inoculation medium for teceleukin Dissolve 6.0 g of peptone, 3.0 g of yeast extract, 1.5 g of meat extract, 1.0 g of glucose, and 13.0 to 20.0 g of agar in 1000 mL of water and sterilize. The pH is 6.5 to 6.6.

Test bacteria inoculation medium slant for teceleukin Sterilized slant culture obtained by adding approximately 9 mL of bacteria inoculation medium for teceleukin to a test tube with an inside diameter of 16 mm.

Testosterone propionate $C_{22}H_{32}O_3$ [Same as the namesake monograph]

Test solution for water determination See the Water Determination <2.48>.

Tetrabromophenolphthalein ethyl ester potassium salt $C_{22}H_{13}O_4Br_4K$ [K 9042, Special class]

Tetrabromophenolphthalein ethyl ester TS Dissolve 0.1 g of tetrabromophenolphthalein ethyl ester potassium salt in acetic acid (100) to make 100 mL. Prepare before use.

Tetra n-butylammonium bromide $[CH_3(CH_2)_3]_4NBr$ White, crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 101 – 105°C

Purity Clarity and color of solution—Dissolve 1.0 g of tetra n-butylammonium bromide in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Dissolve about 0.5 g of tetra n-butylammonium bromide, accurately weighed, in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 32.24 mg of $C_{16}H_{36}NBr$

Tetra n-butylammonium chloride $C_{16}H_{36}ClN$ White crystals, and it is deliquescent.

Water <2.48>: not more than 6.0% (0.1 g).

Content: not less than 95.0%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.25 g of tetra n-butylammonium chloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 27.97 mg of $C_{16}H_{36}ClN$

Tetrabutylammonium hydrogensulfate $C_{16}H_{37}NO_4S$

White crystalline powder.

Content: not less than 98.0%. Assay—Weigh accurately about 0.7 g of tetrabutylammonium hydrogensulfate, dissolve in 100 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of bromocresol green-methyl red TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 33.95 mg of $C_{16}H_{37}NO_4S$

40% Tetrabutylammonium hydroxide TS A solution containing 40 g/dL of tetrabutylammonium hydroxide $[(C_4H_9)_4NOH]$: 259.47].

Content: 36 – 44 g/dL. Assay—Pipet 10 mL of 40% tetrabutylammonium hydroxide TS, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS
= 259.5 mg of $C_{16}H_{37}NO$

0.005 mol/L Tetrabutylammonium hydroxide TS To 10 mL of tetrabutylammonium hydroxide TS add 700 mL of water, adjust to pH 4.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

Tetrabutylammonium hydroxide-methanol TS Methanol solution containing 25 g/dL of tetrabutylammonium hydroxide $[(C_4H_9)_4NOH]$: 259.47]. Colorless to pale yellow solution, having an ammonium-like odor.

Content: 22.5 – 27.5 g/dL. Assay—Pipet 15 mL of tetrabutylammonium hydroxide-methanol TS and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 1 mol/L hydrochloric acid VS
= 259.5 mg of $C_{16}H_{37}NO$

10% Tetrabutylammonium hydroxide-methanol TS

A methanol solution containing 10 g/dL of tetrabutylammonium hydroxide $[(C_4H_9)_4NOH]$: 259.47].

Content: 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of 10% tetrabutylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 25.95 mg of $C_{16}H_{37}NO$

Tetrabutylammonium hydroxide TS A solution containing 13 g/dL of tetrabutylammonium hydroxide $[(C_4H_9)_4NOH]$: 259.47].

Content: 11.7 – 14.3 g/dL. Assay—Pipet a quantity, equivalent to about 0.3 g of tetrabutylammonium hydroxide $[(C_4H_9)_4NOH]$, transfer to a glass-stoppered flask containing 15 mL of water, accurately weighed, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 25.95 mg of $C_{16}H_{37}NO$

Tetrabutylammonium phosphate $(C_4H_9)_4NH_2PO_4$

White powder. It is soluble in water.

Content: not less than 97.0%. Assay—Weigh accurately 1.5 g of tetrabutylammonium phosphate, dissolve in 80 mL of water, and titrate <2.50> with 0.5 mol/L sodium hydroxide

VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.5 mol/L sodium hydroxide VS
= 169.7 mg of $(C_4H_9)_4NH_2PO_4$

Tetracycline $C_{22}H_{24}N_2O_8$ Yellow to dark yellow, crystals or crystalline powder. Sparingly soluble in ethanol, and very slightly soluble in water.

Content: it contains not less than 870 μ g (potency) per mg. Assay—Proceed as directed in the Assay under Tetracycline Hydrochloride. However, use the following formula.

Amount [μ g (potency)] of tetracycline ($C_{22}H_{24}N_2O_8$)
= $W_S \times (A_T/A_S) \times 1000$

W_S : Amount [mg (potency)] of Tetracycline Hydrochloride Reference Standard

Tetracycline Hydrochloride $C_{22}H_{24}N_2O_8 \cdot HCl$ Yellow, crystals or crystalline powder.

Purity Related substances—Dissolve 20 mg of tetracycline hydrochloride in 25 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Proceed the test with 20 μ L of the sample solution as directed in the Purity (2) under Oxytetracycline Hydrochloride, determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than tetracycline is not more than 10%.

Tetraethylammonium hydroxide TS A solution containing 10% of tetraethylammonium hydroxide $[(C_2H_5)_4NOH]$: 147.26]. A clear, colorless liquid, having a strong ammonia odor. It is a strong basic and easily absorbs carbon dioxide from the air.

Content: 10.0 – 11.0% Assay—Weigh accurately about 3 g in a glass-stoppered flask containing 15 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L hydrochloric acid VS
= 14.73 mg of $C_8H_{21}NO$

Tetra-*n*-heptylammonium bromide $[CH_3(CH_2)_6]_4NBr$ White, crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 87 – 89°C.

Content: not less than 98.0%. Assay—Dissolve about 0.5 g of tetra-*n*-heptylammonium bromide, accurately weighed, in 50 mL of diluted acetonitrile (3 in 5), and 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while strongly shaking (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 49.07 mg $C_{28}H_{60}NBr$

Tetrahydrofuran $CH_2(CH_2)_2CH_2O$ [K 9705, Special class]

Tetrahydrofuran for gas chromatography Use tetrahydrofuran prepared by distilling with iron (II) sulfate heptahydrate.

Storage—Preserve in containers, in which the air has been displaced by nitrogen, in a dark, cold place.

Tetrahydrofuran for liquid chromatography C_4H_8O

Clear and colorless liquid.

Density <2.56> (20°C): 0.884 – 0.889 g/mL

Refractive index <2.45> n_D^{20} : 1.406 – 1.409

Purity Ultraviolet absorbing substances—Determine the absorption spectrum of tetrahydrofuran for liquid chromatography as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbances at 240 nm, 254 nm, 280 nm, 290 nm, and between 300 nm and 400 nm are not more than 0.35, 0.20, 0.05, 0.02 and 0.01, respectively.

Peroxide—Perform the test according to the method described in JIS K 9705: not more than 0.01%.

Tetrahydroxyquinone $C_6H_4O_6$ Dark blue crystals. Its color changes to yellow on exposure to light. Soluble in ethanol (95) and sparingly soluble in water.

Tetrahydroxyquinone indicator Mix 1 g of tetrahydroxyquinone with 100 g of sucrose homogeneously.

Tetrakis(2-hydroxypropyl)ethylenediamine for gas chromatography Prepared for gas chromatography.

Tetramethylammonium hydroxide $(CH_3)_4NOH$ Ordinarily, available as an approximately 10% aqueous solution, which is clear and colorless, and has a strong ammonia-like odor. Tetramethylammonium hydroxide is a stronger base than ammonia, and rapidly absorbs carbon dioxide from the air. Use a 10% aqueous solution.

Purity Ammonia and other amines—Weigh accurately a quantity of the solution, corresponding to about 0.3 g of tetramethylammonium hydroxide $[(CH_3)_4NOH]$, in a weighing bottle already containing 5 mL of water. Add a slight excess of 1 mol/L hydrochloric acid TS (about 4 mL), and evaporate on a water bath to dryness. The mass of the residue (tetramethylammonium chloride), dried at 105°C for 2 hours and multiplied by 0.8317, represents the quantity of tetramethylammonium hydroxide $[(CH_3)_4NOH]$, and corresponds to $\pm 0.2\%$ of that found in the Assay.

Residue on evaporation: not more than 0.02% (5 mL, 105°C, 1 hour).

Content: not less than 98% of the labeled amount. Assay—Accurately weigh a glass-stoppered flask containing about 15 mL of water. Add a quantity of the solution, equivalent to about 0.2 g of tetramethylammonium hydroxide $[(CH_3)_4NOH]$, weigh again, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg of $C_4H_{13}NO$

Tetramethylammonium hydroxide-methanol TS A methanol solution containing of 10 g/dL of tetramethylammonium hydroxide $[(CH_3)_4NOH]$: 91.15]

Content: 9.0 – 11.0 g/dL. Assay—Pipet 2 mL of tetramethylammonium hydroxide-methanol TS, transfer to a glass-stoppered flask containing 20 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: bromocresol green-methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 9.115 mg of $C_4H_{13}NO$

Tetramethylammonium hydroxide TS Pipet 15 mL of tetramethylammonium hydroxide, and add dehydrated ethanol (99.5) to make exactly 100 mL.

Tetramethylammonium hydroxide TS, pH 5.5 To 10 mL

of tetramethylammonium hydroxide add 990 mL of water, and adjust the pH to 5.5 with diluted phosphoric acid (1 in 10).

3,3',5,5'-Tetramethylbenzidine dihydrochlorate dihydrate $C_{16}H_{22}Cl_2N_2 \cdot H_2O$ White to slightly reddish-white crystalline powder.

***N,N,N',N'*-Tetramethylethylenediamine** $(CH_3)_2NCH_2CH_2N(CH_3)_2$ Pale yellow clear liquid.

Specific gravity <2.56> d_4^{20} : 0.774 – 0.799

Content: not less than 99.0%.

Tetramethylsilane for nuclear magnetic resonance spectroscopy $(CH_3)_4Si$ Prepared for nuclear magnetic resonance spectroscopy.

Tetra-*n*-pentylammonium bromide $[CH_3(CH_2)_4]_4NBr$ White, crystals or crystalline powder. It is hygroscopic.

Melting point <2.60>: 100 – 101°C

Tetraphenylboron potassium TS Add 1 mL of acetic acid (31) to a solution of potassium biphthalate (1 in 500), then to this solution add 20 mL of a solution of tetraphenylboron sodium (7 in 1000), shake well, and allow to stand for 1 hour. Collect the produced precipitate on filter paper, and wash it with water. To 1/3 quantity of the precipitate add 100 mL of water, warm, with shaking, at about 50°C for 5 minutes, cool quickly, allow to stand for 2 hours with occasional shaking, and filter, discarding the first 30 mL of the filtrate.

Tetraphenylboron sodium See sodium tetraphenylborate.

Tetra-*n*-propylammonium bromide $[CH_3CH_2CH_2]_4NBr$ White, crystals or crystalline powder.

Purity Clarity and color of solution—Dissolve 1.0 g of tetra-*n*-propylammonium bromide in 20 mL of water: the solution is clear and colorless.

Content: not less than 98.0%. Assay—Weigh accurately about 0.4 g of tetra-*n*-propylammonium bromide, dissolve in 50 mL of water, add 5 mL of dilute nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking strongly (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 26.63 mg of $C_{12}H_{28}NBr$

Theophylline $C_7H_8N_4O_2$ White powder. Slightly soluble in water.

Melting point <2.60>: 269 – 274°C

Purity Caffeine, theobromine or paraxanthine—To 0.20 g of theophylline add 5 mL of potassium hydroxide TS or 5 mL of ammonia TS: each solution is clear.

Loss on drying <2.41>: not more than 0.5% (1 g, 105°C, 4 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.25 g of theophylline, previously dried, dissolve it in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium methoxide VS (indicator: 3 drops of thymol blue-*N,N*-dimethylformamide TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium methoxide VS
= 18.02 mg of $C_7H_8N_4O_2$

Thiamine nitrate $C_{12}H_{17}N_5O_4S$ [Same as the namesake monograph]

Thianthol [Same as the monograph Thianthol. Proceed as directed in the Identification (3) under Sulfur, Salicylic Acid and Thianthol Ointment: any spot other than the principal spot does not appear.]

3-Thienylethylpenicillin sodium $C_{14}H_{15}N_2NaO_4S_2$
White to pale yellowish white powder. Very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (95).

Optical rotation <2.49> $[\alpha]_D^{20}$: +265 – +290° (0.5 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

Water <2.48>: Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Content: not less than 90% calculated on the anhydrous basis. Assay—Weigh accurately about 0.1 g of 3-thienylethylpenicillin sodium, dissolve in 35 mL of water, add 0.75 mL of 0.1 mol/L hydrochloric acid TS, and adjust to pH 8.5 with 0.1 mol/L sodium hydroxide TS. To this solution add 2 mL of a penicillinase solution prepared by dissolving penicillinase, equivalent to 513,000 Levy units, in 25 mL of water and neutralizing with dilute sodium hydroxide TS until a pale red color appears with 1 drop of a solution of phenolphthalein in ethanol (95) (1 in 1000) as indicator, and allow to stand at 25°C for 5 minutes. Titrate <2.50> this solution with 0.1 mol/L sodium hydroxide VS until the solution reaches to pH 8.5 (potentiometric titration). Use the water freshly boiled and cooled.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.24 mg of $C_{14}H_{15}N_2NaO_4S_2$

Thimerosal $C_9H_9HgNaO_2S$ White or yellowish crystalline powder. Freely soluble in water.

Melting point <2.60>: 107 – 114°C.

Thioacetamide C_2H_5NS A white crystalline powder or colorless crystals, having a characteristic odor. Freely soluble in water and in ethanol (99.5). Melting point: 112 – 115°C

Thioacetamide-alkaline glycerin TS To 0.2 mL of thioacetamide TS add 1 mL of alkaline glycerin TS, and heat for 20 minutes in a water bath. Prepare before use.

Thioacetamide TS To 0.2 mL of a solution of thioacetamide (1 in 25) add 1 mL of a mixture of 15 mL of sodium hydroxide TS, 5 mL of water and 20 mL of 85% glycerin, and heat in a water bath for 20 seconds. Prepare before use.

Thiodiglycol $S(CH_2CH_2OH)_2$ [β -Thiodiglycol for amino acid autoanalysis] Colorless or pale yellow, clear liquid.

Specific gravity <2.56> d_{20}^{20} : 1.180 – 1.190

Water <2.48>: not more than 0.7%.

Thioglycolate medium I for sterility test See fluid thioglycolate medium.

Thioglycolate medium II for sterility test See alternative thioglycolate medium.

Thioglycolic acid See mercapto acetic acid.

Thionyl chloride $SOCl_2$ A colorless or light yellow, clear liquid, having a pungent odor.

Specific gravity <2.56> d_{20}^{20} : about 1.65 (Method 3)

Content: not less than 95.0%. Assay—Weigh accurately 0.1 g of thionyl chloride in a weighing bottle, put the bottle in a glass-stoppered conical flask containing 50 mL of water cooled to about 5°C, stopper immediately, dissolve the sam-

ple thoroughly, and transfer the solution to a 200-mL beaker. Wash the conical flask and the weighing bottle in it with 30 mL of water, and combine the washings and the solution in the beaker. Add 1 drop of an aqueous solution of polyvinyl alcohol (100 g/L), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.949 mg of $SOCl_2$

Thiopental for assay $C_{11}H_{18}N_2O_2S$ Dissolve 10 g of Thiopental Sodium in 300 mL of water. To this solution add slowly 50 mL of dilute hydrochloric acid with stirring. Take the produced crystals by filtration, wash with water until the filtrate indicates no reaction to chloride, and air-dry. Add diluted ethanol (99.5) (3 in 5), dissolve by heating in a water bath, allow to stand, and take the produced crystals by filtration. Air-dry the crystals in air, and dry again at 105°C for 4 hours. White, odorless crystals.

Melting point <2.60>: 159 – 162°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of thiopental for assay in dehydrated ethanol: the solution is clear and light yellow.

(2) Related substances—Dissolve 0.05 g of thiopental for assay in 15 mL of acetonitrile, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase in the Purity (4) under Thiopental Sodium to make exactly 200 mL, and use this solution as the standard solution. Proceed as directed in Purity (4) under Thiopental Sodium.

Loss on drying <2.41>: not more than 0.20% (1 g, 105°C, 3 hours).

Content: not less than 99.0%. Assay—Weigh accurately about 0.35 g of thiopental for assay, previously dried, dissolve in 5 mL of dehydrated ethanol and 50 mL of chloroform, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.23 mg of $C_{11}H_{18}N_2O_2S$

Thiosemicarbazide $H_2NCSNHNH_2$ [K 8632, Special class]

Thiourea H_2NCSNH_2 [K 8635, Special class]

Thiourea TS Dissolve 10 g of thiourea in water to make 100 mL.

L-Threonine $C_4H_9NO_3$ [Same as the namesake monograph]

Threoprocaterol hydrochloride $C_{16}H_{22}N_2O_3 \cdot HCl$ To procaterol hydrochloride add 10 volumes of 3 mol/L hydrochloric acid TS, heat, and reflux for 3 hours. After cooling, neutralize (pH 8.5) with sodium hydroxide TS, and collect the crystals produced. Suspend the crystals in water, dissolve by acidifying the solution at pH 1 to 2 with addition of hydrochloric acid, neutralize (pH 8.5) by adding sodium hydroxide TS, and separate the crystals produced. Suspend the crystals in 2-propanol, and acidify the solution at pH 1 to 2 by adding hydrochloric acid. The crystals are dissolved and reproduced. Collect the crystals, dry at about 60°C while passing air. White to pale yellowish white, odorless crystals

or crystalline powder. Melting point: about 207°C (with decomposition).

Purity—Dissolve 0.10 g of threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Liquid Chromatography according to the operating conditions in the Purity (3) under Procaterol Hydrochloride Hydrate. Measure each peak area by the automatic integration method, and calculate the amount of threoprocaterol by the area percentage method: it shows the purity of not less than 95.0%. Adjust the detection sensitivity so that the peak height of threoprocaterol obtained from 2 μ L of the solution prepared by diluting 5.0 mL of the sample solution with diluted methanol (1 in 2) to make 100 mL, is 5 to 10% of the full scale, and the time span of measurement is about twice as long as the retention time of threoprocaterol beginning after the peak of solvent.

Thrombin [Same as the namesake monograph]

Thymine $C_5H_6N_2O_2$; 126.11

Identification—Determine the infrared absorption spectrum of thymine, previously dried at 105°C for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3030 cm^{-1} , 1734 cm^{-1} , 1676 cm^{-1} , 1446 cm^{-1} and 814 cm^{-1} .

Purity Related substances—Dissolve 50 mg of thymine in 100 mL of methanol. To 10 mL of this solution add the mobile phase to make 100 mL, and use this solution as the sample solution. Proceed with 10 μ L of the sample solution as directed in the Purity (3) under Aceglutamide Aluminum: any peak does not appear at the retention time of aceglutamide.

Thymol $CH_3C_6H_3(OH)CH(CH_3)_2$ [Same as the namesake monograph]

Thymol blue $C_{27}H_{30}O_5S$ [K 8643, Special class]

Thymol blue-*N,N*-dimethylformamide TS Dissolve 0.1 g of thymol blue in 100 mL of *N,N*-dimethylformamide.

Thymol blue-dioxane TS Dissolve 0.05 g of thymol blue in 100 mL of 1,4-dioxane, and filter if necessary. Prepare before use.

Thymol blue TS Dissolve 0.1 g of thymol blue in 100 mL of ethanol (95), and filter if necessary.

Thymol blue TS, dilute Dissolve 0.05 g of thymol blue in 100 mL of ethanol (99.5), and filter if necessary. Prepare before use.

Thymol for assay [Same as the monograph Thymol. It contains not less than 99.0% of thymol ($C_{10}H_{14}O$).]

Thymolphthalein $C_{28}H_{30}O_4$ [K 8642, Special class]

Thymolphthalein TS Dissolve 0.1 g of thymolphthalein in 100 mL of ethanol (95), and filter if necessary.

Tiaramide hydrochloride for assay $C_{15}H_{18}ClN_3O_3S \cdot HCl$ [Same as the monograph Tiaramide Hydrochloride. When dried, it contains not less than 99.0% of tiaramide hydrochloride ($C_{15}H_{18}ClN_3O_3S \cdot HCl$).]

Tin Sn [K 8580, Special class]

Tin (II) chloride dihydrate $SnCl_2 \cdot 2H_2O$ [K 8136, Spec-

ial class]

Tin (II) chloride-hydrochloric acid TS To 20 g of tin add 85 mL of hydrochloric acid, heat until hydrogen gas no longer are evolved, and allow to cool. Mix 1 volume of this solution and 10 volume of dilute hydrochloric acid. Prepare before use.

Tin (II) chloride-sulfuric acid TS Dissolve 10 g of tin (II) chloride dihydrate in diluted sulfuric acid (3 in 200) to make 100 mL.

Tin (II) chloride TS Dissolve 1.5 g of Tin (II) chloride dihydrate in 10 mL of water containing a small amount of hydrochloric acid. Preserve in glass-stoppered bottles in which a fragment of tin has been placed. Use within 1 month.

Tin (II) chloride TS, acidic Dissolve 8 g of Tin (II) chloride dihydrate in 500 mL of hydrochloric acid. Preserve in glass-stoppered bottles. Use within 3 months.

Tipepidine hibenzate for assay $C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$ [Same as the monograph Tipepidine Hibenzate. When dried, it contains not less than 99.0% of $C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$.]

Titanium dioxide See titanium (IV) oxide.

Titanium dioxide TS See titanium (IV) oxide TS.

Titanium (III) chloride (20) $TiCl_3$ [K 8401, Titanium (III) chloride solution, Special class] Store in light-resistant, glass-stoppered containers.

Titanium (III) chloride-sulfuric acid TS Mix carefully 20 mL of titanium (III) chloride TS and 13 mL of sulfuric acid, add carefully hydrogen peroxide (30) in small portions until a yellow color develops, and heat until white fumes evolve. After cooling, add water, heat again in the same manner, repeat this procedure until the solution is colorless, and add water to make 100 mL.

Titanium (III) chloride TS To titanium (III) chloride (20) add dilute hydrochloric acid to obtain a solution containing 15 g/dL of titanium (III) chloride ($TiCl_3$). Prepare before use.

Content: 14.0 – 16.0 g/dL. **Assay**—To exactly 2 mL of titanium (III) chloride add 200 mL of water and 5 mL of a hydrochloric acid solution (2 in 3), and titrate <2.50> with 0.1 mol/L ferric ammonium sulfate VS under carbon dioxide until a slight red color develops in the solution (indicator: 5 mL of ammonium thiocyanate TS).

Each mL of 0.1 mol/L ferric ammonium sulfate VS = 15.42 mg of $TiCl_3$

Titanium (IV) oxide TiO_2 [K 8703, Special class]

Titanium (IV) oxide TS To 100 mL of sulfuric acid add 0.1 g of titanium (IV) oxide, and dissolve by gradually heating on a flame with occasional gentle shaking.

Titanium trichloride See titanium (III) chloride.

Titanium trichloride-sulfuric acid TS See titanium (III) chloride-sulfuric acid TS.

Titanium trichloride TS See titanium (III) chloride TS.

Titanium yellow $C_{28}H_{19}N_5Na_2O_6S_4$ [K 8639, Special class]

Tocopherol $C_{29}H_{50}O_2$ [Same as the namesake monograph]

Tocopherol acetate $C_{31}H_{52}O_3$ [Same as the namesake monograph]

Tocopherol calcium succinate $C_{66}H_{106}CaO_{10}$ [Same as the namesake monograph]

Tocopherol succinate $C_{33}H_{54}O_5$ Wet 0.5 g of tocopherol calcium succinate with 5 mL of acetic acid (100), add 10 mL of toluene, and warm at 70°C for 30 minutes with occasional shaking. After cooling, add 30 mL of water, shake thoroughly, and allow to stand. Remove the water layer, wash the toluene layer with several 30-mL portions of water until the washings become neutral, and allow to stand. Shake the toluene extract with 3 g of anhydrous sodium sulfate, decant the toluene layer, distil the toluene under reduced pressure, and obtain a light yellow, viscous liquid. When preserved at room temperature for a long time, it becomes a pale yellowish solid.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (286 nm): 38.0 – 42.0 (10 mg, chloroform, 100 mL).

Tolbutamide $C_{12}H_{18}N_2O_3S$ [Same as the namesake monograph]

Toluene $C_6H_5CH_3$ [K 8680, Special class]

***o*-Toluene sulfonamide** $C_7H_9NO_2S$ Colorless crystals or white crystalline powder. Soluble in ethanol (95), and sparingly soluble in water.

Melting point <2.60>: 157 – 160°C

Purity *p*-Toluene sulfonamide—Use a solution of *o*-toluene sulfonamide in ethyl acetate (1 in 5000) as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (6) under Saccharin Sodium Hydrate: any peak other than the peak of *o*-toluene sulfonamide does not appear. Adjust the flow rate so that the retention time of *o*-toluene sulfonamide is about 10 minutes, and adjust the detection sensitivity so that the peak height of *o*-toluene sulfonamide obtained from 10 μ L of the sample solution is about 50% of the full scale. Time span of measurement is about twice as long as the retention time of *o*-toluene sulfonamide beginning after the solvent peak.

Water <2.48>: not more than 0.5% (4 g, use 25 mL of methanol for Karl Fischer method and 5 mL of pyridine for Karl Fischer method).

Content: not less than 98.5%, calculated on the anhydrous basis. *Assay*—Weigh accurately about 0.025 g of *o*-toluene sulfonamide, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS
= 1.712 mg of $C_7H_9NO_2S$

***p*-Toluene sulfonamide** $CH_3C_6H_4SO_2NH_2$ White, crystals or crystalline powder. *Melting point*: about 137°C

Purity Related substances—Dissolve 30 mg of *p*-toluene sulfonamide in acetone to make exactly 200 mL. Proceed with 10 μ L of this solution as directed in the Purity (3) under Tolazamide: any spot other than the principal spot at the *Rf* value of about 0.6 does not appear.

***p*-Toluene sulfonic acid** See *p*-toluenesulfonic acid

monohydrate.

***p*-Toluenesulfonic acid monohydrate**
 $CH_3C_6H_4SO_3H \cdot H_2O$ [K 8681, Special class]

***o*-Toluic acid** $C_8H_8O_2$ White, crystals or crystalline powder.

Melting point <2.60>: 102 – 105°C

Content: not less than 98.0%.

Toluidine blue See toluidine blue O

Toluidine blue O $C_{15}H_{16}ClN_3S$ Dark green powder, soluble in water, and slightly soluble in ethanol (95).

Identification—

(1) A solution (1 in 100) shows a blue to purple color.

(2) A solution in ethanol (95) (1 in 200) shows a blue color.

(3) A solution shows a maximum absorption at around 630 nm.

Triamcinolone acetonide $C_{24}H_{31}FO_6$ [Same as the namesake monograph]

Trichloroacetic acid CCl_3COOH [K 8667, Special class]

Trichloroacetic acid-gelatin-tris buffer solution To 1 volume of a solution of trichloroacetic acid (1 in 5) add 6 volume of gelatin-tris buffer solution, pH 8.0 and 5 volume of water.

Trichloroacetic acid TS Dissolve 1.80 g of trichloroacetic acid, 2.99 g of sodium acetate trihydrate and 1.98 g of acetic acid (31) in water to make 100 mL.

Trichloroacetic acid TS for serrapeptase Dissolve 1.80 g of trichloroacetic acid and 1.80 g of anhydrous sodium acetate in 5.5 mL of 6 mol/L acetic acid TS and water to make 100 mL.

Trichlorofluoromethane CCl_3F A colorless liquid or gas.

Boiling point <2.57>: 23.7°C

Specific gravity <2.56> $d_4^{17.2}$: 1.494

1,1,2-Trichloro-1,2,2-trifluoroethane $CFCl_2 \cdot CF_2Cl$ Colorless volatile liquid. Miscible with acetone and with diethyl ether, and not with water.

Purity Related substances—Perform the test with 0.1 μ L of 1,1,2-trichloro-1,2,2-trifluoroethane as directed under Gas Chromatography <2.02> according to the operating conditions in the Purity (5) under Halothane: any peak other than the peak of 1,1,2-trichloro-1,2,2-trifluoroethane does not appear.

Tricine $C_6H_{13}NO_5$ White crystalline powder. *Melting point*: 182 to 184°C (with decomposition).

Triethanolamine See 2,2',2''-nitrilotrisethanol.

Triethylamine $(C_2H_5)_3N$ Clear colorless liquid, having a strong amines odor. Miscible with methanol, with ethanol (95) and with diethyl ether.

Melting point <2.60>: 89 – 90°C

Specific gravity <2.56> d_4^{20} : 0.722 – 0.730

Triethylamine buffer solution, pH 3.2 To 4 mL of triethylamine add 2000 mL of water, and adjust the pH to 3.2

with phosphoric acid.

Triethylamine-phosphate buffer solution, pH 5.0 To 1.0 mL of triethylamine add 900 mL of water, adjust the pH to 5.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL.

Trifluoroacetic acid CF_3COOH Colorless, clear liquid, having a pungent odor. Miscible well with water.

Boiling point <2.57>: 72 – 73°C

Specific gravity <2.56> d_{20}^{20} : 1.535

Trifluoroacetic acid for nuclear magnetic resonance spectroscopy CF_3COOH Prepared for nuclear magnetic resonance spectroscopy.

Trifluoroacetic acid TS To 1 mL of trifluoroacetic acid add water to make 1000 mL.

Trifluoroacetic anhydride for gas chromatography $(\text{CF}_3\text{CO})_2\text{O}$ Colorless, clear liquid, having a pungent odor.

Boiling point <2.57>: 40 – 45°C

Trimetazidine hydrochloride for assay $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$ [Same as the monograph Trimetazidine Hydrochloride. It contains not less than 99.0% of trimetazidine hydrochloride ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$), calculated on the anhydrous basis.]

Trimethylsilyl imidazole $\text{C}_6\text{H}_{12}\text{N}_2\text{Si}$ Clear, colorless to pale yellow liquid.

Refractive index <2.45> n_D^{20} : 1.4744 – 1.4764

2,4,6-Trinitrobenzenesulfonic acid

$\text{C}_6\text{H}_2(\text{NO}_2)_3\text{SO}_3\text{H} \cdot 2\text{H}_2\text{O}$ Pale yellow to light yellow powder.

Water <2.48>: 11 – 15% (0.1 g, volumetric titration, direct titration).

Content: not less than 98%, calculated on the anhydrous basis. Assay—Weigh accurately about 0.3 g of 2,4,6-trinitrobenzenesulfonic acid, dissolve in 50 mL of a mixture of water and ethanol (99.5) (1:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.32 mg of $\text{C}_6\text{H}_2(\text{NO}_2)_3\text{SO}_3\text{H}$

2,4,6-Trinitrophenol $\text{HOC}_6\text{H}_2(\text{NO}_2)_3$ Light yellow to yellow, moist crystals. It is added 15 to 25% of water for the sake of safety, because it might explode by heating, mechanical shocking and friction when it is dried.

Identification—To 0.1 g add 10 mL of water, dissolve by warming, and add 12 mL of a mixture of 1% copper (II) sulfate solution and ammonia TS (5:1): green precipitates appear.

Content: not less than 99.5%. Assay—Weigh accurately about 0.25 g, previously dried in a desiccator (silica gel) for 24 hours, dissolve in 50 mL of water by warming, and titrate <2.50> with 0.1 mL sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 22.91 mg of $\text{HOC}_6\text{H}_2(\text{NO}_2)_3$

2,4,6-Trinitrophenol-ethanol TS Dissolve 1.8 g of 2,4,6-trinitrophenol in 50 mL of diluted ethanol (99.5) (9 in 10) and

30 mL of water, and add water to make 100 mL.

2,4,6-Trinitrophenol TS Dissolve 1 g of 2,4,6-trinitrophenol in 100 mL of hot water, cool, and filter if necessary.

2,4,6-Trinitrophenol TS, alkaline Mix 20 mL of 2,4,6-trinitrophenol TS with 10 mL of a solution of sodium hydroxide (1 in 20), and add water to make 100 mL. Use within 2 days.

Triphenylchloromethane $(\text{C}_6\text{H}_5)_3\text{CCl}$ White to grayish or yellowish white, crystals or crystalline powder.

Melting point <2.60>: 107 – 115°C

Triphenyltetrazolium chloride See 2,3,5-triphenyl-2H-tetrazolium Chloride.

Triphenyltetrazolium chloride TS See 2,3,5-triphenyl-2H-tetrazolium chloride TS.

2,3,5-Triphenyl-2H-tetrazolium chloride $\text{C}_{19}\text{H}_{15}\text{ClN}_4$
[K 8214, Special class]

2,3,5-Triphenyl-2H-tetrazolium chloride TS Dissolve 0.25 g of 2,3,5-triphenyl-2H-tetrazolium chloride in ethanol (99.5) to make 100 mL. Prepare before use.

Tripotassium citrate monohydrate $\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$ White crystals or crystalline powder. Very soluble in water, and practically insoluble in ethanol (95).

Content: 99.0% or more Assay—Accurately weigh about 0.2 g of tripotassium citrate monohydrate, add 50 mL of acetic acid for nonaqueous titration, dissolve by warming on a water bath, cool, and then titrate <2.50> with 0.1 mol/L of perchloric acid VS (potentiometric titration). Correct by conducting a blank test using the same method.

Each mL of 0.1 mol/L perchloric acid VS
= 32.44 mg of $\text{C}_6\text{H}_5\text{K}_3\text{O}_7 \cdot \text{H}_2\text{O}$

Tris-acetic acid buffer solution, pH 6.5 Dissolve 13.57 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 6.73 g of acetic acid (100) in water to make 1000 mL.

0.5 mol/L Tris buffer solution, pH 6.8 Dissolve 6 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 50 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 6.8, and then add water to make 100 mL. Filter if necessary.

Tris buffer solution, pH 7.0 Dissolve 24.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 7.0 with 0.1 mol/L hydrochloric acid TS.

0.05 mol/L Tris buffer solution, pH 7.0 Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in about 750 mL of water, adjust to pH 7.0 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.1 mol/L Tris buffer solution, pH 8.0 Dissolve 2.42 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 100 mL of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

Tris buffer solution, pH 8.2 Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 0.5 g of polysorbate 20 in 800 mL of water, adjust to pH 8.2 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

Tris buffer solution, pH 8.4 Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 10.2 g of sodium chlo-

ride in 800 mL of water, adjust to pH 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

0.05 mol/L Tris buffer solution, pH 8.6 Dissolve 6.1 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 950 mL of water, add 2 mol/L hydrochloric acid TS to adjust the pH to 8.6, and then add water to make 1000 mL.

Tris buffer solution, pH 8.8 Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 75 mL of water, add 5 mol/L hydrochloric acid TS to adjust the pH to 8.8, and then add water to make 100 mL. Filter if necessary.

Tris buffer solution, pH 9.5 Dissolve 36.3 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 1000 mL of water, and adjust the pH to 9.5 by adding 1 mol/L hydrochloric acid TS.

Tris buffer solution for bacterial endotoxins test Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 800 mL of water for bacterial endotoxins test, add 100 mL of 0.1 mol/L hydrochloric acid TS and water for bacterial endotoxins test to make 1000 mL, and sterilize by heating in an autoclave at 121°C for 90 minutes.

0.2 mol/L Tris-hydrochloride buffer solution, pH 7.4 Dissolve 6.61 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 0.97 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 250 mL.

0.05 mol/L Tris-hydrochloride buffer solution, pH 7.5 Dissolve 6.35 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 1.18 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 1000 mL.

Trishydroxymethylaminomethane See 2-amino-2-hydroxymethyl-1,3-propanediol.

Trisodium citrate dihydrate $C_6H_5Na_3O_7 \cdot 2H_2O$ [K 8288, or same as the monograph Sodium Citrate Hydrate]

Trisodium ferrous pentacyanoamine TS To 1.0 g of sodium pentacyanonitrosylferrate (III) dihydrate add 3.2 mL of ammonia TS, shake, and allow to stand in a tightly stoppered bottle for a night in a refrigerator. Add this solution to 10 mL of ethanol (99.5), filter to collect the yellow precipitate yielded with suction. Wash the precipitate with dehydrated diethyl ether, dry the precipitate, and keep in a desiccator. Dissolve the precipitate in water to make a 1.0 mg/mL solution before use, and keep in a refrigerator. Use within a week after preparation.

Trisodium phosphate dodecahydrate $Na_3PO_4 \cdot 12H_2O$ [K 9012, Special class]

Trypsin for liquid chromatography An enzyme obtained from the bovine pancreas. This one part digests 250 parts of casein in the following reaction system.

Casein solution—To 0.1 g of milk casein add 30 mL of water, disperse the casein well, add 1.0 mL of diluted sodium hydroxide TS (1 in 10) to dissolve, and add water to make 50 mL. Prepare before use.

Sample solution—Dissolve 0.01 g of trypsin for liquid chromatography in 500 mL of water.

Procedure—To 5 mL of the casein solution add 2 mL of the sample solution and 3 mL of water, mix, then allow to stand at 40°C for 1 hour, and add 3 drops of a mixture of ethanol (95), water and acetic acid (100) (10:9:1): no

precipitate appears.

Trypsin inhibitor Produced by purifying soybean. Each mg of trypsin inhibitor inhibits 10,000 to 30,000 BAEE Units of trypsin. One BAEE Unit means a trypsin activity to indicate an absorbance difference of 0.001 at 253 nm when 3.2 mL of the solution is reacted at 25°C and pH 7.6, using *N*- α -benzoyl-L-arginine ethyl ester as substrate.

Trypsin inhibitor TS Dissolve 5 mg of trypsin inhibitor in 0.05 mol/L phosphate buffer solution, pH 7.0 to make 10 mL.

Trypsin TS for test of elcatonin Dissolve 5 mg of trypsin for liquid chromatography in 20 mL of a solution of ammonium hydrogen carbonate (1 in 100). Prepare before use.

Trypsin TS for test of ulinastatin Dissolve crystal trypsin for ulinastatin assay in ice-cooled 1 mmol/L hydrochloric acid TS containing 1 mmol/L calcium chloride dihydrate so that each mL of the solution contains 180 μ g of trypsin. Prepare before use, and preserve in an ice-cooled water bath.

L-Tryptophan $C_{11}H_{12}N_2O_2$ [Same as the namesake monograph]

Turpentine oil [Same as the namesake monograph]

L-Tyrosine $C_9H_{11}NO_3$ White, crystals or crystalline powder. Odorless and tasteless. Freely soluble in formic acid, very slightly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether. It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-10.5 - -12.5^\circ$ (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Loss on drying <2.41>: not more than 0.30% (1 g, 105°C, 3 hours).

Content: not less than 99.0%. **Assay**—Weigh accurately about 0.3 g of L-tyrosine, previously dried, dissolve in 6 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.12 mg of $C_9H_{11}NO_3$

Ubiquinone-9 Yellow to orange, crystalline powder. Odorless and no taste.

Melting point <2.60>: about 44°C

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (275 nm): 163 – 190 (ethanol (99.5))

Uracil $C_4H_4N_2O_2$ Needle crystals. Freely soluble in hot water, and slightly soluble in cold water.

Melting point <2.60>: 335°C

Uranyl acetate See uranyl acetate dihydrate.

Uranyl acetate dihydrate $UO_2(CH_3COO)_2 \cdot 2H_2O$ [K 8360: 1961, Special class]

Uranyl acetate TS Dissolve 1 g of uranyl acetate dihydrate in water to make 20 mL, and filter if necessary.

Uranyl acetate-zinc TS Dissolve 10 g of uranyl acetate dihydrate in 5 mL of acetic acid (31) and 50 mL of water by heating. Separately, dissolve 30 g of zinc acetate dihydrate in 3 mL of acetic acid (31) and 30 mL of water by heating.

While the two solutions are still warm, mix them, cool, and filter.

Urea H_2NCONH_2 [K 8731, Special class]

Urethane See ethyl carbamate.

Ursodeoxycholic acid $\text{C}_{24}\text{H}_{40}\text{O}_4$ [Same as the namesake monograph]

***n*-Valerianic acid** $\text{CH}_3(\text{CH}_2)_3\text{COOH}$ Clear, colorless to pale yellow liquid, having a characteristic odor. Miscible with ethanol (95) and with diethyl ether, and soluble in water.

Distilling range <2.57>: 186 – 188°C, not less than 98 vol%.

Specific gravity <2.56> d_4^{20} : 0.936 – 0.942

L-Valine $\text{C}_5\text{H}_{11}\text{NO}_2$ [Same as the namesake monograph]

H-D-Valyl-L-leucyl-L-arginine *p*-nitroanilide dihydrochloride $\text{C}_{23}\text{H}_{38}\text{N}_8\text{O}_5 \cdot 2\text{HCl}$ White to pale yellow, powder or masses. Sparingly soluble in water.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (316 nm): 214 – 236 (0.01 g, water, 500 mL).

Vanadium pentoxide See vanadium (V) oxide.

Vanadium pentoxide TS See vanadium (V) oxide TS.

Vanadium pentoxide TS, dilute See vanadium (V) oxide TS, dilute.

Vanadium (V) oxide V_2O_5 Orangish yellow to yellow-brown powder.

Identification—Dissolve 0.3 g in 10 mL of ammonia TS and 15 mL of water. To 2 mL of this solution add 20 mL of water, mix, and add gently 1 mL of copper (II) sulfate TS: yellow precipitates appear.

Vanadium (V) oxide TS Add vanadium (V) oxide to phosphoric acid, saturate with vanadium (V) oxide by shaking vigorously for 2 hours, and filter through a glass filter.

Vanadium (V) oxide TS, dilute Dilute 10 mL of vanadium (V) oxide TS with water to make 100 mL. Prepare before use.

Vanillin $\text{C}_6\text{H}_3\text{CHO}(\text{OCH}_3)(\text{OH})$ [K 9544]

Vanillin-hydrochloric acid TS Dissolve 5 mg of vanillin in 0.5 mL of ethanol (95), and to this solution add 0.5 mL of water and 3 mL of hydrochloric acid. Prepare before use.

Vanillin-sulfuric acid-ethanol TS Dissolve 3 g of vanillin in ethanol (99.5) to make 100 mL, and add 0.5 mL of sulfuric acid.

Vanillin-sulfuric acid TS Add cautiously 75 mL of sulfuric acid to 25 mL of ice-cold ethanol (95). After cooling, add 1 g of vanillin to dissolve. Prepare before use.

Vasopressin $\text{C}_{46}\text{H}_{65}\text{N}_{15}\text{O}_{12}\text{S}_2$ A white powder.

Constituent amino acids—Perform the test as directed in the Constituent amino acids under Oxytocin, and calculate the respective molar ratios with respect to glycine: 0.9 – 1.1 for aspartic acid, 0.9 – 1.1 for glutamic acid, 0.9 – 1.1 for proline, 0.8 – 1.1 for tyrosine, 0.9 – 1.1 for phenylalanine, 0.9 – 1.1 for arginine and 0.8 – 1.1 for cystine, and not more than 0.03 for other amino acids.

Vegetable oil Vegetative oils specified in monographs.

Verapamil hydrochloride for assay $\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$ [Same as the monograph Verapamil Hydrochloride. When dried, it contains not less than 99.0% of verapamil hydrochloride ($\text{C}_{27}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl}$).]

Vinblastine sulfate $\text{C}_{46}\text{H}_{58}\text{N}_4\text{O}_9 \cdot \text{H}_2\text{SO}_4$ [Same as the namesake monograph]

Vincristine sulfate $\text{C}_{46}\text{H}_{56}\text{N}_4\text{O}_{10} \cdot \text{H}_2\text{SO}_4$ [Same as the namesake monograph]

Vinyl acetate $\text{C}_4\text{H}_6\text{O}_2$ Clear, colorless liquid.

Specific gravity <2.56>: 0.932 – 0.936

Water <2.48>: not more than 0.2%

Vinyl chloride $\text{C}_2\text{H}_3\text{Cl}$ Colorless gas.

Boiling point <2.57>: -14°C

Melting point <2.60>: -160°C

2-Vinylpyridine $\text{C}_7\text{H}_7\text{N}$ A clear, colorless or dark brown liquid.

Refractive index <2.45> n_D^{20} : 1.546 – 1.552

Specific gravity <2.56> d_4^{20} : 0.975 – 0.982

1-Vinyl-2-pyrrolidone $\text{C}_6\text{H}_9\text{NO}$ Clear liquid.

Purity—Perform the test with 0.5 μL of 1-vinyl-2-pyrrolidone as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of the solutions by the automatic integration method, and calculate the amount of 1-vinyl-2-pyrrolidone by the area percentage method: it is not less than 99.0%.

Operating conditions

Detector: A hydrogen flame-ionization detector.

Column: A hollow, capillary glass column about 0.53 mm in inside diameter and about 30 m in length, having an about 1.0- μm layer of polyethylene glycol 20 M for gas chromatography on the inner side.

Column temperature: Maintain the temperature at 80°C for 1 minute, then raise at the rate of 10°C per minute to 190°C, and hold constant to the temperature for 20 minutes.

Temperature of sample vaporization chamber: A constant temperature of about 190°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 1-vinyl-2-pyrrolidone is about 15 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone from 0.5 μL of 1-vinyl-2-pyrrolidone is about 70% of the full scale.

Time span of measurement: About twice as long as the retention time of 1-vinyl-2-pyrrolidone beginning after the solvent peak.

Water <2.48>—Take 50 mL of methanol for Karl Fischer method and 10 mL of butyrolactone in a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 2.5 g of 1-vinyl-2-pyrrolidone, transfer immediately to a titration flask, and perform the test: water is not more than 0.1%.

V8 protease A protease obtained from *Staphylococcus aureus* strain. When an amount of the enzyme hydrolyzes 1 μmol of *N*-*t*-butoxycarbonyl-L-glutamic acid- α -phenyl ester in 1 minute at pH 7.8 and 37°C is defined as 1 unit, it contains 500 – 1000 units per mg.

V8 protease TS Dissolve V8 protease in water to make a solution of 1 mg/mL. Keep at a cold place and use within 6

days after preparation.

Voglibose for assay $C_{10}H_{21}NO_7$ [Same as the monograph Voglibose].

Warfarin potassium for assay [Same as the monograph Warfarin Potassium. When dried, it contains not less than 99.0% of warfarin potassium ($C_{19}H_{15}KO_4$).]

25% Water containing benzoyl peroxide See Benzoyl peroxide, 25% water containing.

Water for bacterial endotoxins test [Same as the monograph Water for Injection or water produced by other procedures that shows no reaction with the lysate reagent employed, at the detection limit of the reagent.]

Water for injection [Same as the namesake monograph]

Water, sterile purified [Same as the namesake monograph]

Weakly acidic CM-bridged cellulose cation exchanger (H type) Weakly acidic cation exchanger, intensified by crosslinking porous spherical cellulose, into which carboxymethyl groups have been introduced.

Wijs' TS Transfer 7.9 g of iodine trichloride and 8.9 g of iodine to separate flasks, dissolve each with acetic acid (100), mix both solutions, and add acetic acid (100) to make 1000 mL. Preserve in light-resistant, glass containers.

Wogonin for thin-layer chromatography $C_{16}H_{12}O_5$ Yellow crystals or crystalline powder. Slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water. Melting point: 204 – 208°C

Identification—Determine the absorption spectrum of a solution in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 207 nm and 211 nm, and between 273 nm and 277 nm.

Purity Related substances—Dissolve 1 mg in 1 mL of methanol, and perform the test with 10 μ L of this solution as directed in the Identification (3) under Saireito Extract: no spot other than the principal spot (*Rf* value is about 0.4) appears.

Xanthene $C_{13}H_{10}O$ White to light yellow crystals or crystalline powder, having a slight, characteristic odor.

Melting point <2.60>: 98 – 102°C

Water <2.48>: not more than 0.5% (0.15 g).

Xanthene-9-carboxylic acid $C_{14}H_{10}O_3$ Dissolve 0.25 g of propantheline bromide in 5 mL of water and 10 mL of sodium hydroxide TS, heat the mixture to boiling, then continue to heat for 2 minutes. Cool to 60°C, add 5 mL of dilute sulfuric acid, cool, filter the precipitate, and wash thoroughly with water. Recrystallize the residue from dilute ethanol, and dry for 3 hours in a desiccator (in vacuum, silica gel).

Melting point <2.60>: 217 – 222°C

Xanthone $C_{13}H_8O_2$ Light yellow powder. Freely soluble in chloroform, and slightly soluble in hot water and in diethyl ether.

Melting point <2.60>: 174 – 176°C

Purity Related substances—Dissolve 0.050 g of xanthone in chloroform to make exactly 10 mL. Perform the test with 5 μ L of this solution as directed in the Purity under Propantheline Bromide: any spot other than the principal spot at the *Rf*

value of about 0.7 does not appear.

Xanthydrol $C_{13}H_{10}O_2$ White to pale yellow powder. Dissolves in ethanol (95), in diethyl ether, in chloroform, and in acetic acid (100), and is practically insoluble in water.

Melting point <2.60>: 121 – 124°C

Residue on ignition <2.44>: not more than 2.0% (0.5 g).

Xylene $C_6H_4(CH_3)_2$ [K 8271, First class]

o-Xylene $C_6H_4(CH_3)_2$ Colorless, clear liquid.

Distilling range <2.57>: 143 – 146°C, not less than 95 vol%.

Refractive index <2.45> n_D^{20} : 1.501 – 1.506

Specific gravity <2.56> d_4^{20} : 0.875 – 0.885

Xylene cyanol FF $C_{25}H_{27}N_2NaO_7S_2$ [K 8272, Special class]

Xylenol orange $C_{31}H_{30}N_2Na_2O_{13}S$ [K 9563, Special class]

Xylenol orange TS Dissolve 0.1 g of xylenol orange in water to make 100 mL.

Xylitol $C_5H_{12}O_5$ [Same as the namesake monograph]

Xylose See D-xylose.

D-Xylose $C_5H_{10}O_5$ [Same as the monograph D-Xylose of the Japanese Standards of Food Additives]

Yeast extract A peptone-like substance which represents all the soluble product of yeast cells (*Saccharomyces*) prepared under optimum conditions, clarified, and dried by evaporating to a powder. Yeast extract (1 g) represents not less than 7.5 g of yeast. A reddish yellow to brown powder, having a characteristic but not putrescent odor. Soluble in water, forming a yellow to brown solution, having a slight acidic reaction. It contains no added carbohydrate.

Purity (1) Chloride <1.03> (calculated as NaCl): not more than 5%.

(2) Coagulable protein—On heating a solution of yeast extract (1 in 20) to boiling, no precipitate is produced.

Loss on drying <2.41>: not more than 5% (105°C, constant mass).

Residue on ignition <2.44>: not more than 15% (0.5 g).

Nitrogen content <1.08>: 7.2 – 9.5% (105°C, constant mass, after drying).

Yellow beeswax [Same as the namesake monograph]

Zaltoprofen $C_{17}H_{14}O_3S$ [Same as the namesake monograph]

Zaltoprofen for assay $C_{17}H_{14}O_3S$ [Same as the monograph Zaltoprofen. When dried, it contains not less than 99.5% of zaltoprofen ($C_{17}H_{14}O_3S$)].

Zinc Zn [K 8012, Special class]

Zinc acetate See zinc acetate dihydrate.

0.25 mol/L Zinc acetate buffer solution, pH 6.4 Dissolve 54.9 g of zinc acetate dihydrate in 150 mL of acetic acid (100) and 600 mL of water, add 150 mL of ammonia water (28), gently mix, and allow to cool to a room temperature. Adjust to pH 6.4 with ammonia water (28), and add water to make 1000 mL.

Zinc acetate dihydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ [K 8356,

Special class]

Zinc, arsenic-free See zinc for arsenic analysis.

Zinc chloride ZnCl_2 [K 8111, Special class]

Zinc chloride TS Dissolve 10 g of zinc chloride and 10 g of potassium hydrogen phthalate in 900 mL of water, adjust the pH to 4.0 with sodium hydroxide TS, and add water to make 1000 mL.

0.04 mol/L Zinc chloride TS Dissolve 5.453 g of zinc chloride in water to make 1000 mL.

Zinc diethyldithiocarbamate See Test Methods for Plastic Containers <7.02>.

Zinc dibutyldithiocarbamate See Test Methods for Plastic Containers <7.02>

Zinc disodium ethylenediamine tetraacetate See zinc disodium ethylenediamine tetraacetate tetrahydrate.

Zinc disodium ethylenediamine tetraacetate tetrahydrate $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Na}_2\text{O}_8\text{Zn}\cdot 4\text{H}_2\text{O}$ White powder. The pH of a solution of zinc disodium ethylenediamine tetraacetate (1 in 100) is between 6.0 and 9.0.

Purity Clarity and color of solution—Dissolve 0.10 g of zinc disodium ethylenediamine tetraacetate tetrahydrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

Content: not less than 98.0%. **Assay**—Dissolve about 0.5 g of zinc disodium ethylenediamine tetraacetate tetrahydrate, accurately weighed, in water to make exactly 100 mL. Pipet 10 mL of this solution, adjust the pH to about 2 with 80 mL of water and dilute nitric acid, and titrate <2.50> with 0.01 mol/L bismuth nitrate VS until the color of the solution changes from yellow to red (indicator: 2 drops of xylenol orange TS).

Each mL of 0.01 mol/L bismuth nitrate VS
= 4.717 mg of $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Na}_2\text{O}_8\text{Zn}\cdot 4\text{H}_2\text{O}$

Zinc dust See zinc powder.

Zinc for arsenic analysis Zn [K 8012] Use granules of about 800 μm .

Zinc iodide-starch TS To 100 mL of boiling water add a solution of 0.75 g of potassium iodide in 5 mL of water, a solution of 2 g of zinc chloride in 10 mL of water and a smooth suspension of 5 g of starch in 30 mL of water, with stirring. Continue to boil for 2 minutes, then cool.

Sensitivity—Dip a glass rod into a mixture of 1 mL of 0.1 mol/L sodium nitrite VS, 500 mL of water and 10 mL of hydrochloric acid, and touch on zinc iodide-starch paste TS: an apparently blue color appears.

Storage—Preserve in tightly stoppered bottles, in a cold place.

Zincon $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_6\text{S}$ [K 9517, Special class]

Zincon TS Dissolve 0.1 g of zincon in 2 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL.

Zinc powder Zn [K 8013, Special class]

Zinc (standard reagent) Zn [K 8005, Standard reagent for volumetric analysis]

Zinc sulfate See zinc sulfate heptahydrate.

Zinc sulfate for volumetric analysis See zinc sulfate heptahydrate.

Zinc sulfate heptahydrate $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$ [K 8953, Special class]

Zinc sulfate TS Dissolve 10 g of zinc sulfate heptahydrate in water to make 100 mL.

Zirconyl-alizarin red S TS Dissolve 0.2 g of zirconyl nitrate in 5 mL of dilute hydrochloric acid, add 10 mL of alizarin red S TS, and then add water to make 30 mL.

Zirconyl-alizarin S TS See zirconyl-alizarin red S TS.

Zirconyl nitrate See zirconyl nitrate dihydrate.

Zirconyl nitrate dihydrate $\text{ZrO}(\text{NO}_3)_2\cdot 2\text{H}_2\text{O}$ A white crystalline powder. Freely soluble in water.

Identification—(1) To 5 mL of a solution (1 in 20) add 5 mL of sodium hydroxide TS: a white, milky precipitate is formed.

(2) To 10 mL of a solution (1 in 20) add 10 mL of sulfuric acid, cool, and superimpose 2 mL of iron (II) sulfate TS: a brown ring is produced at the zone of contact.

9.42 Solid Supports/Column Packings for Chromatography

Aminopropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Cellulose for thin-layer chromatography Use a high-grade cellulose prepared for thin-layer chromatography.

Cellulose with fluorescent indicator for thin-layer chromatography Use cellulose for thin-layer chromatography containing a suitable fluorescent substance.

Cyanopropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Diethylaminoethyl cellulose for column chromatography Prepared for column chromatography.

Diethylaminoethyl group bound to synthetic polymer for liquid chromatography Produced by binding diethylaminoethyl group to a hydrophilic synthetic polymer, for liquid chromatography. Exchange volume is about 0.1 mg equivalents/ cm^3 .

Dimethylaminopropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Dimethylsilanized silica gel with fluorescent indicator for thin-layer chromatography Dimethylsilanized silica gel for thin-layer chromatography to which a fluorescent indicator is added.

Divinylbenzene-methacrylate co-polymer for liquid chromatography Prepared for liquid chromatography.

Fluorosilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linkage: 8 %) Prepared for

liquid chromatography.

Gel type strong acid ion-exchange resin for liquid chromatography (degree of cross-linkage: 6 %) Prepared for liquid chromatography.

Glycol etherified silica gel for liquid chromatography Glycol group is bound to silica gel for liquid chromatography.

Graphite carbon for gas chromatography Prepared for gas chromatography.

Hexasilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Hydrophilic silica gel for liquid chromatography Dioctylated porous silica gel prepared for liquid chromatography (5–10 μm in particle diameter).

Hydroxypropylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Neutral alumina for chromatography Prepared for chromatography (75 – 180 μm in particle diameter).

Neutral alumina for column chromatography Prepared for column chromatography.

Octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Octadecylsilanized silica gel for thin-layer chromatography Octadecylsilanized silica gel prepared for thin-layer chromatography.

Octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography Octadecylsilanized silica gel for thin-layer chromatography containing fluorescent indicator.

Octadecylsilanized silicone polymer coated silica gel for liquid chromatography Prepared for liquid chromatography.

Octylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Pentaethylenhexaaminated polyvinyl alcohol polymer bead for liquid chromatography Prepared for liquid chromatography.

Phenylated silica gel for liquid chromatography Prepared for liquid chromatography.

Phenylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Polyamide for column chromatography Prepared for column chromatography.

Polyamide for thin-layer chromatography Prepared for thin-layer chromatography.

Polyamide with fluorescent indicator for thin-layer chromatography Add a fluorescent indicator to polyamide for thin-layer chromatography.

Porous acrylonitrile-divinylbenzene copolymer for gas chromatography (pore diameter: 0.06 – 0.08 μm , 100 – 200

m^2/g) A porous acrylonitrile-divinylbenzene copolymer prepared for gas chromatography.

Porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0075 μm , 500 – 600 m^2/g) A porous ethylvinylbenzene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0075 μm , and surface area is 500 to 600 m^2 per g.

Porous polymer beads for gas chromatography Prepared for gas chromatography.

Porous silica gel for liquid chromatography A porous silica gel prepared for liquid chromatography.

Porous styrene-divinylbenzene copolymer for gas chromatography (average pore diameter: 0.0085 μm , 300 – 400 m^2/g) A porous styrene-divinylbenzene copolymer prepared for gas chromatography. The average pore diameter is 0.0085 μm , and surface area is 300 to 400 m^2/g .

Silica gel for gas chromatography A silica gel prepared for gas chromatography.

Silica gel for liquid chromatography A silica gel prepared for liquid chromatography.

Silica gel for thin-layer chromatography A silica gel prepared for thin-layer chromatography.

Silica gel for thin-layer chromatography (particle size 5–7 μm , with fluorescent indication) Prepared for high-performance thin-layer chromatography.

Silica gel with complex fluorescent indicator for thin-layer chromatography A silica gel for thin-layer chromatography containing suitable complex fluorescent indicators.

Silica gel with fluorescent indicator for thin-layer chromatography A silica gel for thin-layer chromatography containing a suitable fluorescent indicator.

Siliceous earth for chromatography A siliceous earth prepared for chromatography.

Siliceous earth for gas chromatography A siliceous earth prepared for gas chromatography.

Strongly acidic ion exchange resin for column chromatography Prepared for column chromatography.

Strongly acidic ion exchange resin for liquid chromatography Prepared for liquid chromatography.

Strongly acidic ion-exchange silica gel for liquid chromatography Prepared for liquid chromatography.

Styrene-divinylbenzene copolymer for liquid chromatography Prepared for liquid chromatography.

Synthetic magnesium silicate for column chromatography Prepared for column chromatography (150 – 250 μm in particle diameter).

Teflon for gas chromatography See Tetrafluoroethylene polymer for gas chromatography.

Tetrafluoroethylene polymer for gas chromatography Prepared for gas chromatography.

Trimethylsilanized silica gel for liquid chromatography Prepared for liquid chromatography.

Weakly acidic ion exchange resin for liquid chromatography Prepared for liquid chromatography.

Zeolite for gas chromatography (0.5 nm in pore diameter)
Zeolite prepared for gas chromatography.

9.43 Filter Papers, Filters for filtration, Test Papers, Crucibles, etc.

Filter paper [R 3801, Filter paper (for chemical analysis), Filter paper for qualitative analysis]

No.1: For bulky gelatinous precipitate

No.2: For moderate-sized precipitate

No.3: For fine precipitate

No.4: Hardened filter paper for fine precipitate

Filter paper for quantitative analysis [R 3801, Filter paper (for chemical analysis), Filter paper for quantitative analysis]

No. 5A: For bulky gelatinous precipitate

No. 5B: For moderate-sized precipitate

No. 5C: For fine precipitate

No. 6: Thin filter paper for fine precipitate

Porcelain crucible [R 1301, Porcelain crucible for chemical analysis]

Sintered glass filter [R 3503, Glass appliance for chemical analysis, Buchner funnel glass filter]

G3: 20–30 μm in pore size

G4: 5–10 μm in pore size

Blue litmus paper See litmus paper, blue.

Congo red paper Immerse filter paper in congo red TS, and air-dry.

Glass fiber See glass wool.

Glass wool [K 8251, Special class]

Lead acetate paper See lead (II) acetate paper.

Lead (II) acetate paper Usually, immerse strips of filter paper, 6 cm \times 8 cm in size, in lead (II) acetate TS, drain off the excess liquid, and dry the paper at 100°C, avoiding contact with metals.

Litmus paper, blue [K 9071, Litmus paper, Blue litmus paper]

Litmus paper, red [K 9071, Litmus paper, Red litmus paper]

Phosgene test paper Dissolve 5 g of 4-dimethylamino-benzaldehyde and 5 g of diphenylamine in 100 mL of ethanol (99.5). Immerse a filter paper 5 cm in width in this solution, and allow to dry spontaneously while the paper is suspended in a dark place under clear air. Then cut off the 5-cm portions from the upper side and lower side of the paper, and cut the remaining paper to a length of 7.5 cm.

Preserve in tight, light-resistant containers. Do not use the paper, which has changed to a yellow color.

Potassium iodate-starch paper Impregnate filter paper

with a mixture of equivalent volumes of a solution of potassium iodate (1 in 20) and freshly prepared starch TS, and dry in a clean room.

Storage—Preserve in a glass-stoppered bottle, protected from light and moisture.

Potassium iodide-starch paper Impregnate filter paper with freshly prepared potassium iodide-starch TS, and dry in a clean room. Store in a glass-stoppered bottle, protected from light and moisture.

Red litmus paper See litmus paper, red.

Turmeric paper Macerate 20 g of powdered dried rhizome of *Curcuma longa* Linné with four 100 mL-portions of cold water, decant the supernatant liquid each time, and discard it. Dry the residue at a temperature not over 100°C. Macerate the dried residue with 100 mL of ethanol (95) for several days, and filter. Immerse filter paper in this ethanol decoction, and allow the ethanol (95) to evaporate spontaneously in clean air.

Sensitivity—Dip a strip of turmeric paper, about 1.5 cm length, in a solution of 1 mg of boric acid in a mixture of 1 mL of hydrochloric acid and 4 mL of water, after 1 minute remove the paper from the liquid, and allow it to dry spontaneously: the yellow color changes to brown. When the strip is moistened with ammonia TS, the color of the strip changes to greenish black.

Zinc iodide-starch paper Impregnate the filter paper for volumetric analysis with freshly prepared zinc iodide-starch TS, and dry in the clean room. Preserve in a glass-stoppered bottle, protected from light and moisture.

9.44 Standard Particles, etc.

α -Alumina for specific surface area determination
 α -Al₂O₃ Prepared for specific surface area determination.

α -Alumina for thermal analysis α -Al₂O₃ Prepared for thermal analysis.

Calibration ball for particle density measurement
Calibration ball with a known volume prepared for measurement of particle density. The volume of the calibration ball must be accurately determined to the nearest 0.001 cm³.

Indium for thermal analysis In Prepared for thermal analysis.

Content: not less than 99.99%.

Nickel for thermal analysis [K 9062 (Nickel), Special class. Content: not less than 99.99%]

Standard particles for calibrating light-shielded automatic fine particle counter Use plastic spherical particles of known size and number.

Tin for thermal analysis Sn [K 8580 (Tin). Content: not less than 99.99%]

Measuring Instruments and Appliances, Thermometers, etc.

9.61 Optical Filters for Wavelength and Transmission Rate Calibration

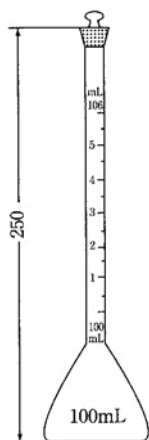
Use optical filters for wavelength calibration and those for transmission rate calibration shown in Table 9.61-1 and Table 9.61-2, respectively. The optical filters for transmission rate calibration are also used for the calibration of absorbances.

Table 9.61-1. Optical Filters for Wavelength Calibration

Type of filter	Range of wavelength calibration (nm)	Product name
Neodymium optical filter for wavelength calibration	400 - 750	JCRM 001
Holmium optical filter for wavelength calibration	250 - 600	JCRM 002

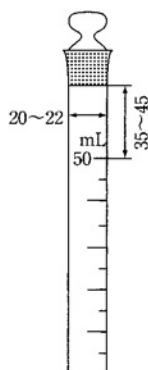
Table 9.61-2. Optical Filters for Transmission Rate Calibration

Type of filter	Transmission rate for calibration (%)	Product name
Optical filter for calibration within the visible wavelength range	1	JCRM 101
	10	JCRM 110
	20	JCRM 120
	30	JCRM 130
	40	JCRM 140
Optical filter for calibration within the ultraviolet wavelength range	10	JCRM 210 A
	30	JCRM 230 A
	50	JCRM 250 A
Optical filter for calibration within the near-ultraviolet wavelength range	10	JCRM 310
	30	JCRM 330
	50	JCRM 350



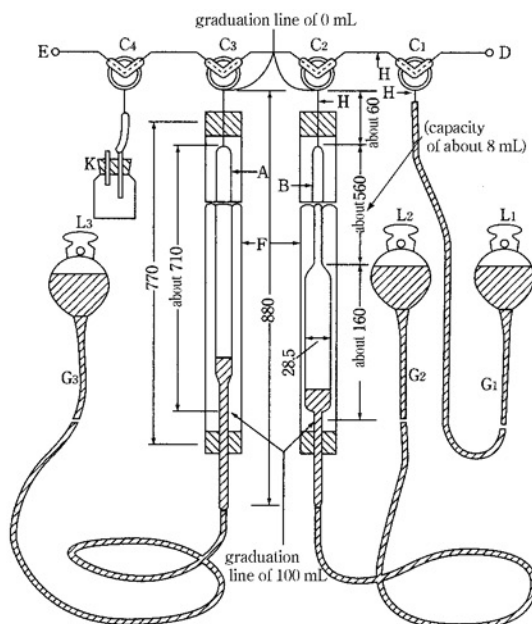
The figures are in mm.

Fig. 9.62-1



The figures are in mm.

Fig. 9.62-2



The figures are in mm.

- A: Gas buret (capacity of 100 mL, about 13.7 mm in inside diameter, graduated in 0.2 mL divisions, and graduated in 0.1 mL divisions at the lower, narrow part).
- B: Gas buret (capacity of 100 mL, about 4.2 mm in inside diameter at the upper stem with graduation in 0.02-mL division, about 28.5 mm in inside diameter at the lower stem with graduation in 1-mL divisions).
- C: (C₁, C₂, C₃ and C₄): Three-way stopcock.
- D: Inlet of sample (bent forward at 20 mm in length).
- E: Outlet of mixed gas (bent forward at 20 mm in length).
- F: Jacket (about 770 mm in length, about 40 mm in outside diameter, almost completely filled with water at room temperature).
- G: Rubber pressure tubing, about 4 mm in inside diameter (G₁: about 80 cm in length; G₂ and G₃: about 120 cm in length).
- H: Heavy-wall capillary tube (about 1 mm in inside diameter).
- K: Receiver.
- L: Leveling bulb (L₁: filled with about 50 mL of mercury; L₂ and L₃: filled with about 150 mL of mercury).

Fig. 9.62-3

9.62 Measuring Instruments, Appliances

Measuring Instruments are the instruments or machines used for measuring mass or volume in the JP tests, and Appliances are the instruments specified in order to make test conditions as consistent as possible in those tests.

Balances and weights (1) Chemical balances—Use

Table 9.62 Specification of Sieves

Sieve number	Nominal size (μm)	Specification of sieves				
		Sieve opening (mm)			Wire (mm)	
		Size (mm)	Permissible variation		Diameter	Permissible variation
			Average	Maximum		
3.5	5600	5.60	± 0.14	0.42	1.66	± 0.040
4	4750	4.75	± 0.118	0.41	1.60	± 0.040
4.7	4000	4.00	± 0.100	0.37	1.40	± 0.040
5.5	3350	3.35	± 0.100	0.32	1.27	± 0.030
6.5	2800	2.80	± 0.084	0.28	1.11	± 0.030
7.5	2360	2.36	± 0.070	0.24	1.03	± 0.030
8.6	2000	2.00	± 0.060	0.20	0.953	± 0.030
10	1700	1.70	± 0.051	0.17	0.840	± 0.025
12	1400	1.40	± 0.042	0.14	0.717	± 0.025
14	1180	1.18	± 0.035	0.14	0.634	± 0.025
16	1000	1.00	± 0.030	0.14	0.588	± 0.025
18	850	0.850	± 0.034	0.127	0.523	± 0.025
22	710	0.710	± 0.028	0.112	0.450	± 0.025
26	600	0.600	± 0.024	0.101	0.390	± 0.020
30	500	0.500	± 0.020	0.089	0.340	± 0.020
36	425	0.425	± 0.017	0.081	0.290	± 0.020
42	355	0.355	± 0.013	0.072	0.250	± 0.020
50	300	0.300	± 0.012	0.065	0.208	± 0.015
60	250	0.250	± 0.0099	0.058	0.173	± 0.015
70	212	0.212	± 0.0087	0.052	0.151	± 0.015
83	180	0.180	± 0.0076	0.047	0.126	± 0.015
100	150	0.150	± 0.0066	0.043	0.104	± 0.015
119	125	0.125	± 0.0058	0.038	0.088	± 0.015
140	106	0.106	± 0.0052	0.035	0.075	± 0.010
166	90	0.090	± 0.0046	0.032	0.063	± 0.010
200	75	0.075	± 0.0041	0.029	0.052	± 0.010
235	63	0.063	± 0.0037	0.026	0.045	± 0.005
282	53	0.053	± 0.0034	0.024	0.037	± 0.005
330	45	0.045	± 0.0034	0.022	0.032	± 0.005
391	38	0.038	± 0.0026	0.018	0.027	± 0.005

balances readable to the extent of 0.1 mg.

(2) Semimicrobalances—Use balances readable to the extent of 0.01 mg.

(3) Microbalances—Use balances readable to the extent of 0.001 mg.

(4) Weights—Use calibrated weights.

Cassia flask Use glass-stoppered flasks, shown in Fig. 9.62-1, made of hard glass and having graduation lines of volume on the neck.

Gas mixer Use the apparatus, shown in Fig. 9.62-3, made of hard glass.

Nessler tube Use colorless, glass-stoppered cylinders 1.0 to 1.5 mm in thickness, shown in Fig. 9.62-2, made of hard glass. The difference of the height of the graduation line of 50 mL from the bottom among cylinders does not exceed 2 mm.

Sieves Sieves conform to the specifications in Table 9.62. Use the sieve number of nominal size as the designation.

Volumetric measures Use volumetric flasks, transfer pipets, push-button micropipets, burets and measuring cylinders conforming to the Japanese Industrial Standard.

9.63 Thermometers

Thermometers Ordinarily, use calibrated thermometers with an immersion line (rod) or calibrated total immersion mercury-filled thermometers according to the Japanese Industrial Standards. Use the thermometers with the immersion line (rod), shown in Table 9.63, for the tests in Congealing Point, Melting Point (Method 1), Boiling Point and Distilling Range.

Table 9.63 Thermometers with Immersion Line

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Liquid	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury
Gas filled above liquid	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon	Nitrogen or Argon
Temperature range	-17 - 50°C	40 - 100°C	90 - 150°C	140 - 200°C	190 - 250°C	240 - 320°C
Minimum graduation	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C	0.2°C
Longer graduation lines at	each 1°C	each 1°C	each 1°C	each 1°C	each 1°C	1°C
Graduation numbered at	each 2°C	each 2°C	each 2°C	each 2°C	each 2°C	2°C
Total length (mm)	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300	280 - 300
Stem diameter (mm)	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3	6.0 ± 0.3
Bulb length (mm)	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18	12 - 18
Distance from bottom of bulb to graduation at the lowest temperature (mm)	75 - 90	75 - 90	75 - 90	75 - 90	75 - 90	75 - 90
Distance from top of thermometer to graduation at the highest temperature (mm)	35 - 65	35 - 65	35 - 65	35 - 65	35 - 65	35 - 65
Distance from bottom of bulb to immersion line (mm)	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62	58 - 62
From of top of thermometer	loop	loop	loop	loop	loop	loop
Test temperature	-15°C, 15°C, 45°C	45°C, 70°C, 95°C	95°C, 120°C, 145°C	145°C, 170°C, 195°C	195°C, 220°C, 245°C	245°C, 280°C, 315°C
Maximum scale error at any point	0.2°C	0.2°C	0.2°C	0.2°C	0.3°C (0.2°C, at 195°C of test temperature)	0.4°C (0.5°C, at 315°C of test temperature)

Official Monographs

Absorptive Ointment

吸水軟膏

Method of preparation

White Petrolatum	400 g
Cetanol	100 g
White Beeswax	50 g
Sorbitan Sesquioleate	50 g
Lauromacrogol	5 g
Ethyl Parahydroxybenzoate or Methyl Parahydroxybenzoate	1 g
Butyl Parahydroxybenzoate or Propyl Parahydroxybenzoate	1 g
Purified Water	a sufficient quantity

To make 1000 g

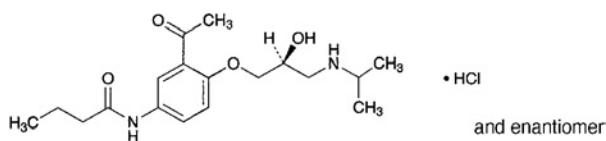
Melt White Petroleum, Cetanol, White Beeswax, Sorbitan Sesquioleate and Lauromacrogol by heating on a water bath, mix and maintain at about 75°C. Add Methyl Parahydroxybenzoate or Ethyl Parahydroxybenzoate and Propyl Parahydroxybenzoate or Butyl Parahydroxybenzoate to Purified Water, dissolve by warming at 80°C. Combine both solutions, mix to make emulsion, cool, and stir thoroughly until it congeals.

Description Absorptive Ointment is white in color and is lustrous. It has a slightly characteristic odor.

Containers and storage Containers—Tight containers.

Acebutolol Hydrochloride

アセブトロール塩酸塩



$C_{18}H_{28}N_2O_4 \cdot HCl$: 372.89

N-{3-Acetyl-4-[(2*RS*)-2-hydroxy-3-(1-methylethyl)aminopropoxy]phenyl}butanamide monohydrochloride [34381-68-5]

Acebutolol Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{18}H_{28}N_2O_4 \cdot HCl$.

Description Acebutolol Hydrochloride occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Acebutolol Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Acebutolol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acebutolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Acebutolol Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 141 – 145°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Acebutolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Acebutolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Acebutolol Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 25 mL, and pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

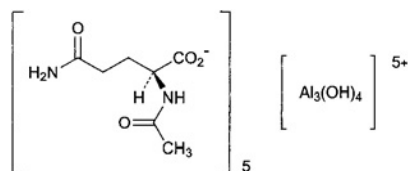
Assay Weigh accurately about 0.25 g of Acebutolol Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.29 mg of $C_{18}H_{28}N_2O_4 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Aceglutamide Aluminum

アセグルタミドアルミニウム



$C_{35}H_{59}Al_3N_{10}O_{24}$: 1084.84

Pentakis[(2*S*)-2-acetylamino-4-carbamoylbutanoato]tetrahydroxotrialuminium
[12607-92-0]

Aceglutamide Aluminum contains not less than 85.4% and not more than 87.6% of aceglutamide ($C_7H_{12}N_2O_4$: 188.18), and not less than 7.0% and not more than 8.0% of aluminum (Al: 26.98), calculated on the dried basis.

Description Aceglutamide Aluminum occurs as a white powder, having astringent bitter taste.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification (1) Dissolve 0.03 g each of Aceglutamide Aluminum and Aceglutamide Reference Standard in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of bromocresol green in ethanol (95) (1 in 1000), then spray evenly diluted ammonia solution (28) (1 in 100): the spots from the sample solution and the standard solution show a light yellow and have the same R_f value.

(2) A solution of Aceglutamide Aluminum in dilute hydrochloric acid (1 in 20) responds to the Qualitative Tests <1.09> for aluminum salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-5.5 - -7.5^\circ$ (2 g calculated on the dried basis, water, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Put 1.0 g of Aceglutamide Aluminum in a porcelain crucible, cover the crucible loosely, and heat gently to carbonize. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat gently until the white fumes no more evolve, and heat to incinerate at 500 to 600°C. If the incineration is not accomplished, add 2 mL of nitric acid and 1 mL of sulfuric acid, heat in the same manner as above, then ignite at 500 to 600°C to incinerate. After cooling, add 2 mL of hydrochloric acid, proceed with this solution according to Method 2, and perform the test. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution with the same amount of the reagents, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20

ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aceglutamide Aluminum according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Aceglutamide Aluminum in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-acetamidoglutarimide in the mobile phase to make exactly 100 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 2-acetamidoglutarimide from the sample solution is not more than that from the standard solution (2), the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution are not more than 3/10 times the peak area of aceglutamide from the standard solution (1), and the total of the peak areas other than aceglutamide and 2-acetamidoglutarimide from the sample solution is not more than the peak area of aceglutamide from the standard solution (1).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of aceglutamide.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution (1) add the mobile phase to make exactly 50 mL. Confirm that the peak area of aceglutamide obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of aceglutamide obtained from 20 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay (1).

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of aceglutamide is not more than 2.0%.

Loss on drying <2.41> Not more than 5.0% (1 g, 130°C, 5 hours).

Assay (1) Aceglutamide—Weigh accurately about 50 mg of Aceglutamide Aluminum, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Aceglutamide Reference Standard, dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of aceglutamide to that of the internal standard.

Amount (mg) of aceglutamide ($C_7H_{12}N_2O_4$)

$$= W_s \times (Q_T/Q_S)$$

W_s : Amount (mg) of Aceglutamide Reference Standard

Internal standard solution—A solution of thymine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted perchloric acid (1 in 1000) and methanol (99:1).

Flow rate: Adjust the flow rate so that the retention time of aceglutamide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, aceglutamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aceglutamide to that of the internal standard is not more than 1.0%.

(2) **Aluminum**—Weigh accurately about 3.0 g of Aceglutamide Aluminum, add 20 mL of dilute hydrochloric acid, and heat on a water bath for 60 minutes. After cooling, add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <2.50> with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red (indicator: 2 mL of dithizone TS). Perform a blank determination in the same manner.

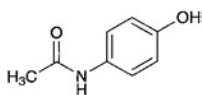
Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 1.349 mg of Al

Containers and storage Containers—Tight containers.

Acetaminophen

Paracetamol

アセトアミノフェン



$C_8H_9NO_2$: 151.16

N-(4-Hydroxyphenyl)acetamide [103-90-2]

Acetaminophen, when dried, contains not less than 98.0% of $C_8H_9NO_2$.

Description Acetaminophen occurs as white crystals or

crystalline powder.

It is freely soluble in methanol and in ethanol (95), sparingly soluble in water, and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification Determine the infrared absorption spectra of Acetaminophen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Acetaminophen Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 172°C

Purity (1) Chloride <1.03>—Dissolve 4.0 g of Acetaminophen in 100 mL of water by heating, cool with shaking in ice water, allow to stand until ordinary temperature is attained, add water to make 100 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) **Sulfate** <1.14>—To 25 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(3) **Heavy metals** <1.07>—Proceed with 2.0 g of Acetaminophen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) **Arsenic** <1.11>—Prepare the test solution with 1.0 g of Acetaminophen according to Method 3, and perform the test (not more than 2 ppm).

(5) **Related substances**—Dissolve 50 mg of Acetaminophen in 1 mL of methanol, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the peak area of acetaminophen from the sample solution is not larger than the peak area of acetaminophen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate, pH 4.7 and methanol (4:1)

Flow rate: Adjust the flow rate so that the retention time of acetaminophen is about 5 minutes.

Selection of column: Dissolve 0.01 g each of Acetaminophen and *p*-aminophenol in 1 mL of methanol, add the mobile phase to make 50 mL, to 1 mL of this solution

add the mobile phase to make 10 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of *p*-aminophenol and acetaminophen in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of acetaminophen obtained from 10 μ L of the standard solution is about 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of acetaminophen beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.3% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Acetaminophen and Acetaminophen Reference Standard, previously dried, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 3 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at the wavelength of maximum absorption at about 244 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\text{Amount (mg) of } C_8H_9NO_2 = W_S \times (A_T/A_S)$$

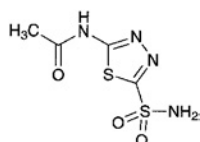
W_S : Amount (mg) of Acetaminophen Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Acetazolamide

アセタゾラミド



$C_4H_6N_4O_3S_2$: 222.25

N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide [59-66-5]

Acetazolamide contains not less than 98.0% and not more than 102.0% of $C_4H_6N_4O_3S_2$, calculated on the dried basis.

Description Acetazolamide occurs as a white to pale yellowish white, crystalline powder. It is odorless, and has a slight bitter taste.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: about 255°C (with decomposition).

Identification (1) To 0.1 g of Acetazolamide add 5 mL of sodium hydroxide TS, then add 5 mL of a solution of 0.1 g of hydroxylammonium chloride and 0.05 g of copper (II) sulfate pentahydrate in 10 mL of water: a light yellow color develops. Then heat this solution for 5 minutes: a deep yellow color is produced gradually.

(2) To 0.02 g of Acetazolamide add 2 mL of dilute hydrochloric acid, boil for 10 minutes, cool, and add 8 mL of water: this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) To 0.2 g of Acetazolamide add 0.5 g of granulated zinc and 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moistened lead (II) acetate paper.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Acetazolamide in 10 mL of sodium hydroxide TS: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—To 1.5 g of Acetazolamide add 75 mL of water, and warm at 70°C for 20 minutes with occasional shaking. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Sulfate <1.14>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Acetazolamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Silver-reducing substances—Wet 5 g of Acetazolamide with 5 mL of aldehyde-free ethanol, and add 125 mL of water, 10 mL of nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS. Stir for 30 minutes by protecting from light, filter through a glass filter (G3), and wash the residue on the glass filter with two 10-mL portions of water. Combine the filtrate with the washings, to the solution add 5 mL of ferric ammonium sulfate TS, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS: not less than 4.8 mL of 0.1 mol/L ammonium thiocyanate VS is consumed.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.15 g of Acetazolamide, and dissolve in 400 mL of water in a water bath by heating. After cooling, add water to make exactly 1000 mL. Pipet 5 mL of the solution, add 10 mL of 1 mol/L hydrochloric acid TS, and then add water to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_4H_6N_4O_3S_2 = (A/474) \times 200,000$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Acetic Acid

酢酸

Acetic Acid contains not less than 30.0 w/v% and

not more than 32.0 w/v% of $C_2H_4O_2$: 60.05.

Description Acetic Acid is a clear, colorless liquid. It has a pungent, characteristic odor and an acid taste.

It is miscible with water, with ethanol (95) and with glycerin.

Specific gravity d_{20}^{20} : about 1.04

Identification Acetic Acid changes blue litmus paper to red, and responds to the Qualitative Tests <1.09> for acetate.

Purity (1) Chloride—To 20 mL of Acetic Acid add 40 mL of water, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no opalescence is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07>—Evaporate 10 mL of Acetic Acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 30 mL of Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 5 mL of Acetic Acid, add 30 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 60.05 mg of $C_2H_4O_2$

Containers and storage Containers—Tight containers.

Glacial Acetic Acid

氷酢酸

$C_2H_4O_2$: 60.05

Acetic acid [64-19-7]

Glacial Acetic Acid contains not less than 99.0% of $C_2H_4O_2$.

Description Glacial Acetic Acid is a clear, colorless, volatile liquid, or colorless or white, crystalline masses. It has a pungent, characteristic odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

Boiling point: about 118°C

Specific gravity d_{20}^{20} : about 1.049

Identification A solution of Glacial Acetic Acid (1 in 3) changes blue litmus paper to red, and responds to the Qualitative Tests <1.09> for acetate.

Congearing point <2.42> Not below 14.5°C.

Purity (1) Chloride—To 10 mL of Glacial Acetic Acid add water to make 100 mL, and use this solution as the sample solution. To 10 mL of the sample solution add 5 drops of silver nitrate TS: no opalescence is produced.

(2) Sulfate—To 10 mL of the sample solution obtained in (1) add 1 mL of barium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07>—Evaporate 2.0 mL of Glacial Acetic Acid on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2.0 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Potassium permanganate-reducing substances—To 20 mL of the sample solution obtained in (1) add 0.10 mL of 0.1 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(5) Non-volatile residue—Evaporate 10 mL of Glacial Acetic Acid on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

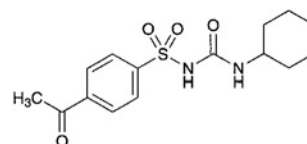
Assay Place 10 mL of water in a glass-stoppered flask, and weigh accurately. Add about 1.5 g of Glacial Acetic Acid, weigh accurately again, then add 30 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 60.05 mg of $C_2H_4O_2$

Containers and storage Containers—Tight containers.

Acetohexamide

アセトヘキサミド



$C_{15}H_{20}N_2O_4S$: 324.40

4-Acetyl-N-(cyclohexylcarbamoyl)benzenesulfonamide
[968-81-0]

Acetohexamide, when dried, contains not less than 98.0% and not more than 101.0% of $C_{15}H_{20}N_2O_4S$.

Description Acetohexamide occurs as a white to yellowish white powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 185°C (with decomposition).

Identification (1) Dissolve 0.10 g of Acetohexamide in 100 mL of methanol. To 5 mL of the solution add 20 mL of 0.5 mol/L hydrochloric acid TS and 75 mL of methanol, and use the solution as the sample solution (1). Determine the absorption spectrum of the sample solution (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>, using methanol as the blank, and compare the spectrum with the

Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to exactly 10 mL of the sample solution (1) add methanol to make exactly 50 mL, and use the solution as the sample solution (2). Determine the absorption spectrum of the sample solution (2) as directed under Ultraviolet-visible Spectrophotometry <2.24>, using methanol as the blank, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acetohexamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <1.03>—Dissolve 1.5 g of Acetohexamide in 40 mL of *N,N*-dimethylformamide, add 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL (not more than 0.011%).

(2) **Sulfate <1.14>**—Dissolve 2.0 g of Acetohexamide in 40 mL of *N,N*-dimethylformamide, and add 1 mL of dilute hydrochloric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and *N,N*-dimethylformamide to make 50 mL (not more than 0.010%).

(3) **Heavy metals <1.07>**—Proceed with 1.0 g of Acetohexamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) **Related substances (i) Cyclohexylamine**—Dissolve exactly 1.0 g of Acetohexamide in exactly 30 mL of 0.5 mol/L sodium hydroxide TS, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the sample solution. Separately, dissolve exactly 50 mg of cyclohexylamine in 0.5 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, and add 0.5 mol/L sodium hydroxide TS to make exactly 300 mL. Pipet 30 mL of this solution, add exactly 5 mL of hexane, shake vigorously for 60 minutes, allow to stand for 5 minutes, and use the upper layer as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area of cyclohexylamine by the automatic integration method: the peak area of cyclohexylamine is not more than that with the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A fused-silica column 0.53 mm in inside diameter and 30 m in length, coated the inner surface with methylsilicone polymer for gas chromatography 1.5 μ m in thickness.

Column temperature: A constant temperature of about 90°C.

Injection port temperature: A constant temperature of about 150°C.

Detector temperature: A constant temperature of about

210°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of cyclohexylamine is about 4 minutes.

Split ratio: 1:1

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cyclohexylamine is not less than 8000.

System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cyclohexylamine is not more than 5%.

(ii) **Dicyclohexylurea**—Dissolve exactly 1.0 g of Acetohexamide in exactly 10 mL of 0.5 mol/L sodium hydroxide TS, add exactly 20 mL of methanol, shake, then add exactly 5 mL of diluted hydrochloric acid (1 in 10), shake vigorously for 15 minutes, and centrifuge. Filter 10 mL or more of the supernatant liquid through a membrane filter with pore size of not larger than 0.5 μ m. Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dissolve exactly 50 mg of dicyclohexylurea in methanol to make exactly 100 mL. Pipet 2 mL of this solution, and add methanol to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 10 mL of 0.5 mol/L sodium hydroxide TS, shake, then add exactly 5 mL of diluted hydrochloric acid (1 in 10), shake, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of dicyclohexylurea by the automatic integration method: the peak area of dicyclohexylurea is not more than that with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.5 g of sodium hydroxide in 1000 mL of 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 6.5 with 0.5 mol/L sodium hydroxide TS. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of dicyclohexylurea is about 10 minutes.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of dicyclohexylurea is not less than 10,000.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dicyclohexylurea is not more than 2.0%.

(iii) **Other related substances**—Dissolve 0.10 g of Acetohexamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet two 1 mL portions of

this solution, add acetone to make exactly 10 mL and 25 mL, respectively, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol, ammonia solution (28) and cyclohexane (6:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than 4.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

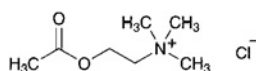
Assay Weigh accurately about 0.3 g of Acetohexamide, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination using a solution prepared by adding 19 mL of water to 30 mL of *N,N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 32.44 mg of $C_{15}H_{20}N_2O_4S$

Containers and storage Containers—Well-closed containers.

Acetylcholine Chloride for Injection

注射用アセチルコリン塩化物



$C_7H_{16}ClNO_2$: 181.66

2-Acetoxy-*N,N,N*-trimethylethylammonium chloride
[60-31-1]

Acetylcholine Chloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 98.0% and not more than 102.0% of acetylcholine chloride ($C_7H_{16}ClNO_2$), and not less than 19.3% and not more than 19.8% of chlorine (Cl: 35.45), calculated on the dried basis.

It contains not less than 93% and not more than 107% of the labeled amount of acetylcholine chloride ($C_7H_{16}ClNO_2$).

Method of preparation Prepare as directed under Injections.

Description Acetylcholine Chloride for Injection occurs as white crystals or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is extremely hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Acetylcholine Chloride for Injection, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Acetylcholine Chloride for Injection (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 149 – 152°C. Seal Acetylcholine Chloride for Injection in a capillary tube for melting point immediately after drying both of the sample and the tube at 105°C for 3 hours, and determine the melting point.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Acetylcholine Chloride for Injection in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.10 g of Acetylcholine Chloride for Injection in 10 mL of freshly boiled and cooled water, and add 1 drop of bromothymol blue TS, and 0.30 mL of 0.01 mol/L sodium hydroxide VS: the solution is blue in color.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Acetylcholine Chloride for Injection according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Acetylcholine chloride—Weigh accurately the contents of not less than 10 Acetylcholine Chloride for Injections. Weigh accurately about 0.5 g of the contents, dissolve in 15 mL of water, then add exactly 40 mL of 0.1 mol/L sodium hydroxide VS, stopper loosely, and heat on a water bath for 30 minutes. Cool quickly, and titrate <2.50> the excess sodium hydroxide with 0.05 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.17 mg of $C_7H_{16}ClNO_2$

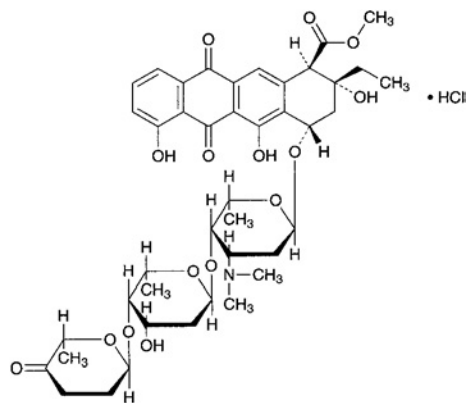
(2) Chlorine—Titrate <2.50> the solution, which has been titrated in (1), with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 3.545 mg of Cl

Containers and storage Containers—Hermetic containers.

Aclarubicin Hydrochloride

アクリルビシン塩酸塩



$C_{42}H_{53}NO_{15} \cdot HCl$: 848.33

Methyl (1*R*,2*R*,4*S*)-4-{2,6-dideoxy-4-*O*-[(2*R*,6*S*)-6-methyl-5-oxo-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]- α -*L*-*lyxo*-hexopyranosyl-(1 \rightarrow 4)-2,3,6-trideoxy-3-dimethylamino- α -*L*-*lyxo*-hexopyranosyloxy}-2-ethyl-2,5,7-trihydroxy-6,11-dioxo-1,2,3,4-tetrahydrotetracene-1-carboxylate monohydrochloride [75443-99-0]

Aclarubicin Hydrochloride is the hydrochloride of an anthracycline substance having antitumor activity produced by the growth of *Streptomyces galilaeus*.

It contains not less than 920 μ g (potency) and not more than 975 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Aclarubicin Hydrochloride is expressed as mass (potency) of aclarubicin ($C_{42}H_{53}NO_{15}$: 811.87).

Description Aclarubicin Hydrochloride occurs as a yellow to pale orange-yellow powder.

It is very soluble in chloroform and in methanol, freely soluble in water, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aclarubicin Hydrochloride in diluted methanol (4 in 5) (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aclarubicin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Aclarubicin Hydrochloride in methanol (1 in 200) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-146 - -162^\circ$ (50 mg calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.05 g of Aclarubicin Hydrochloride in 10 mL of water is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Aclarubicin Hydrochloride in 10 mL of water: the solution is clear and yellow to pale orange-yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Aclarubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Aclarubicin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the area percentage method: the amount of aklavinone having the relative retention time of about 0.6 to aclarubicin is not more than 0.2%, aclacinomycin L1 having the relative retention time of about 0.75 to aclarubicin is not more than 0.5%, 1-deoxypyrrromycin having the relative retention time of about 1.7 to aclarubicin is not more than 1.5% and aclacinomycin S1 having the relative retention time of about 2.3 to aclarubicin is not more than 0.5%, and the total amount of the peaks other than aclarubicin and the peaks mentioned above is not more than 1.0% of the peak area of aclarubicin.

Operating conditions—

Detector: A visible absorption photometer (wavelength: 436 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of chloroform, methanol, acetic acid (100), water and triethylamine (6800:2000:1000:200:1).

Flow rate: Adjust the flow rate so that the retention time of aclarubicin is about 5 minutes.

Time span of measurement: As long as about 4 times of the retention time of aclarubicin beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of aclarubicin obtained from 20 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L of the solution for system suitability test.

System performance: Dissolve 5 mg of Aclarubicin Hydrochloride in 10 mL of 0.1 mol/L hydrochloric acid TS, and allow to stand for 60 minutes. To 1.0 mL of this solution add 1.0 mL of 0.2 mol/L sodium hydroxide TS, 1.0 mL of phosphate buffer solution, pH 8.0 and 1.0 mL of chloroform, shake vigorously, and take the chloroform layer. When the procedure is run with 20 μ L of the chloroform under the above operating conditions, aclarubicin and 1-deoxypyrrromycin are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 5 times with 20 μ L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of

aclarubicin is not more than 2.0%.

Water <2.48> Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aclarubicin Hydrochloride, equivalent to about 20 mg (potency), and dissolve in diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Aclarubicin Reference Standard, equivalent to about 20 mg (potency), add 0.6 mL of diluted hydrochloric acid (1 in 250) and diluted methanol (4 in 5) to make exactly 100 mL. Pipet 15 mL of this solution, add diluted methanol (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S , at 433 nm.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of aclarubicin (C}_{42}\text{H}_{53}\text{NO}_{15}) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Aclarubicin Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant and at 5°C or below.

Acrinol and Zinc Oxide Oil

アクリノール・チンク油

Method of preparation

Acrinol, very finely powdered	10 g
Zinc Oxide Oil	990 g
To make 1000 g	

Prepare by mixing the above ingredients.

Description Acrinol and Zinc Oxide Oil is a yellowish white, slimy substance. Separation of a part of its ingredients occurs on prolonged standing.

Identification (1) Shake well 1 g of Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, filter after thorough shaking, and to the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(3) Shake well 0.2 g of Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centrifuge, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of acrinol in 50 mL of ethanol (95)

and 2.5 mL of acetic acid (100), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and standard solution exhibit a blue fluorescence and show the same R_f value.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Compound Acrinol and Zinc Oxide Oil

複方アクリノール・チンク油

Method of preparation

Acrinol, very finely powdered	10 g
Zinc Oxide Oil	650 g
Ethyl Aminobenzoate, finely powdered	50 g
White Beeswax	20 g
Hydrophilic Petrolatum	270 g
To make 1000 g	

Prepare by mixing the above ingredients.

Description Compound Acrinol and Zinc Oxide Oil is light yellow to yellow in color.

Identification (1) Shake well 1 g of Compound Acrinol and Zinc Oxide Oil with 10 mL of diethyl ether, 2 mL of acetic acid (100) and 10 mL of water, and separate the water layer. Shake the layer with 5 mL of hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color is produced (acrinol).

(2) Place 1 g of Compound Acrinol and Zinc Oxide Oil in a crucible, melt by warming, heat, gradually raising the temperature until the mass is thoroughly charred, and then ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

(3) Shake well 0.2 g of Compound Acrinol and Zinc Oxide Oil with 20 mL of ethanol (95) and 1 mL of acetic acid (100), centrifuge, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of acrinol and 25 mg of ethyl aminobenzoate in 50 mL of ethanol (95) and in 2.5 mL of acetic acid (100), respectively, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and standard solution (1) exhibit a blue fluorescence, and show the same R_f value. Also examine under ultraviolet

light (main wavelength: 254 nm): the spots from the sample solution and standard solution (2) exhibit a purple color, and show the same *R_f* value.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Acrinol and Zinc Oxide Ointment

アクリノール・亜鉛華軟膏

Method of preparation

Acrinol, very finely powdered	10 g
Zinc Oxide Ointment	990 g
To make 1000 g	

Prepare as directed under Ointments, with the above ingredients.

Description Acrinol and Zinc oxide Ointment is yellow in color.

Identification (1) Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 2 to 3 drops of sodium nitrite TS, and allow to stand: a dark red color develops in the water layer (acrinol).

(2) Ignite 0.5 g of Acrinol and Zinc Oxide Ointment to char, and dissolve the residue in 5 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for zinc salt.

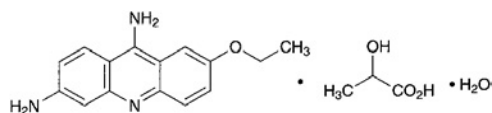
(3) Shake 0.5 g of Acrinol and Zinc Oxide Ointment with 5 mL of diethyl ether, 1 mL of acetic acid (100) and 5 mL of water, separate the water layer, and use the water layer as the sample solution. Dissolve 5 mg of acrinol in 1 mL of acetic acid (100) and 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95) and acetic acid (100) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots from the sample solution and the standard solution exhibit a blue fluorescence and show the same *R_f* value.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Acrinol Hydrate

Ethacridine Lactate

アクリノール水和物



$C_{15}H_{15}N_3O \cdot C_3H_6O_3 \cdot H_2O$: 361.39
2-Ethoxy-6,9-diaminoacridine monolactate monohydrate
[1837-57-6]

Acrinol Hydrate contains not less than 98.5% and not more than 101.0% of acrinol ($C_{15}H_{15}N_3O \cdot C_3H_6O_3$: 343.38), calculated on the anhydrous basis.

Description Acrinol Hydrate occurs as a yellow, crystalline powder.

It is sparingly soluble in water, in methanol and in ethanol (99.5).

Melting point: about 245°C (with decomposition).

The pH of a solution of Acrinol Hydrate (1 in 100) is between 5.5 and 7.0.

Identification (1) Determine the absorption spectrum of a solution of Acrinol Hydrate (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Acrinol Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Acrinol Hydrate (1 in 100) add 5 mL of dilute sulfuric acid, shake well, allow to stand for about 10 minutes at room temperature, and filter: the filtrate responds to the Qualitative Tests <1.09> for lactate.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Acrinol Hydrate in 80 mL of water by warming on a water bath, cool, and add 10 mL of sodium hydroxide TS and water to make 100 mL. Shake well, allow to stand for 30 minutes, filter, to 40 mL of the filtrate add 7 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare 50 mL of the control solution with 4 mL of sodium hydroxide TS, 7 mL of dilute nitric acid, 0.30 mL of 0.01 mol/L hydrochloric acid VS and sufficient water (not more than 0.026%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Acrinol Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Volatile fatty acids—Dissolve 0.5 g of Acrinol Hydrate in a mixture of 20 mL of water and 5 mL of dilute sulfuric acid, shake well, filter, and heat the filtrate: no odor of volatile fatty acids is perceptible.

(4) Related substances—Dissolve 10 mg of Acrinol Hy-

drate in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 10 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than acrinol is not larger than 3 times the peak area of acrinol obtained with the standard solution (2), and the total area of the peaks other than acrinol is not larger than the peak area of acrinol with the standard solution (1).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.8 g of sodium dihydrogen phosphate in 900 mL of water, adjust to pH 2.8 with phosphoric acid, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography, and add 1.0 g of sodium 1-octanesulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of acrinol is about 15 minutes.

Time span of measurement: About 3 times as long as the retention time of acrinol beginning after the solvent peak.

System suitability—

Test for required detectability: Confirm that the peak area of acrinol obtained with 10 μ L of the standard solution (2) is equivalent to 7 to 13% of that with 10 μ L of the standard solution (1).

System performance: When the procedure is run with 10 μ L of the standard solution (1) under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of acrinol are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of acrinol is not more than 1.5%.

Water <2.48> 4.5 – 5.5% (0.2 g, volumetric titration, direct titration)

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.27 g of Acrinol Hydrate, dissolve in 5 mL of formic acid, add 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

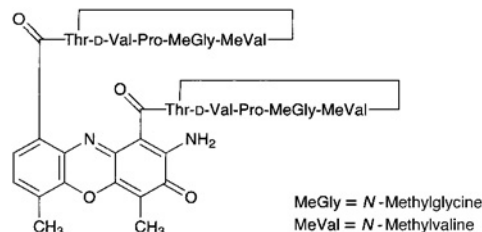
Each mL of 0.1 mol/L perchloric acid VS
= 34.34 mg of acrinol (C₁₅H₁₅N₃O·C₃H₆O₃)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Actinomycin D

アクチノマイシン D



C₆₂H₈₆N₁₂O₁₆: 1255.42
[50-76-0]

Actinomycin D is a peptide substance having antitumor activity produced by the growth of *Streptomyces parvulus*.

It, when dried, contains not less than 950 μ g (potency) and not more than 1030 μ g (potency) per mg. The potency of Actinomycin D is expressed as mass (potency) of actinomycin D (C₆₂H₈₆N₁₂O₁₆).

Description Actinomycin D occurs as an orange-red to red crystalline powder.

It is freely soluble in acetone, sparingly soluble in acetonitrile and in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Actinomycin D in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Actinomycin D Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g each of Actinomycin D and Actinomycin D Reference Standard in 10 mL of acetone, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and methanol (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spot from the sample solution is the same as that from the standard solution.

Optical rotation <2.49> [α]_D²⁰: -292 – -317° (after drying, 10 mg, methanol, 10 mL, 100 mm).

Loss on drying <2.41> Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Actinomycin D and Actinomycin D Reference Standard, previously dried, equivalent to about 60 mg (potency), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the

test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of actinomycin D, A_T and A_S , of both solutions.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{62}\text{H}_{86}\text{N}_{12}\text{O}_{16} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Actinomycin D Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L acetic acid-sodium acetate TS and acetonitrile (25:23).

Flow rate: Adjust the flow rate so that the retention time of actinomycin D is about 23 minutes.

System suitability—

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of actinomycin D are not less than 2000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of actinomycin D is not more than 2.0%.

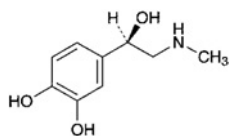
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Adrenaline

Epinephrine

アドレナリン



$\text{C}_9\text{H}_{13}\text{NO}_3$: 183.20

4-[(1*R*)-1-Hydroxy-2-(methylamino)ethyl]benzene-1,2-diol [51-43-4]

Adrenaline, when dried, contains not less than 98.0% of $\text{C}_9\text{H}_{13}\text{NO}_3$.

Description Adrenaline occurs as a white to grayish white, crystalline powder. It has no odor.

It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in methanol, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

It gradually changes to brown in color by air and by light.

Identification (1) Dissolve 0.01 g of Adrenaline in 10 mL

of diluted acetic acid (31) (1 in 500), and use this solution as the sample solution. To 1 mL of the sample solution add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to red.

(2) Place 1 mL each of the sample solution obtained in (1) in test tubes A and B. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and add 10 mL of phosphate buffer solution, pH 6.5, to B. To each of the test tubes add 1 mL of iodine TS, allow to stand for 5 minutes, and add 2 mL each of sodium thiosulfate TS: a red color develops in test tube A, and a deep red color develops in test tube B.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-50.0 - -53.5^\circ$ (after drying, 1 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Adrenaline in 10 mL of dilute hydrochloric acid: the solution is clear, and is not more colored than Matching Fluid A.

(2) Adrenalone—Dissolve 50 mg of Adrenaline in 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.40.

(3) Noradrenaline—Dissolve 10.0 mg of Adrenaline in 2.0 mL of a L-tartaric acid solution (1 in 200). Pipet 1 mL of the solution, add 3.0 mL of pyridine, then add 1.0 mL of freshly prepared sodium naphthoquinone sulfonate TS, and allow to stand in a dark place for 30 minutes. To this solution add 5.0 mL of pyridine containing 0.05 g of L-ascorbic acid: the solution is not more colored than the following control solution.

Control solution: Dissolve 2.0 mg of Noradrenaline Bitartrate Reference Standard and 90 mg of Adrenaline Bitartrate Reference Standard in methanol to make exactly 10 mL. Pipet 1 mL of this solution, and proceed in the same manner.

Loss on drying <2.41> Not more than 1.0% (2 g, in vacuum, silica gel, 18 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Adrenaline, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 18.32 \text{ mg of } \text{C}_9\text{H}_{13}\text{NO}_3 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Adrenaline Injection

Epinephrine Injection

アドレナリン注射液

Adrenaline Injection is aqueous solution for injection.

It contains not less than 0.085 w/v% and not more

than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20).

Method of preparation Dissolve Adrenaline in diluted Hydrochloric Acid (9 in 10,000), and prepare as directed under Injections.

Description Adrenaline Injection is a colorless, clear liquid.

It changes gradually to pale red and then to brown on exposure to air and light.

pH: 2.3 – 5.0

Identification (1) To 1 mL of Adrenaline Injection add 4 mL of water and 1 drop of iron (III) chloride TS: a deep green color is produced, and it gradually changes to red.

(2) Place 1 mL each of Adrenaline Injection in test tubes A and B, and proceed as directed in the Identification (2) under Adrenaline.

Extractable volume <6.05> It meets the requirement.

Assay Pipet 30 mL of Adrenaline Injection into a separator, add 25 mL of carbon tetrachloride, shake vigorously for 1 minute, allow the liquids to separate, and discard the carbon tetrachloride. Repeat this procedure three times. Rinse the stopper and mouth of the separator with a small amount of water. Add 0.2 mL of starch TS, then while swirling the separator add iodine TS dropwise until a persistent blue color develops, and immediately add sodium thiosulfate TS to discharge the blue color. Add 2.1 g of sodium hydrogen carbonate to the liquid in the separator, preventing it from coming in contact with the mouth of the separator, and shake until most of the sodium hydrogen carbonate dissolves. Rapidly inject 1.0 mL of acetic anhydride into the contents of the separator. Immediately stopper the separator loosely, and allow to stand until the evolution of gas ceases. Shake vigorously, allow to stand for 5 minutes, extract with six 25-mL portions of chloroform, and filter each chloroform extract through a pledget of absorbent cotton. Evaporate the combined chloroform extracts on a water bath in a current of air to 3 mL, completely transfer this residue by means of small portions of chloroform to a tared beaker, and heat again to evaporate to dryness. Dry the residue at 105°C for 30 minutes, cool in a desiccator (silica gel), and accurately measure the mass W (mg) of the dried residue. Dissolve in chloroform to make exactly 5 mL, and determine the optical rotation <2.49> $[\alpha]_D^{20}$ using a 100-mm cell.

$$\begin{aligned} \text{Amount (mg) of adrenaline (C}_9\text{H}_{13}\text{NO}_3) \\ = W \times \{0.5 + (0.5 \times |[\alpha]_D^{20}|)/93\} \times 0.5923 \end{aligned}$$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Adrenaline Solution

Epinephrine Solution

アドレナリン液

Adrenaline Solution contains not less than 0.085 w/v% and not more than 0.115 w/v% of adrenaline ($C_9H_{13}NO_3$: 183.20)

Method of preparation

Adrenaline	1 g
Sodium Chloride	8.5 g
Diluted Hydrochloric Acid (9 in 100)	10 mL
Stabilizer	a suitable quantity
Preservative	a suitable quantity
Purified Water	a sufficient quantity
To make 1000 mL	

Prepare by mixing the above ingredients.

Description Adrenaline Solution is clear, colorless or slightly reddish liquid.

It changes gradually to pale red and then to brown on exposure to air and light.

pH: 2.3 – 5.0

Identification Proceed as directed in the Identification under Adrenaline Injection.

Assay Proceed as directed in the Assay under Adrenaline Injection.

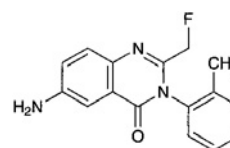
$$\begin{aligned} \text{Amount (mg) of adrenaline (C}_9\text{H}_{13}\text{NO}_3) \\ = W \times \{0.5 + (0.5 \times |[\alpha]_D^{20}|)/93\} \times 0.5923 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Afloqualone

アフロクアロン



$C_{16}H_{14}FN_3O$: 283.30

6-Amino-2-fluoromethyl-3-(2-tolyl)-3H-quinazolin-4-one
[56287-74-2]

Afloqualone, when dried, contains not less than 98.5% of $C_{16}H_{14}FN_3O$.

Description Afloqualone occurs as white to light yellow crystals or crystalline powder.

It is soluble in acetonitrile, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

Melting point: about 197°C (with decomposition).

Identification (1) Conduct this procedure without exposure to light, using light-resistant containers. Determine the absorption spectrum of a solution of Afloqualone in ethanol (99.5) (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Afloqualone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Acidity or alkalinity—Take 1.0 g of Afloqualone in a light-resistant vessel, add 20 mL of freshly boiled and cooled water, shake well, and filter. To 10 mL of the filtrate add 2 drops of bromothymol blue TS: a yellow color develops. The color changes to blue by adding 0.20 mL of 0.01 mol/L sodium hydroxide TS.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Afloqualone in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 10 mg of Afloqualone in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the total of the peak areas other than the peak area of afloqualone from the sample solution is not more than the peak area of afloqualone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.2 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, adjust to pH 5.5 with diluted phosphoric acid (1 in 10). To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of afloqualone is about 5.5 minutes.

Time span of measurement: About 4 times as long as the retention time of afloqualone beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 5 mL of the standard solution, add the mobile phase to make exactly 25 mL, and confirm that the peak area of afloqualone obtained from 20 μ L of this solution is equivalent to 15 to 25% of that of afloqualone obtained from 20 μ L of the standard solution.

System performance: Dissolve 0.01 g of Afloqualone in a suitable amount of the mobile phase, add 5 mL of a solution

of propyl parahydroxybenzoate in the mobile phase (1 in 2000) and the mobile phase to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, afloqualone and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of afloqualone is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1.0 g, platinum crucible).

Assay Weigh accurately about 0.4 g of Afloqualone, previously dried, dissolve in 10 mL of hydrochloric acid and 40 mL of water, and add 10 mL of a solution of potassium bromide (3 in 10). After cooling at 15°C or below, titrate <2.50> with 0.1 mol/L sodium nitrite VS according to the potentiometric titration or amperometric titration under the Electrometric Titration method.

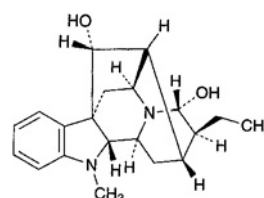
Each mL of 0.1 mol/L sodium nitrite
= 28.33 mg of C₁₆H₁₄FN₃O

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ajmaline

アジマリン



C₂₀H₂₆N₂O₂: 326.43
(17*R*,21*R*)-Ajmalan-17,21-diol [4360-12-7]

Ajmaline, when dried, contains not less than 96.0% of C₂₀H₂₆N₂O₂.

Description Ajmaline occurs as a white to pale yellow, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic anhydride and in chloroform, sparingly soluble in methanol, in ethanol (95), in acetone and in diethyl ether, and very slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Melting point: about 195°C (with decomposition).

Identification (1) Dissolve 0.05 g of Ajmaline in 5 mL of methanol, and use this solution as the sample solution. Add 3 mL of nitric acid to 1 mL of the sample solution: a deep red color develops.

(2) Spot the sample solution of (1) on filter paper, and spray Dragendorff's TS: an orange color develops.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (249 nm): 257 – 271 (after drying, 2 mg, ethanol (95), 100 mL).

$E_{1\text{cm}}^{1\%}$ (292 nm): 85 – 95 (after drying, 2 mg, ethanol (95), 100 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +136 – +151° (after drying, 0.5 g, chloroform, 50 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of Ajmaline in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and diethylamine (5:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.6 g, in vacuum, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.3 g of Ajmaline, previously dried, dissolve in 50 mL of acetic anhydride and 50 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 16.32 mg of $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ajmaline Tablets

アジマリン錠

Ajmaline Tablets contain not less than 90% and not more than 110% of the labeled amount of ajmaline ($\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$: 326.43).

Method of preparation Prepare as directed under Tablets, with Ajmaline.

Identification (1) Shake a quantity of powdered Ajmaline Tablets, equivalent to 0.1 g of Ajmaline according to the labeled amount, with 30 mL of chloroform, and filter. Evaporate the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification under Ajmaline.

(2) Dissolve 0.01 g of the residue of (1) in 100 mL of ethanol (95). To 10 mL of this solution add ethanol (95) to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 247 nm and 251 nm and between 291 nm and 294 nm, and a minimum between 269 nm and 273 nm.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Ajmaline Tablets at 100 revolutions per minute according to the Paddle method using 900 mL of 2 nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution 60 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm . Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg of ajmaline for assay, previously dried in vacuum at 80°C for 3 hours, dissolve in 2 nd fluid for dissolution test to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 288 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Ajmaline Tablets in 60 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of ajmaline ($\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$)
= $W_S \times (A_T/A_S) \times (1/C) \times 180$

W_S : Amount (mg) of ajmaline for assay.

C : Labeled amount (mg) of ajmaline ($\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Ajmaline Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of ajmaline ($\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$), add 15 mL of ammonia solution (28), and extract with four 25-mL portions of chloroform. Combine the chloroform extracts, wash with 10 mL of water, add 5 g of anhydrous sodium sulfate, shake well, and filter. Wash the container and the residue with two 10-mL portions of chloroform, and filter. Evaporate the combined filtrate on a water bath to dryness, dissolve the residue in 50 mL of acetic anhydride and 50 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

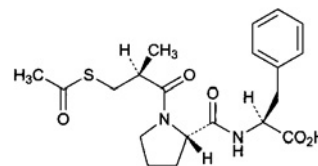
Each mL of 0.05 mol/L perchloric acid VS
= 16.32 mg of $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Alacepril

アラセプリル



$\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: 406.50

(2S)-2-[(2S)-1-[(2S)-3-(Acetylsulfanyl)-2-methylpropanoyl]pyrrolidine-2-carbonyl]amino-3-phenylpropanoic acid
[74258-86-9]

Alacepril, when dried, contains not less than 98.5% and not more than 101.0% of $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$.

Description Alacepril occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) To 20 mg of Alacepril add 0.1 g of sodium hydroxide, and heat gradually to melt: the gas evolved changes the color of a moistened red litmus paper to blue. After cooling, to the melted substance add 2 mL of water, shake, and add 1 mL of lead (II) acetate TS: a brown to black precipitate is formed.

(2) Determine the infrared absorption spectrum of Alacepril, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-81 - -85^\circ$ (after drying, 0.25 g, ethanol (95), 25 mL, 100 mm).

Melting point <2.60> 153 – 157°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <1.14>—Dissolve 0.5 g of Alacepril in 30 mL of methanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Alacepril according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 50 mg of Alacepril in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than alacepril from the sample solution is not larger than 2/5 times the peak area of alacepril from the standard solution, and the total area of the peaks other than the peak of alacepril from the sample solution is not larger than the peak area of alacepril from the standard solution. For this calculation, use the areas of the peaks, having the relative retention time of about 2.3 and about 2.6 with respect to alacepril, after multiplying by their sensitivity factors, 1.5 and 1.9, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated

silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100), acetonitrile, methanol and tetrahydrofuran (6:2:1:1).

Flow rate: Adjust the flow rate so that the retention time of alacepril is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of alacepril beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 4 mL of the standard solution add ethanol (95) to make exactly 10 mL. Confirm that the peak area of alacepril obtained with 10 μ L of this solution is equivalent to 30 to 50% of that with 10 μ L of the standard solution.

System performance: Dissolve 20 mg of Alacepril in 50 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 80,000). When the procedure is run with 10 μ L of this solution under the above operating conditions, alacepril and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alacepril is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Alacepril, previously dried, dissolve in 75 mL of a mixture of methanol and water (2:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.65 mg of C₂₀H₂₆N₂O₅S

Containers and storage Containers—Tight containers.

Alacepril Tablets

アラセプリル錠

Alacepril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of alacepril (C₂₀H₂₆N₂O₅S: 406.50).

Method of preparation Prepare as directed under Tablets, with Alacepril.

Identification Shake well a quantity of powdered Alacepril Tablets, equivalent to 0.1 g of Alacepril according to the labeled amount, with 10 mL of ethanol (95), filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of alacepril in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of ethanol

(99.5) and hexane (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the principal spot from the sample solution and the spot from the standard solution show the same color tone and *R_f* value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Alacepril Tablets add 2 mL of water, disperse the particle with the aid of ultrasonic wave, and add exactly 2 mL of the internal standard solution every 10 mg of alacepril (C₂₀H₂₆N₂O₅S) according to the labeled amount. To this solution add a suitable amount of methanol, extract for 15 minutes with the aid of ultrasonic wave while occasional shaking, and shake more 15 minutes. Add methanol to make exactly *V* mL so that each mL of the solution contains about 0.5 mg of alacepril (C₂₀H₂₆N₂O₅S), centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of alacepril for assay, previously dried at 105°C for 3 hours, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q_T* and *Q_S*, of the peak area of alacepril to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of alacepril (C}_{20}\text{H}_{26}\text{N}_{2}\text{O}_{5}\text{S)} \\ &= W_S \times (Q_T/Q_S) \times (V/50) \end{aligned}$$

W_S: Amount (mg) of alacepril for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (3 in 20,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Alacepril Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 30 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V'* mL so that each mL contains about 14 μg of alacepril (C₂₀H₂₆N₂O₅S) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 14 mg of alacepril for assay, previously dried at 105°C for 3 hours, dissolve in 2 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, *A_{T1}* and *A_{S1}*, at 230 nm, and *A_{T2}* and *A_{S2}*, at 300 nm, of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rates for a 12.5-mg tablet and a 25-mg tablet in 30 minutes are not less than 75%, respectively, and that for a 50-mg tablet in 30 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of

alacepril (C₂₀H₂₆N₂O₅S)

$$= W_S \times \frac{A_{T1} - A_{T2}}{A_{S1} - A_{S2}} \times \frac{V'}{V} \times \frac{1}{C} \times 90$$

W_S: Amount (mg) of alacepril for assay

C: Labeled amount (mg) of alacepril (C₂₀H₂₆N₂O₅S) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Alacepril Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of alacepril (C₂₀H₂₆N₂O₅S), moisten with 2 mL of water, add exactly 3 mL of the internal standard solution and 40 mL of methanol, extract for 15 minutes with the aid of ultrasonic wave, cool, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 50 mg of alacepril for assay, previously dried at 105°C for 3 hours, add exactly 3 mL of the internal standard solution, dissolve with methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q_T* and *Q_S*, of the peak area of alacepril to that of the internal standard.

$$\text{Amount (mg) of alacepril (C}_{20}\text{H}_{26}\text{N}_{2}\text{O}_{5}\text{S)} = W_S \times (Q_T/Q_S)$$

W_S: Amount (mg) of alacepril for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100), acetonitrile, methanol and tetrahydrofuran (13:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of alacepril is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, alacepril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of alacepril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Albumin Tannate

タンニン酸アルブミン

Albumin Tannate is a compound of tannic acid and a protein.

The label states the origin of the protein of Albumin Tannate.

Description Albumin Tannate occurs as a light brown powder. It is odorless, or has a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS with turbidity.

Identification (1) To 0.1 g of Albumin Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. After cooling, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-purple to bluish black color is produced. On standing, a bluish black precipitate is produced.

(2) To 0.1 g of Albumin Tannate add 5 mL of nitric acid: an orange-yellow color develops.

Purity (1) Acidity—Shake 1.0 g of Albumin Tannate with 50 mL of water for 5 minutes, and filter. To 25 mL of the filtrate add 1.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(2) Fats—To 2.0 g of Albumin Tannate add 20 mL of petroleum benzene, shake vigorously for 15 minutes, and filter. Evaporate 10 mL of the filtrate on a water bath: the mass of the residue is not more than 50 mg.

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (0.5 g).

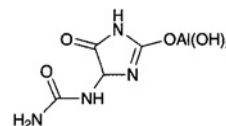
Digestion test To 1.00 g of Albumin Tannate add 0.25 g of saccharated pepsin and 100 mL of water, shake well, and allow to stand for 20 minutes at 40 ± 1°C in a water bath. Add 1.0 mL of dilute hydrochloric acid, shake, and allow to stand for 3 hours at 40 ± 1°C. Cool rapidly to ordinary temperature, and filter. Wash the residue with three 10-mL portions of water, dry in a desiccator (silica gel) for 18 hours, and dry at 105°C for 5 hours: the mass of the residue is 0.50 to 0.58 g.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Aldioxa

アルジオキサ



$C_4H_7AlN_4O_5$: 218.10

Dihydroxo(5-oxo-4-ureido-4,5-dihydro-1*H*-imidazol-2-yl)oxoaluminum [5579-81-7]

Aldioxa is a condensation product of allantoin and aluminum hydroxide.

When dried, it contains not less than 65.3% and not more than 74.3% of allantoin ($C_4H_6N_4O_3$: 158.12), and not less than 11.1% and not more than 13.0% of aluminum (Al: 26.98).

Description Aldioxa occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Melting point: about 230°C (with decomposition).

Identification (1) To 0.2 g of Aldioxa add 10 mL of dilute hydrochloric acid, boil for 5 minutes, and add 10 mL of a solution of phenylhydrazinium chloride (1 in 100). After cooling, mix well with 0.5 mL of potassium hexacyanoferrate (III) TS, and shake with 1 mL of hydrochloric acid: a red color develops.

(2) To 0.2 g of Aldioxa add 10 mL of dilute hydrochloric acid, dissolve by warming, and cool: the solution responds to the Qualitative Tests <1.09> for aluminum salt.

Purity (1) Chloride <1.03>—To 0.10 g of Aldioxa add 6 mL of dilute nitric acid, boil to dissolve with shaking for 5 minutes, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(2) Sulfate <1.14>—To 0.20 g of Aldioxa add 6 mL of dilute hydrochloric acid, boil to dissolve with shaking for 5 minutes, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Nitrate—To 0.10 g of Aldioxa add carefully 5 mL of water and 5 mL of sulfuric acid, dissolve by shaking, cool, and superimpose 2 mL of iron (II) sulfate TS: no brown ring is produced at the zone of contact.

(4) Heavy metals <1.07>—To 1.0 g of Aldioxa add 3 mL of hydrochloric acid and 3 mL of water, heat gently to boil with shaking, and evaporate on a water bath to dryness. To the residue add 30 mL of water, shake under warming, cool, filter, and to the filtrate add 2 mL of dilute acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3 mL of hydrochloric acid add 3 mL of water,

evaporate on a water bath to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid (31) and water to make 50 mL (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aldioxa according to Method 2, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 4.0% (1 g, 105°C, 2 hours).

Assay (1) Allantoin—Weigh accurately about 0.1 g of Aldioxa, previously dried, dissolve in 50 mL of dilute sulfuric acid by heating, cool, and add water to make exactly 100 mL. Pipet 10 mL of this solution, and perform the test as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.3953 mg of $C_4H_6N_4O_3$

(2) Aluminum—Weigh accurately about 0.2 g of Aldioxa, previously dried, dissolve carefully in 50 mL of dilute hydrochloric acid by heating, cool, and add dilute hydrochloric acid to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Aluminum Stock Solution, dilute with water so that each mL of the solution contains not less than 16.0 μg and not more than 64.0 μg of aluminum (Al: 26.98), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the aluminum content of the sample solution from the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene

Supporting gas—Nitrous oxide

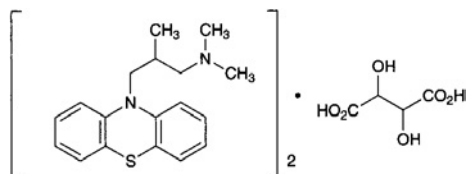
Lamp: An aluminum hollow cathode lamp

Wavelength: 309.2 nm

Containers and storage Containers—Well-closed containers.

Alimemazine Tartrate

アリメマジン酒石酸塩



$(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$: 746.98

N,N,2-Trimethyl-3-(10*H*-phenothiazin-10-yl)propylamine hemitartrate [41375-66-0]

Alimemazine Tartrate, when dried, contains not less than 98.0% of $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$.

Description Alimemazine Tartrate occurs as a white powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl

ether.

The pH of a solution of Alimemazine Tartrate (1 in 50) is between 5.0 and 6.5.

It is gradually colored by light.

Identification (1) To 2 mL of a solution of Alimemazine Tartrate (1 in 100) add 1 drop of iron (III) chloride TS: a red-brown color is produced, and immediately a yellow precipitate is formed.

(2) Dissolve 1 g of Alimemazine Tartrate in 5 mL of water, add 3 mL of sodium hydroxide TS, extract with two 10-mL portions of diethyl ether [use the aqueous layer obtained in the Identification (4)]. Shake the combined diethyl ether extracts with 3 g of anhydrous sodium sulfate, filter, and evaporate the diethyl ether with the aid of a current of air. Dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 16 hours: it melts <2.60> between 66°C and 70°C.

(3) Determine the absorption spectrum of a solution of Alimemazine Tartrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) The aqueous layer, obtained in the identification (2), when neutralized with dilute acetic acid, responds to the Qualitative Tests <1.09> (1) and (2) for tartrate.

Melting point <2.60> 159 – 163°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Alimemazine Tartrate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Alimemazine Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Alimemazine Tartrate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Alimemazine Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through brown to green-brown (indicator: 2 mL of *p*-naphtholbenzene TS). Perform a blank determination, and make any necessary correction.

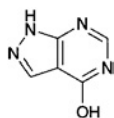
Each mL of 0.1 mol/L perchloric acid VS
= 37.35 mg of $(C_{18}H_{22}N_2S)_2 \cdot C_4H_6O_6$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Allopurinol

アロプリノール



$C_5H_4N_4O$: 136.11

1*H*-Pyrazolo[3,4-*d*]pyrimidin-4-ol [315-30-0]

Allopurinol, when dried, contains not less than 98.0% of $C_5H_4N_4O$.

Description Allopurinol occurs as white to pale yellowish white crystals or crystalline powder. It is odorless.

It is slightly soluble in *N,N*-dimethylformamide, very slightly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: not lower than 320°C (with decomposition).

Identification (1) Dissolve 0.1 g of Allopurinol in 50 mL of water by warming. To 5 mL of this solution add 1 mL of ammonia TS and 1 mL of silver nitrate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Allopurinol in 50 mL of water by warming. To 5 mL of this solution add 0.5 mL of copper (II) sulfate TS: a blue precipitate is produced.

(3) Determine the absorption spectrum of a solution of Allopurinol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Allopurinol in 10 mL of sodium hydroxide TS: the solution is clear, and has no more color than Matching Fluid D.

(2) Sulfate <1.14>—To 2.0 g of Allopurinol add 100 mL of water, and boil for 5 minutes. Cool, add water to make 100 mL, and filter. To 25 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Allopurinol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Allopurinol according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.05 g of Allopurinol in 10 mL of ammonia TS, and use this solution as the sample solution. Pipet 1 mL of this solution, add ammonia TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with ammonia TS-saturated

1-butanol to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

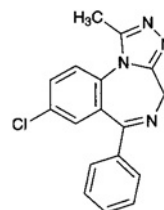
Assay Weigh accurately about 0.16 g of Allopurinol, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide by warming. Cool, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). To 70 mL of *N,N*-dimethylformamide add 12 mL of water, perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 13.61 mg of $C_5H_4N_4O$

Containers and storage Containers—Tight containers.

Alprazolam

アルプラゾラム



$C_{17}H_{13}ClN_4$: 308.76

8-Chloro-1-methyl-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine [28981-97-7]

Alprazolam, when dried, contains not less than 98.5% of $C_{17}H_{13}ClN_4$.

Description Alprazolam occurs as white crystals or crystalline powder.

It is freely soluble in chloroform, soluble in methanol and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in water.

It dissolves in dilute nitric acid.

Identification (1) Determine the absorption spectrum of a solution of Alprazolam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.05 g of Alprazolam in 0.7 mL of deuteriochloroform for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits a single signal A at around δ 2.6 ppm,

doublet signals B and C at around δ 4.0 ppm and δ 5.4 ppm, and a broad signal D between δ 7.1 ppm and 7.9 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:8.

(3) Perform the test with Alprazolam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 228 – 232°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Alprazolam in 10 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Alprazolam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances – Dissolve 50 mg of Alprazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, then pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of acetone, hexane, ethyl acetate and ethanol (95) (4:2:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

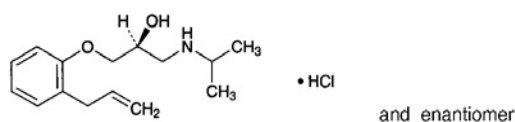
Assay Weigh accurately about 0.25 g of Alprazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.44 mg of C₁₇H₁₃ClN₄

Containers and storage Containers—Well-closed containers.

Alprenolol Hydrochloride

アルプレノロール塩酸塩



C₁₅H₂₃NO₂.HCl: 285.81
(2*RS*)-1-(2-Allylphenoxy)-3-
[(1-methylethyl)amino]propan-2-ol monohydrochloride
[13707-88-5]

Alprenolol Hydrochloride, when dried, contains not

less than 99.0% of C₁₅H₂₃NO₂.HCl.

Description Alprenolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 2 mL of a solution of Alprenolol Hydrochloride (1 in 100) add 0.05 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer.

(2) Dissolve 0.05 g of Alprenolol Hydrochloride in 5 mL of water, add 1 to 2 drops of bromine TS, and shake: the color of the test solution disappears.

(3) Determine the absorption spectrum of a solution of Alprenolol Hydrochloride in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Alprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Alprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Melting point <2.60> 108 – 112°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Alprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Alprenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Alprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Alprenolol Hydrochloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 2.5 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone, acetic acid (100) and water (60:42:5:3) to a distance of about 10 cm, air-dry the plate, and then dry at 80°C for 30 minutes. After cooling, allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot on the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum,

silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Alprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

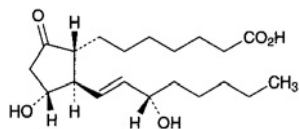
Each mL of 0.1 mol/L perchloric acid VS
= 28.58 mg of $C_{15}H_{23}NO_2 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Alprostadil

Prostaglandin E₁

アルプロスタジール



$C_{20}H_{34}O_5$: 354.48

7-[(1R,2R,3R)-3-Hydroxy-2-[(1E,3S)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl]heptanoic acid
[745-65-3]

Alprostadil, when dried, contains not less than 97.0% and not more than 103.0% of $C_{20}H_{34}O_5$.

Description Alprostadil occurs as white crystals or crystalline powder.

It is freely soluble in ethanol (99.5) and in tetrahydrofuran, slightly soluble in acetonitrile, and practically insoluble in water.

Identification (1) The absorption spectrum of a solution of Alprostadil in ethanol (99.5) (1 in 100,000) determined as directed under Ultraviolet-visible Spectrophotometry <2.24> shows no absorption between 210 nm and 350 nm. Separately, to 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum in the same way. Compare the spectrum so obtained with the Reference Spectrum or the spectrum of a solution of Alprostadil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Alprostadil, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Alprostadil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-53 - -61^\circ$ (after drying, 25 mg, tetrahydrofuran, 5 mL, 100 mm).

Melting point <2.60> 114 - 118°C

Purity Related substances—Dissolve 4 mg of Alprostadil in 2 mL of a mixture of acetonitrile for liquid chromatography and water (9:1), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, and add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks, having the relative retention time of about 0.70 and 1.26 with respect to alprostadil, is not larger than 1/2 times the peak area of alprostadil with the standard solution, the area of the peaks, having the relative retention time of about 0.88 and 1.18 with respect to alprostadil, is not larger than the peak area of alprostadil with the standard solution, the area of the peaks other than alprostadil and the peaks mentioned above is not larger than 1/10 times the peak area of alprostadil with the standard solution and the total area of the peaks other than alprostadil is not larger than 2 times the peak area of alprostadil with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of alprostadil beginning after the solvent peak. **System suitability**—

Test for required detectability: Measure exactly 2 mL of the standard solution, add the mixture of acetonitrile for liquid chromatography and water (9:1) to make exactly 20 mL. Confirm that the peak area of alprostadil obtained with 5 μ L of this solution is equivalent to 7 to 13% of that with 5 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of alprostadil is not more than 1.5%.

Loss on drying <2.41> Not more than 1.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 5 mg each of Alprostadil and Alprostadil Reference Standard, previously dried, dissolve in exactly 5 mL of the internal standard solution, add a mixture of acetonitrile for liquid chromatography and water (9:1) to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of alprostadil to that of the internal standard.

Amount (mg) of $C_{20}H_{34}O_5$ = $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of Alprostadil Reference Standard

Internal standard solution—A solution of dimethyl phthalate in the mixture of acetonitrile for liquid chromatography and

water (9:1) (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 196 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 9.07 g of potassium dihydrogen phosphate in water to make 1000 mL. Adjust the pH to 6.3 with a solution prepared by dissolving 9.46 g of disodium hydrogen phosphate in water to make 1000 mL, and dilute to 10 times its volume with water. To 360 mL of this solution add 110 mL of acetonitrile for liquid chromatography and 30 mL of methanol for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of alprostadil is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, alprostadil and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of alprostadil to that of the internal standard is not more than 1.0%.

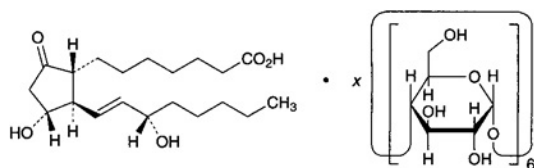
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Alprostadil Alfadex

Prostaglandin E₁ α -Cyclodextrin Clathrate Compound

アルプロスタジール アルファデクス



$C_{20}H_{34}O_5 \cdot x C_{36}H_{60}O_{30}$

7-[(1R,2R,3R)-3-Hydroxy-2-[(1E,3S)-3-hydroxyoct-1-en-1-yl]-5-oxocyclopentyl]heptanoic acid— α -cyclodextrin [55648-20-9]

Alprostadil Alfadex is a α -cyclodextrin clathrate compound of alprostadil.

It contains not less than 2.8% and not more than 3.2% of alprostadil ($C_{20}H_{34}O_5$; 354.48), calculated on the anhydrous basis.

Description Alprostadil Alfadex occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (95), in ethyl acetate and in diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (1). Separately, to 0.02 g of Alprostadil Alfadex add 5 mL of ethyl acetate, shake, and centrifuge. Use the supernatant liquid as the sample solution (2). Evaporate the solvent from these solutions under reduced pressure, add 2 mL of sulfuric acid to the residue, and shake for 5 minutes: the liquid obtained from the sample solution (1) shows an orange-yellow color, while the liquid obtained from the sample solution (2) does not show that color.

(2) Dissolve 0.02 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent from the supernatant liquid under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), add 5 mL of 1,3-dinitrobenzene TS, then add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) under ice-cooling, and allow to stand for 20 minutes in a dark place under ice-cooling: a purple color develops.

(3) Dissolve 0.05 g of Alprostadil Alfadex in 1 mL of iodine TS, by heating on a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Alprostadil Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits no absorption between 220 nm and 400 nm. Separately, to 10 mL of the solution add 1 mL of potassium hydroxide-ethanol TS, allow to stand for 15 minutes, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> $[\alpha]_D^{20}$: +126 – +138° (0.1 g calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Alprostadil Alfadex in 20 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Alprostadil Alfadex in 10 mL of water: the solution is colorless. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry within 30 minutes after preparation of the solution: the absorbance at 450 nm is not larger than 0.10.

(2) Prostaglandin A₁—Dissolve 0.10 g of Alprostadil Alfadex in 5 mL of water, add exactly 5 mL of the internal standard solution and ethanol (95) to make 15 mL, and use this solution as the sample solution. Separately, dissolve 1.5 mg of prostaglandin A₁ in ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 5 mL of the internal standard solution, 2 mL of ethanol (95) and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the operating conditions described in the Assay, and calculate the ratios, Q_T and Q_S , of the peak area of prostaglandin A₁ to that of the internal standard: Q_T is not larger than Q_S .

Internal standard solution—A solution of propyl parahydroxybenzoate in dilute ethanol (1 in 15,000).

(3) Related substances—Dissolve 0.10 g of Alprostadil

Alfadex in 3 mL of water, add exactly 3 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid obtained as the sample solution. Separately, dissolve 1.0 mg of Prostaglandin A₁ in ethyl acetate to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of phosphomolybdic acid in ethanol (95) (1 in 4) on the plate, and heat at 100°C for 5 minutes: the spots other than the principal spot from the sample solution, and the spots other than the spot corresponding to the spot from the standard solution are all not more intense than the spot from the standard solution.

Water <2.48> Not more than 6.0% (0.2 g, direct titration).

Assay Weigh accurately about 0.1 g of Alprostadil Alfa-dex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Alprostadil Reference Standard, dissolve in 5 mL of ethanol (95), add exactly 5 mL of the internal standard solution and water to make 15 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of alprostadil to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of alprostadil (C}_{20}\text{H}_{34}\text{O}_5\text{)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Alprostadil Reference Standard

Internal standard solution—A solution of propyl para-hydroxybenzoate in dilute ethanol (1 in 15,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of alprostadil is about 6 minutes.

Selection of column: Dissolve about 0.1 g of Alprostadil Alfadex in 5 mL of water, add 5 mL of a solution of prostaglandin A₁ in ethanol (95) (3 in 200,000) and 5 mL of the internal standard solution. Proceed with 10 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of alprostadil, the internal standard and prostaglandin A₁ in this order and complete separation of these peaks.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5°C.

Expiration date 24 months after preparation.

Dried Aluminum Hydroxide Gel

乾燥水酸化アルミニウムゲル

Dried Aluminum Hydroxide Gel contains not less than 50.0% of aluminum oxide (Al₂O₃: 101.96).

Description Dried Aluminum Hydroxide Gel occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Most of it dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

Identification To 0.2 g of Dried Aluminum Hydroxide Gel add 20 mL of dilute hydrochloric acid, warm, and centrifuge: the supernatant liquid responds to the Qualitative Tests <1.09> for aluminum salt.

Purity (1) Acidity or alkalinity—To 1.0 g of Dried Aluminum Hydroxide Gel add 25 mL of water, shake well, and centrifuge: the supernatant liquid is neutral.

(2) Chloride <1.03>—To 1.0 g of Dried Aluminum Hydroxide Gel add 30 mL of dilute nitric acid, heat gently to boil while shaking, cool, add water to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.284%).

(3) Sulfate <1.14>—To 1.0 g of Dried Aluminum Hydroxide Gel add 15 mL of dilute hydrochloric acid, heat gently to boil while shaking, cool, add water to make 250 mL, and centrifuge. To 25 mL of the supernatant liquid add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Nitrate—To 0.10 g of Dried Aluminum Hydroxide Gel add 5 mL of water, then carefully add 5 mL of sulfuric acid, shake well to dissolve, and cool. Superimpose the solution on 2 mL of iron (II) sulfate TS: no brown-colored ring is produced at the zone of contact.

(5) Heavy metals <1.07>—Dissolve 2.0 g of Dried Aluminum Hydroxide Gel in 10 mL of dilute hydrochloric acid by heating, filter if necessary, and add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid to dryness, and add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Arsenic <1.11>—To 0.8 g of Dried Aluminum Hydroxide Gel add 10 mL of dilute sulfuric acid, heat gently to boil while shaking, cool, and filter. Take 5 mL of the filtrate, use this solution as the test solution, and perform the test (not more than 5 ppm).

Acid-consuming capacity Weigh accurately about 0.2 g of Dried Aluminum Hydroxide Gel, and transfer to a glass-stoppered flask. Add exactly 100 mL of 0.1 mol/L

hydrochloric acid VS, stopper the flask, shake at $37 \pm 2^\circ\text{C}$ for 1 hour, and filter. Measure exactly 50 mL of the filtrate, and titrate <2.50> while thoroughly stirring, the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS until the pH of the solution becomes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 250 mL per g of Dried Aluminum Hydroxide Gel.

Assay Weigh accurately about 2 g of Dried Aluminum Hydroxide Gel, add 15 mL of hydrochloric acid, heat on a water bath with shaking for 30 minutes, cool, and add water to make exactly 500 mL. Pipet 20 mL of this solution, add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid (31)-ammonium acetate buffer solution, pH 4.8, boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <2.50> with 0.05 mol/L zinc acetate VS until the color of the solution changes from light dark green to light red. (indicator: 2 mL of dithizone TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

Containers and storage Containers—Tight containers.

Dried Aluminum Hydroxide Gel Fine Granules

乾燥水酸化アルミニウムゲル細粒

Dried Aluminum Hydroxide Gel Fine Granules contain not less than 47.0% of aluminum oxide (Al_2O_3 : 101.96).

Method of preparation Prepare with Dried Aluminum Hydroxide Gel as directed under Powders.

Identification To 0.2 g of Dried Aluminum Hydroxide Gel Fine Granules add 20 mL of dilute hydrochloric acid, warm and centrifuge: the supernatant liquid responds to the Qualitative Tests <1.09> for aluminum salt.

Acid-consuming capacity Proceed as directed for Acid-consuming capacity under Dried Aluminum Hydroxide Gel: the volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 235 mL per g of Dried Aluminum Hydroxide Gel Fine Granules.

Assay Proceed as directed in the Assay under Dried Aluminum Hydroxide Gel.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.549 mg of Al_2O_3

Containers and storage Containers—Tight containers.

Natural Aluminum Silicate

天然ケイ酸アルミニウム

Description Natural Aluminum Silicate occurs as a white or slightly colored powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Natural Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), with some decomposition, leaving a large amount of insoluble substance.

Identification (1) To 0.5 g of Natural Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests <1.09> for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Natural Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

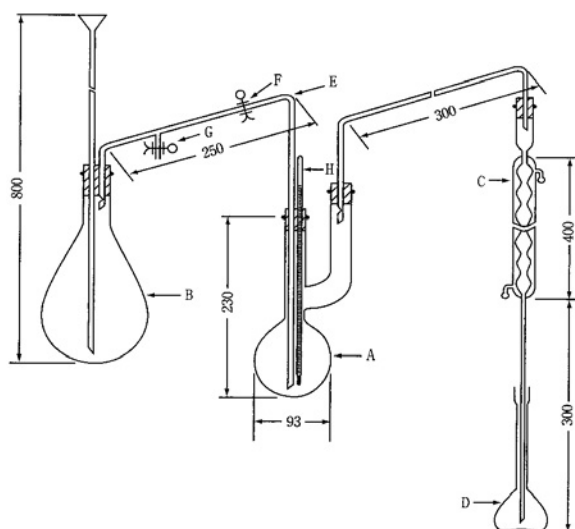
Purity (1) Acidity or alkalinity—Shake 5.0 g of Natural Aluminum Silicate with 100 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.

(2) Chloride <1.03>—To 5.0 g of Natural Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute nitric acid, dilute to 50 mL with water, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—To the residue obtained in (6) add 3 mL of dilute hydrochloric acid, heat on a water bath for 10 minutes, dilute to 50 mL with water, and filter. To 2.0 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Heavy metals <1.07>—To 1.5 g of Natural Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then cool, centrifuge, remove the supernatant liquid, wash the residue with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise, until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking and redissolve the precipitate. Heat the mixture with 0.45 g of hydroxylammonium chloride, cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test, using 50 mL of this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(5) Arsenic <1.11>—To 1.0 g of Natural Aluminum Silicate, add 5 mL of dilute hydrochloric acid, heat gently to boil while shaking well, cool rapidly, and centrifuge. Mix the



The figures are in mm.

- A: Distilling flask of about 300-mL capacity.
 B: Steam generator of about 1000-mL capacity, containing a few boiling tips to prevent bumping
 C: Condenser
 D: Receiver: 200-mL volumetric flask
 E: Steam-introducing tube having an internal diameter of about 8 mm
 F, G: Rubber tube with a clamp
 H: Thermometer

residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).

(6) Soluble salts—Evaporate 50 mL of the supernatant liquid obtained in (1) on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 40 mg.

(7) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for

fluoride under Oxygen Flask Combustion Method <1.06>. No corrective solution is used in this procedure.

$$\begin{aligned} \text{Amount (mg) of fluoride (F: 19.00) in the test solution} \\ = \text{amount (mg) of fluoride in 5 mL of} \\ \text{the standard solution} \\ \times (A_T/A_S) \times (200/V) \end{aligned}$$

The content of fluoride (F) is not more than 0.01%.

Loss on drying <2.41> Not more than 20.0% (1 g, 105°C, 3 hours).

Adsorptive power To 0.10 g of Natural Aluminum Silicate add 20 mL of a solution of methylene blue trihydrate (3 in 2000), shake for 15 minutes, allow to stand for 5 hours at $37 \pm 2^\circ\text{C}$, and centrifuge. Dilute 1.0 mL of the supernatant liquid with water to 200 mL. Place 50 mL of the solution in a Nessler tube and observe horizontally or vertically against a white background: the color of the solution is not deeper than that of the following control solution.

Control solution: Dilute 1.0 mL of a solution of methylene blue trihydrate (3 in 2000) with water to 400 mL, and use 50 mL of this solution.

Containers and storage Containers—Well-closed containers.

Synthetic Aluminum Silicate

合成ケイ酸アルミニウム

Description Synthetic Aluminum Silicate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Synthetic Aluminum Silicate (1 g) dissolves when heated in 20 mL of a solution of sodium hydroxide (1 in 5), leaving a small amount of insoluble substance .

Identification (1) To 0.5 g of Synthetic Aluminum Silicate add 3 mL of diluted sulfuric acid (1 in 3), heat until white fumes evolve, cool, add 20 mL of water, and filter. Render the filtrate slightly acid with ammonia TS: the solution responds to the Qualitative Tests <1.09> for aluminum salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Synthetic Aluminum Silicate, and fuse again: an infusible material appears in the bead, producing, upon cooling, an opaque bead with a web-like structure.

Purity (1) Acidity or alkalinity—Shake 1.0 g of Synthetic Aluminum Silicate with 20 mL of water, and centrifuge: the supernatant liquid so obtained is neutral.

(2) Chloride <1.03>—To 5.0 g of Synthetic Aluminum Silicate add 100 mL of water, boil gently for 15 minutes while shaking, then cool, add water to restore the original volume, and centrifuge. To 10 mL of the supernatant liquid add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—To 2.0 mL of the supernatant liquid obtained in (2) add 1 mL of dilute hydrochloric acid and

water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Heavy metals <1.07>—To 3.0 g of Synthetic Aluminum Silicate add 50 mL of water and 5 mL of hydrochloric acid, boil gently for 20 minutes while shaking, then after cooling, centrifuge, remove the supernatant liquid, wash the precipitate with two 10-mL portions of water, centrifuging each time, combine these washings with the filtrate, and add ammonia solution (28) dropwise until a precipitate just appears. Add dropwise dilute hydrochloric acid with vigorous shaking to redissolve the precipitate. Heat the solution with 0.45 g of hydroxylammonium chloride, and after cooling, add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Perform the test with 50 mL of this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(5) Arsenic <1.11>—To 1.0 g of Synthetic Aluminum Silicate add 10 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 20.0% (1 g, 105°C, 3 hours).

Acid-consuming capacity <6.04> Weigh accurately about 1 g of Synthetic Aluminum Silicate, transfer to a glass-stoppered flask, add 200 mL of 0.1 mol/L hydrochloric acid VS, exactly measured, stopper the flask, and shake at $37 \pm 2^\circ\text{C}$ for 1 hour. Filter, pipet 50 mL of the filtrate, and titrate <2.50> by stirring well the excess hydrochloric acid with 0.1 mol/L sodium hydroxide VS until the pH of the solution changes to 3.5. The volume of 0.1 mol/L hydrochloric acid VS consumed is not less than 50.0 mL per g of Synthetic Aluminum Silicate.

Containers and storage Containers—Well-closed containers.

Aluminum Monostearate

モノステアリン酸アルミニウム

Aluminum Monostearate is mainly aluminum compounds of stearic acid ($\text{C}_{18}\text{H}_{36}\text{O}_2$; 284.48) and palmitic acid ($\text{C}_{16}\text{H}_{32}\text{O}_2$; 256.42).

Aluminum Monostearate, when dried, contains not less than 7.2% and not more than 8.9% of aluminum (Al; 26.98).

Description Aluminum Monostearate occurs as a white to yellowish white powder. It is odorless or has a faint, characteristic odor.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Heat 3 g of Aluminum Monostearate with 30 mL of hydrochloric acid in a water bath with occasional shaking for 10 minutes. After cooling, shake the mixture vigorously with 50 mL of water and 30 mL of diethyl ether for 3 minutes, and allow to stand. To the separated aqueous layer add sodium hydroxide TS until the solution becomes slightly turbid, and filter: the filtrate responds to the Qualitative Tests <1.09> for aluminum salt.

(2) Wash the diethyl ether layer separated in (1) with two 20-mL portions of water, and evaporate the diethyl ether layer on a water bath: the residue melts <1.13> at above 54°C .

Acid value for fatty acid <1.13> 193 – 210. Weigh accurately about 1 g of fatty acid obtained in the Identification (2), transfer a 250-mL glass-stoppered flask, add 100 mL of a mixture of diethyl ether and ethanol (95) (2:1), warm to dissolve, add several drops of phenolphthalein TS, and proceed as directed under Acid Value.

Purity (1) Free fatty acid—Mix 1.0 g of Aluminum Monostearate with about 50 mL of a mixture of neutralized ethanol and diethyl ether (1:1), filter through dry filter paper, wash the vessel and the filter paper with a small amount of a mixture of neutralized ethanol and diethyl ether (1:1), combine the filtrate and the washings, and add 2.1 mL of 0.1 mol/L potassium hydroxide VS: a red color develops.

(2) Water-soluble salts—Heat 2.0 g of Aluminum Monostearate with 80 mL of water in a loosely stoppered conical flask on a water bath for 30 minutes with occasional shaking. After cooling, filter through dry filter paper, wash the residue with a small amount of water, combine the washings with the filtrate, add water to make 100 mL, evaporate 50 mL of this solution on a water bath, and heat strongly at 600°C : the mass of the residue is not more than 10.0 mg.

(3) Heavy metals <1.07>—Heat 1.0 g of Aluminum Monostearate over a small flame with caution at the beginning, and continue the heating, gradually raising the temperature, to ash. After cooling, add 10 mL of diluted hydrochloric acid (1 in 2), evaporate on a water bath, and boil the residue with 20 mL of water for 1 minute. Cool, filter, wash the residue with water, combine the filtrate and the washings, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Evaporate 10 mL of diluted hydrochloric acid (1 in 2) on a water bath to dryness, add 2 mL of dilute acetic acid and 5.0 mL of Standard Lead Solution, dilute with water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(4) Arsenic <1.11>—Mix 1.0 g of Aluminum Monostearate with 2 g of magnesium nitrate hexahydrate, ignite over a small flame, moisten the residue after cooling with 0.5 mL of nitric acid, and heat. Heat again the residue with 10 mL of dilute sulfuric acid until white fumes evolve, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C , 3 hours).

Assay Weigh accurately about 1 g of Aluminum Monostearate, previously dried, ignite gently to ash, and cool. Add dropwise 0.5 mL of nitric acid, evaporate on a water bath by heating, and then heat strongly between 900°C

and 1100°C to a constant mass. After cooling, weigh rapidly the ignited residue, and designate the mass as aluminum oxide (Al₂O₃: 101.96).

Amount (mg) of aluminum (Al)
= amount (mg) of aluminum oxide (Al₂O₃) × 0.5293

Containers and storage Containers—Well-closed containers.

Aluminum Potassium Sulfate Hydrate

Alum

硫酸アルミニウムカリウム水和物

AlK(SO₄)₂·12H₂O: 474.39

Aluminum Potassium Sulfate Hydrate contains not less than 99.5% of AlK(SO₄)₂·12H₂O.

Description Aluminum Potassium Sulfate Hydrate occurs as colorless or white crystals or powder. It is odorless. It has a slightly sweet, strongly astringent taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Aluminum Potassium Sulfate Hydrate (1 in 20) is acid.

Identification A solution of Aluminum Potassium Sulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for aluminum salt, to the Qualitative Tests <1.09> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <1.09> (1) and (3) for sulfate.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Iron <1.10>—Prepare the test solution with 1.0 g of Aluminum Potassium Sulfate Hydrate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.6 g of Aluminum Potassium Sulfate Hydrate, according to Method 1, and perform the test (not more than 3.3 ppm).

Assay Weigh accurately about 4.5 g of Aluminum Potassium Sulfate Hydrate, and dissolve in water to make exactly 200 mL. Take exactly 20 mL of this solution, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <2.50> with 0.05 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 23.72 mg of AlK(SO₄)₂·12H₂O

Containers and storage Containers—Tight containers.

Dried Aluminum Potassium Sulfate

Burnt Alum

乾燥硫酸アルミニウムカリウム

AlK(SO₄)₂: 258.21

Dried Aluminum Potassium Sulfate, when dried, contains not less than 98.0% of AlK(SO₄)₂.

Description Dried Aluminum Potassium Sulfate occurs as white masses or white powder. It is odorless. It has a slightly sweet, astringent taste.

It is freely soluble in hot water and practically insoluble in ethanol (95).

It dissolves slowly in water.

Identification A solution of Dried Aluminum Potassium Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for aluminum salt, to the Qualitative Tests <1.09> (1), (3) and (4) for potassium salt, and to the Qualitative Tests <1.09> (1) and (3) for sulfate.

Purity (1) Water-insoluble substances—To 2.0 g of Dried Aluminum Potassium Sulfate add 40 mL of water, shake frequently, and allow to stand for 48 hours. Collect the insoluble residue on a glass filter (G4), wash with 50 mL of water, and dry at 105°C for 2 hours: the mass of the residue is not more than 50 mg.

(2) Heavy metals <1.07>—Dissolve 0.5 g of Dried Aluminum Potassium Sulfate in 45 mL of water, and filter, if necessary. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 40 ppm).

(3) Iron <1.10>—Prepare the test solution with 0.54 g of Dried Aluminum Potassium Sulfate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 37 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of Dried Aluminum Potassium Sulfate, according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 15.0% (2 g, 200°C, 4 hours).

Assay Weigh accurately about 1.2 g of Dried Aluminum Potassium Sulfate, previously dried, add 80 mL of water, and heat on a water bath with occasional shaking for 20 minutes. Cool, add water to make exactly 100 mL, and filter, if necessary. Discard the first 30 mL of the filtrate, take exactly the subsequent 20 mL of the filtrate, and add exactly 30 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8, boil for 5 minutes, and cool. Add 55 mL of ethanol (95), and titrate <2.50> with 0.05 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.91 mg of AlK(SO₄)₂

Containers and storage Containers—Tight containers.

Alum Solution

ミヨウバン水

Alum Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of aluminum potassium sulfate Hydrate [AlK(SO₄)₂·12H₂O: 474.39].

Method of preparation

Aluminum Potassium Sulfate Hydrate	3 g
Mentha Water	50 mL
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Dissolve and mix the above ingredients.

Description Alum Solution is a clear, colorless liquid. It has the odor of the mentha oil and an astringent taste.

Identification (1) To 5 mL of Alum Solution add 3 mL of ammonium chloride TS and 1 mL of ammonia TS: a white, gelatinous precipitate is produced, which changes to red upon the addition of 5 drops of alizarin red S TS (aluminum sulfate).

(2) Place 100 mL of Alum Solution in an evaporating dish, evaporate on a water bath to dryness, and dissolve the residue in 5 mL of water: the solution responds to the Qualitative Tests <1.09> for potassium salt.

(3) Alum Solution responds to the Qualitative Tests <1.09> (1) and (2) for sulfate.

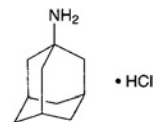
Assay Pipet 50 mL of Alum Solution, add exactly 30 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and further add 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.8. Boil for 5 minutes, cool, add 55 mL of ethanol (95), and titrate <2.50> with 0.02 mol/L zinc acetate VS (indicator: 2 mL of dithizone TS), until the color of the solution changes from light dark green to light red. Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 9.488 mg of AlK(SO₄)₂·12H₂O

Containers and storage Containers—Tight containers.

Amantadine Hydrochloride

アマンタジン塩酸塩



C₁₀H₁₇N·HCl: 187.71

Tricyclo[3.3.1.1^{3,7}]dec-1-ylamine monohydrochloride
[665-66-7]

Amantadine Hydrochloride, when dried, contains not less than 99.0% of C₁₀H₁₇N·HCl.

Description Amantadine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in formic acid, freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 0.1 g of Amantadine Hydrochloride add 1 mL of pyridine and 0.1 mL of acetic anhydride, dissolve by boiling for 1 minute, add 10 mL of dilute hydrochloric acid, and cool in ice water. Filter the crystals separated, wash with water, and dry at 105°C for 1 hour: the residue melts <2.60> between 147°C and 151°C.

(2) Determine the infrared absorption spectrum of Amantadine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Amantadine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Amantadine Hydrochloride in 5 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Amantadine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Amantadine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Amantadine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Amantadine Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS and 10 mL of chloroform, and shake. Filter the chloroform layer through absorbent cotton with 3 g of anhydrous sodium sulfate on a funnel, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: each peak area other than that of amantadine from the sample solution is not larger

than 1/3 of the peak area of amantadine from the standard solution, and the total area of each peak is not larger than the peak area of amantadine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μm in particle diameter) coated with a mixture (L) of branched hydrocarbon of petroleum hexamethyltetracosane group for gas chromatography and potassium hydroxide at the ratios of 2% and 1%, respectively.

Column temperature: Inject at a constant temperature of about 125°C, maintain the temperature for 5 minutes, raise at the rate of 5°C per minute to 150°C, and maintain at a constant temperature of about 150°C for 15 minutes.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of amantadine is about 11 minutes.

Selection of column: Dissolve 0.15 g of naphthalene in 5 mL of the sample solution, and add chloroform to make 100 mL. Proceed with 2 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of naphthalene and amantadine in this order with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of amantadine obtained from 2 μL of the standard solution composes about 10% of the full scale.

Time span of measurement: About twice as long as the retention time of amantadine beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

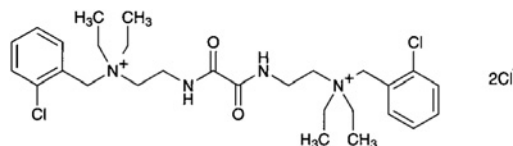
Assay Weigh accurately about 0.2 g of Amantadine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add acetic acid (100) to make 70 mL, and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.77 mg of $\text{C}_{10}\text{H}_{17}\text{N}\cdot\text{HCl}$

Containers and storage Containers—Well-closed containers.

Ambenonium Chloride

アンベノニウム塩化物



$\text{C}_{28}\text{H}_{42}\text{Cl}_4\text{N}_4\text{O}_2$: 608.47

2,2'-[(1,2-Dioxoethane-1,2-diyl)diimino]bis[N-(2-chlorobenzyl)-N,N-diethylethylammonium] dichloride
[115-79-7]

Ambenonium Chloride contains not less than 98.5% of $\text{C}_{28}\text{H}_{42}\text{Cl}_4\text{N}_4\text{O}_2$, calculated on the dried basis.

Description Ambenonium Chloride occurs as a white powder.

It is freely soluble in water, in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in acetic anhydride.

It is hygroscopic.

Melting point: about 205°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ambenonium Chloride in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ambenonium Chloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ambenonium Chloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ambenonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ambenonium Chloride according to Method 4, and perform the test. Use a solution of magnesium nitrate in ethanol (95) (1 in 5). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ambenonium Chloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, formic acid and water (12:6:5) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more

intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 11.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

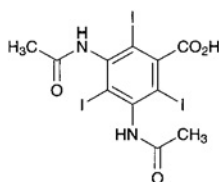
Assay Weigh accurately about 0.3 g of Ambenonium Chloride, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 30.42 \text{ mg of } C_{28}H_{42}Cl_4N_4O_2 \end{aligned}$$

Containers and storage Containers—Tight containers.

Amidotrizoic Acid

アミドトリゾ酸



$C_{11}H_9I_3N_2O_4$; 613.91

3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid [117-96-4]

Amidotrizoic Acid, calculated on the dried basis, contains not less than 98.0% of $C_{11}H_9I_3N_2O_4$.

Description Amidotrizoic Acid occurs as a white, crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Heat 0.1 g of Amidotrizoic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared absorption spectrum of Amidotrizoic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Amidotrizoic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Amidotrizoic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, and add 0.4 mL of a solution of 1-naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Determine the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.15.

(3) Soluble halides—Dissolve 2.5 g of Amidotrizoic Acid

in 20 mL of water and 2.5 mL of ammonia TS, add 20 mL of dilute nitric acid and water to make 100 mL, allow to stand for 15 minutes with occasional shaking, and filter. Discard the first 10 mL of the filtrate, transfer the subsequent 25 mL of the filtrate to a Nessler tube, and add ethanol (95) to make 50 mL. Proceed as directed under Chloride Limit Test <1.03> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS, add 6 mL of dilute nitric acid and water to make 25 mL, then ethanol (95) to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Amidotrizoic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, add 5 mL of chloroform, shake well, and allow to stand: the solution is colorless in the chloroform layer.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Amidotrizoic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 0.6 g of Amidotrizoic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

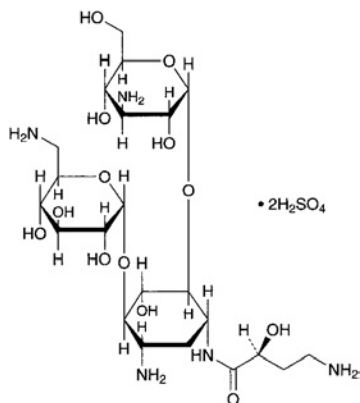
Assay Transfer about 0.5 g of Amidotrizoic Acid, accurately weighed, to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, connect to a reflux condenser, boil for 30 minutes, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <2.50> with 0.1 mol/L silver nitrate VS until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

$$\begin{aligned} \text{Each mL of 0.1 mol/L silver nitrate VS} \\ = 20.46 \text{ mg of } C_{11}H_9I_3N_2O_4 \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Amikacin Sulfate

アミカシン硫酸塩



$C_{22}H_{43}N_5O_{13} \cdot 2H_2SO_4$; 781.76

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-
[6-amino-6-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-1-*N*-
[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptomycin
disulfate [39831-55-5]

Amikacin Sulfate is the sulfate of a derivative of kanamycin.

It contains not less than 691 μ g (potency) and not more than 791 μ g (potency) per mg, calculated on the dried basis. The potency of Amikacin Sulfate is expressed as mass (potency) of amikacin ($C_{22}H_{43}N_5O_{13}$; 585.60).

Description Amikacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the infrared absorption spectrum of Amikacin Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Amikacin Sulfate Reference Standard previously dried: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g each of Amikacin Sulfate and Amikacin Sulfate Reference Standard in 4 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia water (28), methanol and tetrahydrofuran (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the principal spots from the sample solution and the standard solution exhibit a red-purple color and show the same *R_f* value.

(3) A solution of Amikacin Sulfate (1 in 100) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +76 – +84° (1 g, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Amikacin Sulfate in 100 mL of water: the pH of the solution is between 6.0 and 7.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Amikacin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Amikacin Sulfate in 4 mL of a water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ammonia water (28), methanol and tetrahydrofuran (1:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 4.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Amikacin Sulfate and Amikacin Sulfate Reference Standard, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL. Pipet 200 μ L each of these solutions in the test tube with glass stopper, add exactly 3 mL of pyridine and exactly 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100), stopper tightly, and heat in a water bath at 70°C for 30 minutes. After cooling, add exactly 2 mL each of acetic acid (100), and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the heights, *H_T* and *H_S*, of the peak of amikacin derivative.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of amikacin } (C_{22}H_{43}N_5O_{13}) \\ = W_S \times (H_T/H_S) \times 1000 \end{aligned}$$

W_S: Amount [mg (potency)] of Amikacin Sulfate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.72 g of potassium dihydrogenphosphate in 800 mL of water, adjust to pH 6.5 with a solution of potassium hydroxide (1 in 40), and add water to make 1000 mL. To 280 mL of this solution add 720 mL of methanol, and mix.

Flow rate: Adjust the flow rate so that the retention time of amikacin derivative is about 9 minutes.

System suitability—

System performance: Dissolve about 5 mg (potency) of Amikacin Sulfate and about 5 mg (potency) of Kanamycin

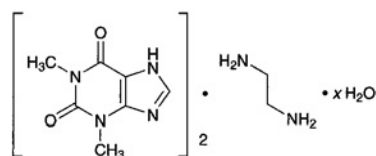
Sulfate in 5 mL of water. Transfer 200 μ L of this solution in a glass-stoppered test tube, add 3 mL of pyridine and 2 mL of a solution of 2,4,6-trinitrobenzenesulfonic acid (1 in 100), stopper tightly, heat in a water bath at 70°C for 30 minutes. After cooling, add 2 mL of acetic acid (100). When the procedure is run with 20 μ L of this solution under the above operating conditions, amikacin derivative and kanamycin derivative are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of amikacin derivative is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Aminophylline Hydrate

アミノフィリン水和物



$C_{14}H_{16}N_8O_4 \cdot C_2H_8N_2 \cdot xH_2O$
1,3-Dimethyl-1*H*-purine-2,6 (3*H*,7*H*)-dione
hemi(ethylenediamine) hydrate [5877-66-5, dihydrate]

Aminophylline Hydrate contains not less than 84.0% and not more than 86.0% of theophylline ($C_7H_8N_4O_2$: 180.16), and not less than 14.0% and not more than 15.0% of ethylenediamine ($C_2H_8N_2$: 60.10), calculated on the anhydrous basis.

Description Aminophylline Hydrate occurs as white to pale yellow granules or powder. It is odorless or slightly ammonia-like odor, and has a bitter taste.

It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) and in diethyl ether.

To 1 g of Aminophylline Hydrate add 5 mL of water, and shake: it dissolves almost completely. Separation of crystals begins in 2 to 3 minutes, and these crystals dissolve on the addition of a small amount of ethylenediamine.

It is gradually affected by light, and gradually loses ethylenediamine in air.

Identification (1) Dissolve 0.75 g of Aminophylline Hydrate in 30 mL of water, and use this solution as the sample solution. To 20 mL of the sample solution add 1 mL of dilute hydrochloric acid: a precipitate is gradually formed. Filter the precipitate, recrystallize from water, and dry at 105°C for 1 hour: the crystals so obtained melt <2.60> between 271°C and 275°C.

(2) Dissolve 0.1 g of the crystals obtained in (1) in 50 mL of water, and to 2 mL of this solution add tannic acid TS dropwise: a white precipitate is produced, and this precipitate dissolves upon dropwise addition of tannic acid TS.

(3) To 0.01 g of the crystals obtained in (1) add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert the dish containing the residue over a

vessel containing 2 to 3 drops of ammonia TS: the color of the residue changes to red-purple, which is destroyed on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of water, add 3 mL of ammonia-ammonium chloride buffer solution, pH 8.0, and 1 mL of copper (II) sulfate-pyridine TS, and mix. Add 5 mL of chloroform to the mixture, and shake: the chloroform layer develops a green color.

(5) To 5 mL of the sample solution obtained in (1) add 2 drops of copper (II) sulfate TS: a purple color develops. Add 1 mL of copper (II) sulfate TS: the color changes to blue, and green precipitates are formed on standing.

pH <2.54> Dissolve 1.0 g of Aminophylline Hydrate in 25 mL of water: the pH of the solution is between 8.0 and 9.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Aminophylline Hydrate in 10 mL of hot water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Aminophylline Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Water <2.48> Not more than 7.9% (0.3 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Theophylline—Weigh accurately about 0.25 g of Aminophylline Hydrate, and dissolve in 50 mL of water and 8 mL of ammonia TS by gentle warming on a water bath. Add exactly 20 mL of 0.1 mol/L silver nitrate VS, warm on a water bath for 15 minutes, allow to stand between 5°C and 10°C for 20 minutes, collect the precipitate by suction, and wash with three 10-mL portions of water. Combine the filtrate and washings, and add dilute nitric acid to make neutral. Add 3 mL of dilute nitric acid, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 18.02 mg of theophylline ($C_7H_8N_4O_2$)

(2) Ethylenediamine—Weigh accurately about 0.5 g of Aminophylline Hydrate, dissolve in 30 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.005 mg of ethylenediamine ($C_2H_8N_2$)

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Aminophylline Injection

アミノフィリン注射液

Aminophylline Injection is an aqueous solution for injection.

It contains not less than 75% and not more than 86% of the labeled amount of theophylline ($C_7H_8N_4O_2$: 180.16), and not less than 13% and not more than 20% of ethylenediamine ($C_2H_8N_2$: 60.10).

The concentration of Aminophylline Injection is expressed as the quantity of aminophylline dihydrate ($C_{16}H_{24}N_{10}O_4 \cdot 2H_2O$: 456.46).

Method of preparation Prepare as directed under Injections, with Aminophylline Hydrate. It may be prepared with Theophylline and its equivalent Ethylenediamine, instead of Aminophylline Hydrate.

It may contain not more than 60 mg of Ethylenediamine as a stabilizer for each g of Aminophylline Hydrate.

Description Aminophylline Injection is a clear and colorless liquid. It has a slightly bitter taste.

It gradually changes in color by light.

pH: 8.0 – 10.0

Identification To a volume of Aminophylline Injection, equivalent to 0.75 g of Aminophylline Hydrate according to the labeled amount, add water to make 30 mL. Proceed with this solution as directed in the Identification under Aminophylline Hydrate.

Extractable volume <6.05> It meets the requirement.

Assay (1) Theophylline—To an accurately measured volume of Aminophylline Injection, equivalent to about 0.2 g of theophylline ($C_7H_8N_4O_2$) (about 0.25 g of Aminophylline Hydrate), add 15 mL of water, 8 mL of ammonia TS and 20 mL of silver nitrate TS, and warm on a water bath for 15 minutes. Cool to between 5°C and 10°C for 20 minutes, filter the precipitate through a glass filter (G4), and wash with three 10-mL portions of water. Dissolve the precipitate in 5 mL of nitric acid, and wash the filter with three 10-mL portions of water. Combine the nitric acid solution and washings, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 18.02 mg of theophylline ($C_7H_8N_4O_2$)

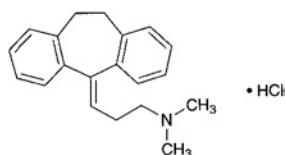
(2) Ethylenediamine—To an accurately measured volume of Aminophylline Injection, equivalent to about 30 mg of ethylenediamine ($C_2H_8N_2$) (about 0.2 g of Aminophylline Hydrate), add water to make 30 mL, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 to 3 drops of bromophenol blue TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 3.005 mg of ethylenediamine ($C_2H_8N_2$)

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Amitriptyline Hydrochloride

アミトリプチリン塩酸塩



$C_{20}H_{23}N \cdot HCl$: 313.86

3-(10,11-Dihydro-5H-dibenzo[*a,d*]cyclohepten-5-

ylidene)-*N,N*-dimethylpropylamine monohydrochloride
[549-18-8]

Amitriptyline Hydrochloride, when dried, contains not less than 99.0% of $C_{20}H_{23}N \cdot HCl$.

Description Amitriptyline Hydrochloride occurs as colorless crystals or a white to pale yellow crystalline powder. It has a bitter taste and a numbing effect.

It is freely soluble in water, in ethanol (95) and in acetic acid (100), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Amitriptyline Hydrochloride (1 in 20) is between 4.0 and 5.0.

Identification (1) Dissolve 5 mg of Amitriptyline Hydrochloride in 3 mL of sulfuric acid: a red color develops. Add 5 drops of potassium dichromate TS to this solution: it turns dark brown.

(2) Acidify 1 mL of a solution of Amitriptyline Hydrochloride (1 in 500) with 0.5 mL of dilute nitric acid, and add 1 drop of silver nitrate TS: a white, opalescent precipitate is produced.

(3) Determine the absorption spectrum of a solution of Amitriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Amitriptyline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 195 – 198°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Amitriptyline Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Amitriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Amitriptyline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.39 mg of $C_{20}H_{23}N \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Amitriptyline Hydrochloride Tablets

アミトリプチリン塩酸塩錠

Amitriptyline Hydrochloride Tablets contain not less than 90% and not more than 110% of the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$; 313.86).

Method of preparation Prepare as directed under Tablets, with Amitriptyline Hydrochloride.

Identification (1) Weigh a quantity of powdered Amitriptyline Hydrochloride Tablets, equivalent to 0.1 g of Amitriptyline Hydrochloride according to the labeled amount. Add 10 mL of chloroform, shake thoroughly, and filter. Evaporate the filtrate on a water bath to about 2 mL, add diethyl ether until turbidity is produced, and allow to stand. Filter the crystals formed through a glass filter (G4), and proceed as directed in the Identification (1) and (2) under Amitriptyline Hydrochloride.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 238 nm and 240 nm, and a minimum between 228 nm and 230 nm.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Amitriptyline Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of 2 nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution 60 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add 2 nd fluid for dissolution test to make exactly V' mL so that each mL contains about 11 μg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of Amitriptyline Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 2 nd fluid for dissolution test to make exactly 250 mL. Pipet 5 mL of this solution, add 2 nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate of Amitriptyline Hydrochloride Tablets in 60 minutes should be not less than 70%.

Dissolution rate (%) with respect to the labeled amount of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S : Amount (mg) of Amitriptyline Hydrochloride Reference Standard.

C : Labeled amount (mg) of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Amitriptyline Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of amitriptyline hydrochloride ($C_{20}H_{23}N.HCl$), and add 75 mL of diluted methanol (1 in 2). After shaking for 30 minutes, add diluted methanol (1 in 2) to make exactly 100 mL, and filter. Discard the first 20-mL portion of the filtrate, measure exactly the subsequent 5-mL portion, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Amitriptyline Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Measure exactly 5 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

$$\begin{aligned} &\text{Amount (mg) of amitriptyline hydrochloride} \\ &(\text{C}_{20}\text{H}_{23}\text{N.HCl}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Amitriptyline Hydrochloride Reference Standard

Containers and storage Containers—Tight containers.

Ammonia Water

アンモニア水

Ammonia Water contains not less than 9.5 w/v% and not more than 10.5 w/v% of ammonia (NH_3 ; 17.03).

Description Ammonia Water occurs as a clear, colorless liquid, having a very pungent, characteristic odor.

It is alkaline.

Specific gravity d_{20}^{20} : 0.95 – 0.96

Identification (1) Hold a glass rod moistened with hydrochloric acid near the surface of Ammonia Water: dense white fumes are produced.

(2) Hold moistened red litmus paper near the surface of Ammonia Water: it turns blue.

Purity (1) Residue on evaporation—Evaporate 10.0 mL of Ammonia Water to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Heavy metals <1.07>—Evaporate 5.0 mL of Ammonia Water to dryness on a water bath, add 1 mL of dilute hydrochloric acid to the residue, and evaporate to dryness. Dissolve the residue in 2 mL of dilute acetic acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(3) Potassium permanganate-reducing substances—To 10.0 mL of Ammonia Water add 40 mL of dilute sulfuric acid while cooling, and add 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color of the potassium permanganate does not disappear within 10 minutes.

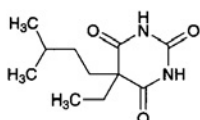
Assay Measure exactly 5 mL of Ammonia Water, add 25 mL of water, and titrate <2.50> with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 17.03 mg of NH₃

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Amobarbital

アモバルビタール



C₁₁H₁₈N₂O₃: 226.27
5-Ethyl-5-(3-methylbutyl)pyrimidine-
2,4,6(1H,3H,5H)-trione [57-43-2]

Amobarbital, when dried, contains not less than 99.0% of C₁₁H₁₈N₂O₃.

Description Amobarbital occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, sparingly soluble in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

The pH of a saturated solution of Amobarbital is between 5.0 and 5.6.

Identification (1) Boil 0.2 g of Amobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 0.05 g of Amobarbital in 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7, and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. Shake the mixture: a red-purple color is produced in the chloroform layer.

(3) To 0.4 g of Amobarbital add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture on a water bath for 30 minutes under a reflux condenser, and allow to stand for 1 hour. Filter the crystals produced, wash with 7 mL of sodium hydroxide TS and a small portion of water, recrystallize from ethanol, and dry at 105°C for 30 minutes: the crystals so obtained melt <2.60> between 168°C and 173°C or between 150°C and 154°C.

Melting point <2.60> 157 – 160°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Amobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.30 g of Amobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and

water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Dissolve 0.40 g of Amobarbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Amobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Amobarbital. The solution is not more colored than Matching Fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

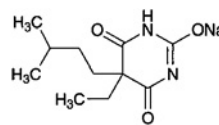
Assay Weigh accurately about 0.5 g of Amobarbital, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 22.63 mg of C₁₁H₁₈N₂O₃

Containers and storage Containers—Well-closed containers.

Amobarbital Sodium for Injection

注射用アモバルビタールナトリウム



C₁₁H₁₇N₂NaO₃: 248.25
Monosodium 5-ethyl-5-(3-methylbutyl)-4,6-
dioxo-1,4,5,6-tetrahydropyrimidin-2-olate [64-43-7]

Amobarbital Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of amobarbital sodium (C₁₁H₁₇N₂NaO₃), and not less than 92.5% and not more than 107.5% of the labeled amount of amobarbital sodium (C₁₁H₁₇N₂NaO₃).

Method of preparation Prepare as directed under Injections.

Description Amobarbital Sodium for Injection occurs as white crystals or a crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether and in chloroform.

The pH of its solution (1 in 10) is between 10.0 and 11.0. It is hygroscopic.

Identification (1) Dissolve 1.5 g of Amobarbital Sodium for Injection in 20 mL of water, and add 10 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Collect the precipitate, wash with four 10-mL portions of water, and dry at 105°C for 3 hours: it melts <2.60> between 157°C and 160°C. With this precipitate, proceed as directed in the Identification under Amobarbital.

(2) Ignite 0.5 g of Amobarbital Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Amobarbital Sodium for Injection in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 0.5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.018%).

(3) Sulfate <1.14>—Dissolve 2.0 g of Amobarbital Sodium for Injection in 49 mL of water, add 1 mL of acetic acid (100), shake, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 0.5 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(4) Heavy metals <1.07>—Dissolve 2.0 g of Amobarbital Sodium for Injection in 45 mL of water, add 5 mL of dilute hydrochloric acid, shake vigorously, and warm on a water bath for 2 minutes with occasional shaking. Cool, add 30 mL of water, shake, and filter. Discard the first 10 mL of the filtrate, add 1 drop of phenolphthalein TS to the subsequent 40 mL of the filtrate, add ammonia TS until a slight red color develops, and add 2.5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.5 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, add ammonia TS until a pale red color develops, and add 2.5 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(5) Neutral or basic substances—Dissolve about 1 g of Amobarbital Sodium for Injection, accurately weighed, in 10 mL of water and 5 mL of sodium hydroxide TS, then add 40 mL of chloroform, and shake well. Separate the chloroform layer, wash with two 5-mL portions of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 0.30%.

(6) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Amobarbital Sodium for Injection: the so-

lution is not more colored than Matching Fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

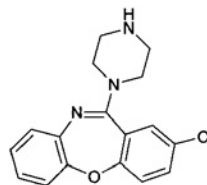
Assay Weigh accurately the contents of not less than 10 samples of Amobarbital Sodium for Injection. Weigh accurately about 0.5 g of the contents, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform, then with three 25-mL portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water, and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Combine the filtrate and the washings, and add 10 mL of ethanol (95). Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination with a mixture of 160 mL of chloroform and 30 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 24.83 mg of $C_{11}H_{17}N_2NaO_3$

Containers and storage Containers—Hermetic containers.

Amoxapine

アモキサピン



$C_{17}H_{16}ClN_3O$: 313.78

2-Chloro-11-(piperazin-1-yl)dibenzo[*b,f*][1,4]oxazepine
[14028-44-5]

Amoxapine, when dried, contains not less than 98.5% of $C_{17}H_{16}ClN_3O$.

Description Amoxapine occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Amoxapine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption as the same wavelengths.

(2) Determine the infrared absorption spectrum of Amoxapine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Amoxapine as directed under Flame Coloration Test <1.04> (2); a green color appears.

Melting point <2.60> 178 – 182°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Amoxapine according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(2) Related substances—Dissolve 0.5 g of Amoxapine in 10 mL of a mixture of ethanol (95) and acetic acid (100) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 10 mL. Pipet 1 mL of this solution, add a mixture of ethanol (95) and acetic acid (100) (9:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and acetic acid (100) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.4% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

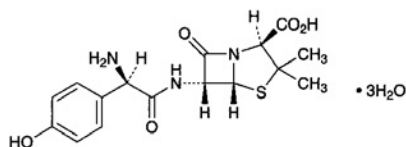
Assay Weigh accurately about 0.3 g of Amoxapine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 15.69 mg of C₁₇H₁₆ClN₃O

Containers and storage Containers—Tight containers.

Amoxicillin Hydrate

アモキシシリン水和物



C₁₆H₁₉N₃O₅S·3H₂O: 419.45
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)-acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [61336-70-7]

Amoxicillin Hydrate contains not less than 950 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Amoxicillin Hydrate is expressed as mass (potency) of amoxicillin (C₁₆H₁₉N₃O₅S: 365.40).

Description Amoxicillin Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Amoxicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Amoxicillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +290 – +315° (0.1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity (1) Heavy metals <1.07>—To 1.0 g of Amoxicillin Hydrate add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), mix, and heat on a water bath to dryness. Carbonize the residue by gently heating. After cooling, add 1 mL of sulfuric acid, heat carefully, then heat at 500 – 600°C to incinerate. After cooling, add 1 mL of hydrochloric acid to the residue, and heat on a water bath to dryness. Then add 10 mL of water to the residue, and heat on a water bath to dissolve. After cooling, add ammonia TS to adjust the pH to 3 – 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 2 mL of a solution of magnesium sulfate heptahydrate (1 in 4), then proceed in the same manner as for preparation of the test solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Amoxicillin Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Amoxicillin Hydrate in 50 mL of a solution of sodium tetraborate decahydrate (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than amoxicillin obtained from the sample solution is not more than the peak area of amoxicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

Time span of measurement: About 4 times as long as the

retention time of amoxicillin.

System suitability—

Test for required detection: To exactly 1 mL of the standard solution add a solution of sodium tetraborate decahydrate (1 in 200) to make exactly 10 mL. Confirm that the peak area of amoxicillin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of amoxicillin obtained from 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Water <2.48> Not less than 11.0% and not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Amoxicillin Hydrate and Amoxicillin Reference Standard, equivalent to about 30 mg (potency), dissolve each in a solution of boric acid (1 in 200) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of amoxicillin of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of amoxicillin (C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S)} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Amoxicillin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.361 g of sodium acetate trihydrate in 750 mL of water, adjust the pH to 4.5 with acetic acid (31), and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of amoxicillin is about 8 minutes.

System suitability—

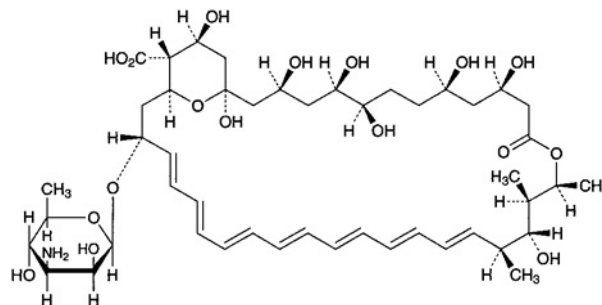
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of amoxicillin is not less than 2500.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of amoxicillin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Amphotericin B

アムホテリシン B



$C_{47}H_{73}NO_{17}$: 924.08

(1*R*,3*S*,5*R*,6*R*,9*R*, 11*R*, 15*S*, 16*R*, 17*R*, 18*S*, 19*E*, 21*E*, 23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*S*,37*S*)-33-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriacontane-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [1397-89-3]

Amphotericin B is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces nodosus*.

It contains not less than 840 μ g (potency) per mg, calculated on the dried basis. The potency of Amphotericin B is expressed as mass (potency) of amphotericin B ($C_{47}H_{73}NO_{17}$).

Description Amphotericin B occurs as a yellow to orange powder.

It is freely soluble in dimethylsulfoxide and practically insoluble in water and in ethanol (95).

Identification (1) Dissolve 5 mg of Amphotericin B in 10 mL of dimethylsulfoxide. To 1 mL of this solution add 5 mL of phosphoric acid: a blue color develops between the two layers, and the solution becomes blue by shaking. After addition of 15 mL of water it becomes yellow to light yellow-brown by shaking.

(2) Dissolve 25 mg of Amphotericin B in 5 mL of dimethylsulfoxide, and add methanol to make 50 mL. To 1 mL of this solution add methanol to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Amphotericin B Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity Amphotericin A—Weigh accurately about 50 mg each of Amphotericin B and Amphotericin B Reference Standard, add exactly 10 mL each of dimethylsulfoxide to dissolve, and add methanol to make exactly 50 mL. Pipet 4 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution (1), respectively. Separately, weigh accurately about 20 mg of Nystatin Reference Standard, add exactly 40 mL of dimethylsulfoxide to dissolve, then add methanol to make exactly 200 mL. Pipet 4 mL of this solu-

tion, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained in the same manner as the sample solution as the blank, and determine the absorbances at 282 nm and at 304 nm. Calculate the amount of amphotericin A by the following equation: not more than 5% for Amphotericin B used for injections, and not more than 15% for Amphotericin B not used for injections.

$$\begin{aligned} & \text{Amount (\% of amphotericin A)} \\ &= \frac{W_S \times \{(A_{Sa1} \times A_{T2}) - (A_{Sa2} \times A_{T1})\} \times 25}{W_T \times \{(A_{Sa1} \times A_{Sb2}) - (A_{Sa2} \times A_{Sb1})\}} \end{aligned}$$

W_S : Amount (mg) of Nystatin Reference Standard

W_T : Amount (mg) of the sample

A_{Sa1} : Absorbance at 282 nm of the standard solution (1)

A_{Sb1} : Absorbance at 282 nm of the standard solution (2)

A_{Sa2} : Absorbance at 304 nm of the standard solution (1)

A_{Sb2} : Absorbance at 304 nm of the standard solution (2)

A_{T1} : Absorbance at 282 nm of the sample solution

A_{T2} : Absorbance at 304 nm of the sample solution

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Saccharomyces cerevisiae* ATCC 9763

(ii) Culture medium—Use the medium 2) under (1) Agar media for seed and base layer.

(iii) Preparation of cylinder-agar plate—Proceed as directed in 5 under the Cylinder plate method, using Petri dish plates not dispensing the agar medium for base layer and dispensing 8.0 mL of the seeded agar medium.

(iv) Standard solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B Reference Standard equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add dimethylsulfoxide to make solutions so that each mL contains 200 µg (potency) and 50 µg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solution—Use light-resistant vessels. Weigh accurately an amount of Amphotericin B equivalent to about 20 mg (potency), dissolve in dimethylsulfoxide to make exactly 20 mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 µg (potency) and 50 µg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Amphotericin B for Injection

注射用アムホテリシン B

Amphotericin B for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of amphotericin B (C₄₇H₇₃NO₁₇: 924.08).

Method of preparation Prepare as directed under Injections, with Amphotericin B.

Description Amphotericin B for Injection occurs as yellow to orange, powder or masses.

Identification To an amount of Amphotericin B for Injection, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

pH <2.54> Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B according to the labeled amount, in 10 mL of water. To 1 mL of this solution add water to make 50 mL: 7.2 – 8.0.

Purity Clarity and color of solution—Dissolve an amount of Amphotericin B for Injection, equivalent to 50 mg (potency) of Amphotericin B according to the labeled amount, in 10 mL of water: the solution is clear and yellow to orange.

Loss on drying <2.41> Not more than 8.0% (0.3 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 3.0 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test. However, use the average of the limits specified in the potency definition for *T*.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B for Injection, equivalent to about 50 mg (potency) according to the labeled amount, dissolve in dimethylsulfoxide to make exactly 50 mL, and use this solution as the sample stock solu-

tion. Measure exactly a suitable quantity of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains about 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers. Storage—Store in a cold place.

Amphotericin B Syrup

アムホテリシン B シロップ

Amphotericin B Syrup contain not less than 90.0% and not more than 115.0% of the labeled amount of amphotericin B ($\text{C}_{47}\text{H}_{73}\text{NO}_{17}$: 924.08).

Method of preparation Prepare as directed under Syrup, with Amphotericin B.

Identification To an amount of Amphotericin B Syrup, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

pH <2.54> 5.0 – 7.0.

Microbial limits <4.05> Perform the test as directed under Microbial Limit Test: the total microbial count is not more than 100 per mL, and the total fungus and yeast count is not more than 50 per mL.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately an amount of Amphotericin B Syrup, equivalent to about 0.1 g (potency) according to the labeled amount, add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains about 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Amphotericin B Tablets

アムホテリシン B 錠

Amphotericin B Tablets contain not less than 90.0% and not more than 120.0% of the labeled amount of amphotericin B ($\text{C}_{47}\text{H}_{73}\text{NO}_{17}$: 924.08).

Method of preparation Prepare as directed under Tablets, with Amphotericin B.

Identification To an amount of pulverized Amphotericin B Tablets, equivalent to 25 mg (potency) of Amphotericin B according to the labeled amount, add 5 mL of dimethylsulfoxide and 45 mL of methanol, and shake. To 1 mL of this solution add methanol to make 50 mL, and filter, if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 361 nm and 365 nm, between 380 nm and 384 nm and between 403 nm and 407 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test. However, use the average of the limits specified in the potency definition for *T*.

Loss on drying <2.41> Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, preparation of cylinder-agar plate and standard solutions—Proceed as directed in the Assay under Amphotericin B.

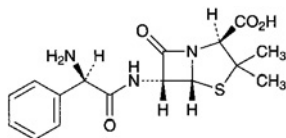
(ii) Sample solutions—Prepare using light-resistant containers. Weigh accurately and powder not less than 20 tablets of Amphotericin B Tablets. Weigh accurately a part of the powder, equivalent to about 0.1 g (potency) according to the labeled amount, add about 70 mL of dimethylsulfoxide, shake, then add dimethylsulfoxide to make exactly 100 mL, centrifuge, and use the supernatant liquid as the sample stock solution. Measure exactly a suitable amount of the sample stock solution, add dimethylsulfoxide to make solutions so that each mL contains 200 μg (potency) and 50 μg (potency). Pipet 1 mL each of these solutions, add 0.2 mol/L phosphate buffer solution, pH 10.5 to make exactly 20 mL, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Anhydrous Ampicillin

Anhydrous Aminobenzylpenicillin

無水アンピシリン



$C_{16}H_{19}N_3O_4S$: 349.40
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [69-53-4]

Anhydrous Ampicillin contains not less than 960 μg (potency) and not more than 1005 μg (potency) per mg, calculated on the anhydrous basis. The potency of Anhydrous Ampicillin is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$).

Description Anhydrous Ampicillin occurs as white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Anhydrous Ampicillin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Anhydrous Ampicillin in 100 mL of water is between 4.0 and 5.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Anhydrous Ampicillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Anhydrous Ampicillin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Anhydrous Ampicillin in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than ampicillin from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and

flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: As long as about 10 times of the retention time of ampicillin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 2.0% (2.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Anhydrous Ampicillin and Ampicillin Reference Standard, equivalent to about 50 mg (potency), add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ampicillin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of ampicillin } (C_{16}H_{19}N_3O_4S) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Ampicillin Reference Standard

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

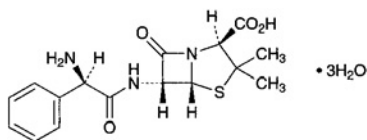
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ampicillin Hydrate

Aminobenzylpenicillin Hydrate

アンピシリン水和物



$C_{16}H_{19}N_3O_4S \cdot 3H_2O$: 403.45

(2*S*,5*R*,6*R*)-6-[(2*R*)-2-Amino-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [7177-48-2]

Ampicillin Hydrate contains not less than 960 μ g (potency) and not more than 1005 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Hydrate is expressed as mass (potency) of ampicillin ($C_{16}H_{19}N_3O_4S$: 349.40).

Description Ampicillin Hydrate occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification Determine the infrared absorption spectrum of Ampicillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ampicillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +280 – +305° (0.5 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Ampicillin Hydrate in 400 mL of water is between 3.5 and 5.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ampicillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ampicillin hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Ampicillin hydrate in the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than that of ampicillin obtained from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the standard solution.

(4) *N,N*-Dimethylaniline—Weigh accurately about 1 g of Ampicillin Hydrate, dissolve in 5 mL of sodium hydroxide TS, add exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the sample solution. Separately, weigh accurately about 50 mg of *N,N*-dimethylaniline, dissolve in 2 mL of hydrochloric acid and 20 mL of water, add water to make exactly 50 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, determine the ratios, Q_T and Q_S , of the peak area of *N,N*-dimethylaniline to that of the internal standard, and calculate the amount of *N,N*-dimethylaniline by the following equation: not more than 20 ppm.

$$\begin{aligned} \text{Amount (ppm) of } N,N\text{-dimethylaniline} \\ = (W_S/W_T) \times (Q_T/Q_S) \times 400 \end{aligned}$$

W_S : Amount (g) of *N,N*-dimethylaniline

W_T : Amount (g) of the sample

Internal standard solution—A solution of naphthalene in cyclohexane (1 in 20,000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (180 – 250 μ m in particle diameter) coated with 50% phenyl-50% methyl polysiloxane for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 120°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of *N,N*-dimethylaniline is about 5 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard stock solution, and add water to make exactly 250 mL. Pipet 1 mL of this solution, add 5 mL of sodium hydroxide TS and exactly 1 mL of the internal standard solution, shake vigorously for 1 minute, and use the upper layer liquid obtained after allowing it to stand for the test. Confirm that when the procedure is run with 1 μ L of the upper layer liquid under the above operating conditions, the

ratio of the peak area of *N,N*-dimethylaniline to that of the internal standard is equivalent to 15 – 25% of the ratio of the peak area of *N,N*-dimethylaniline to that of the internal standard obtained from the standard solution.

System performance: Dissolve 50 mg of *N,N*-dimethylaniline in cyclohexane to make 50 mL. To 1 mL of this solution add the internal standard solution to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 1 μ L of the solution for system suitability test under the above operating conditions, *N,N*-dimethylaniline and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 1 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of *N,N*-dimethylaniline to that of the internal standard is not more than 2.0%.

Water <2.48> 12.0 – 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ampicillin Hydrate and Ampicillin Reference Standard, equivalent to about 50 mg (potency), dissolve in a suitable volume of the mobile phase, add exactly 5 mL each of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ampicillin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of ampicillin } \text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S} \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Ampicillin Reference Standard

Internal standard solution—A solution of guaifenesin in the mobile phase (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 40.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating

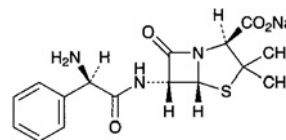
conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ampicillin Sodium

Aminobenzylpenicillin Sodium

アンピシリンナトリウム



$\text{C}_{16}\text{H}_{18}\text{N}_3\text{NaO}_4\text{S}$: 371.39

Monosodium (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [69-52-3]

Ampicillin Sodium contains not less than 850 μ g (potency) and not more than 950 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Ampicillin Sodium is expressed as mass (potency) of ampicillin ($\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$: 349.40).

Description Ampicillin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is very soluble in water, and sparingly soluble in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Ampicillin Sodium, previously dried in a desiccator (reduced pressure not exceeding 0.67 kPa, 60°C) for 3 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Ampicillin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +246 – +272° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Ampicillin Sodium in 10 mL of water is between 8.0 and 10.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ampicillin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ampicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Ampicillin Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this

solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than ampicillin obtained from the sample solution is not more than the peak area of ampicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.94 g of diammonium hydrogen phosphate in 850 mL of water, add 100 mL of acetonitrile, adjust the pH to 5.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 6 minutes.

Time span of measurement: About 10 times as long as the retention time of ampicillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of ampicillin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of ampicillin obtained from 10 μ L of the standard solution.

System performance: Dissolve 50 mg of Ampicillin Reference Standard in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200) and the mobile phase to make 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μ L of the solution for system suitability test under the above operating conditions, ampicillin and guaifenesin are eluted in this order with the resolution between these peaks being not less than 35.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of ampicillin to that of guaifenesin is not more than 1.0%.

Water <2.48> Not more than 2.0% (0.2 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer, having pH 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 5 μ g (potency) and 1.25 μ g (potency), and use these solutions as the high concentration

standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Ampicillin Sodium, equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 5 μ g (potency) and 1.25 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Amyl Nitrite

亜硝酸アミル

C₅H₁₁NO₂: 117.15

Amyl Nitrite is the nitrous acid ester of 3-methylbutanol-1 and contains a small quantity of 2-methylbutanol-1 and the nitrous acid esters of other homologues.

It contains not less than 90.0 % of C₅H₁₁NO₂.

Description Amyl Nitrite is a clear, light yellowish liquid, and has a characteristic, fruity odor.

It is miscible with ethanol (95), and with diethyl ether.

It is practically insoluble in water.

It is affected by light and by heat.

It is volatile at ordinary temperature and flammable even at a low temperature.

Boiling point: about 97°C

Identification Determine the infrared spectrum of Amyl Nitrite as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{20}^{20} : 0.871 – 0.880

Purity (1) Acidity—To 5 mL of Amyl Nitrite add a mixture of 1.0 mL of 1 mol/L sodium hydroxide VS, 10 mL of water and 1 drop of phenolphthalein TS, shake, and allow to stand for 1 minute: the light red color of the water layer does not disappear.

(2) Water—Allow 2.0 mL of Amyl Nitrite to stand in ice water: no turbidity is produced.

(3) Aldehyde—To 3 mL of a mixture of equal volumes of silver nitrate TS and aldehyde free-ethanol add ammonia TS dropwise until the precipitate first formed is redissolved. Add 1.0 mL of Amyl Nitrite, and warm between 60°C and 70°C for 1 minute: a brown to black color is not produced.

(4) Residue on evaporation—Evaporate 10.0 mL of Amyl Nitrite on a water bath in a draft, carefully protecting from flame, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Weigh accurately a volumetric flask containing 10 mL of ethanol (95), add about 0.5 g of Amyl Nitrite, and weigh accurately again. Add exactly 25 mL of 0.1 mol/L sil-

ver nitrate VS, then add 15 mL of potassium chlorate solution (1 in 20) and 10 mL of dilute nitric acid, stopper the flask immediately, and shake it vigorously for 5 minutes. Dilute with water to make exactly 100 mL, shake, and filter through dry filter paper. Discard the first 20 mL of the filtrate, measure exactly 50 mL of the subsequent filtrate, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 35.14 mg of $C_5H_{11}NO_2$

Containers and storage Containers—Hermetic containers not exceeding 10-ml capacity.

Storage—Light-resistant, in a cold place, and remote from fire.

Dental Antiformin

Dental Sodium Hypochlorite Solution

歯科用アンチホルミン

Dental Antiformin contains not less than 3.0 w/v% and not more than 6.0 w/v% of sodium hypochlorite (NaClO: 74.44).

Description Dental Antiformin is a slightly light yellow-green, clear liquid. It has a slight odor of chlorine.

It gradually changes by light.

Identification (1) Dental Antiformin changes red litmus paper to blue, and then decolorizes it.

(2) To Dental Antiformin add dilute hydrochloric acid: it evolves the odor of chlorine, and the gas changes potassium iodide starch paper moistened with water to blue.

(3) Dental Antiformin responds to the Qualitative Tests <1.09> (1) for sodium salt.

Assay Measure exactly 3 mL of Dental Antiformin in a glass-stoppered flask, add 50 mL of water, 2 g of potassium iodide and 10 mL of acetic acid (31), and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.722 mg of NaClO

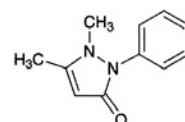
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 10°C.

Antipyrine

Phenazone

アンチピリン



$C_{11}H_{12}N_2O$: 188.23

1,5-Dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one
[60-80-0]

Antipyrine, when dried, contains not less than 99.0% of $C_{11}H_{12}N_2O$.

Description Antipyrine occurs as colorless or white crystals, or a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and sparingly soluble in diethyl ether.

A solution of Antipyrine (1 in 10) is neutral.

Identification (1) To 5 mL of a solution of Antipyrine (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 2 mL of a solution of Antipyrine (1 in 100) add 4 drops of dilute iron (III) chloride TS: a yellow-red color develops. Then add 10 drops of dilute sulfuric acid: the color changes to light yellow.

(3) To 5 mL of a solution of Antipyrine (1 in 100) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

(4) To 0.1 g of Antipyrine add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, boil the mixture, and cool: a yellow-red precipitate is produced.

Melting point <2.60> 111 – 113°C

Purity (1) Chloride <1.03>—Perform the test with 1.0 g of Antipyrine. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Antipyrine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Antipyrine: the solution remains colorless.

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

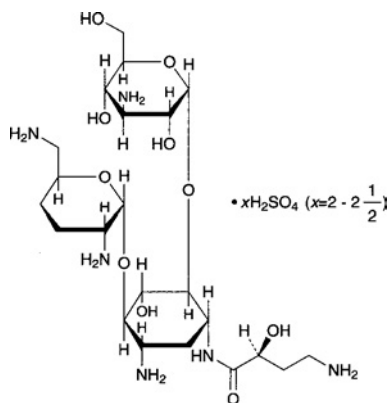
Assay Dissolve about 0.2 g of Antipyrine, previously dried and accurately weighed, in 20 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Dissolve the precipitate in 10 mL of chloroform, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS
= 9.411 mg of $C_{11}H_{12}N_2O$

Containers and storage Containers—Well-closed containers.

Arbekacin Sulfate

アルベカシン硫酸塩



$C_{22}H_{44}N_6O_{10} \cdot xH_2SO_4$ ($x = 2 - 2\frac{1}{2}$)

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,3,4,6-tetrahydro- α -D-*erythro*-hexopyranosyl-(1 \rightarrow 4)]-1-N-[(2*S*)-4-amino-2-hydroxybutanoyl]-2-deoxy-D-streptamine sulfate [51025-85-5, Arbekacin]

Arbekacin Sulfate is the sulfate of a derivative of dibekacin.

It contains not less than 670 μ g (potency) and not more than 750 μ g (potency) per mg, calculated on the dried basis. The potency of Arbekacin Sulfate is expressed as mass (potency) of arbekacin ($C_{22}H_{44}N_6O_{10}$: 552.62).

Description Arbekacin Sulfate occurs as a white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 10 mg each of Arbekacin Sulfate and Arbekacin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spot obtained from the sample solution and the spot from the standard solution are purple-brown in color and their R_f values are the same.

(2) A solution of Arbekacin Sulfate (1 in 50) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +69 – +79° (0.25 g after drying, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.75 g of Arbekacin Sulfate in 10 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Arbekacin Sulfate in 5 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Arbekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Dibekacin—Weigh accurately about 20 mg of Arbekacin Sulfate, add exactly 10 mL of the internal standard solution to dissolve, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Dibekacin Sulfate Reference Standard, equivalent to about 10 mg (potency), and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of dibekacin to that of the internal standard. Calculate the amount of dibekacin by the following equation: not more than 2.0%.

$$\begin{aligned} \text{Amount (\%)} & \text{ of dibekacin} \\ & = (W_S/W_T) \times (Q_T/Q_S) \times (1/10) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Dibekacin Sulfate Reference Standard

W_T : Amount (mg) of the sample

Internal standard solution—A solution of bekanamycin sulfate (1 in 2000).

Operating conditions—

Detector: Fluorometric detector (excitation wavelength: 340 nm, detection wavelength: 460 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Reaction coil: A column about 0.3 mm in inside diameter and about 3 m in length.

Reaction coil temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8.70 g of sodium 1-pentane sulfonate and 8.52 g of anhydrous sodium sulfate in 980 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 230 mL of this solution add 20 mL of methanol.

Reagent: Dissolve 12.36 g of boric acid in 960 mL of water, add 10 mL of a solution of *o*-phthalaldehyde in ethanol (99.5) (1 in 25), adjust the pH to 10.5 with 8 mol/L potassium hydroxide TS, and add water to make 1000 mL. To this solution add 1 mL of 2-mercaptoethanol.

Reaction temperature: A constant temperature of about 50°C.

Flow rate of the mobile phase: 0.5 mL per minute.

Flow rate of the reagent: 1 mL per minute.

System suitability—

System performance: Dissolve 20 mg each of Arbekacin Sulfate, bekanamycin sulfate and dibekacin sulfate in 200 mL

of water. When the procedure is run with 5 μL of this solution under the above operating conditions, bekanamycin, arbekacin and dibekacin are eluted in this order, and the resolution between the peaks, bekanamycin and arbekacin is not less than 5 and arbekacin and dibekacin is not less than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dibekacin to that of the internal standard is not more than 2.0%.

(4) **Related substances**—Dissolve 20 mg of Arbekacin Sulfate in 20 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than arbekacin and dibekacin obtained from the sample solution is not more than the peak area of arbekacin from the standard solution.

Operating conditions—

Detector, column, column temperature, reaction coil, reaction coil temperature, mobile phase, reagent, reaction temperature, flow rate of mobile phase, and flow rate of reagent: Proceed as directed in the operating conditions in the Purity (3).

Time span of measurement: About 1.5 times as long as the retention time of arbekacin.

System suitability—

System performance: Dissolve 10 mg each of Arbekacin Sulfate and dibekacin sulfate in 200 mL of water. When the procedure is run with 5 μL of this solution under the above operating conditions, arbekacin and dibekacin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of arbekacin is not more than 5.0%.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) **Test organism**—*Bacillus subtilis* ATCC 6633

(ii) **Culture medium**—Use the medium i in 1) under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) **Standard solutions**—Weigh accurately an amount of Arbekacin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard

solution, respectively.

(iv) **Sample solutions**—Weigh accurately an amount of Arbekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Arbekacin Sulfate Injection

アルベカシン硫酸塩注射液

Arbekacin Sulfate Injection is an aqueous injection.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of arbekacin sulfate ($\text{C}_{22}\text{H}_{44}\text{N}_6\text{O}_{10}$: 552.62).

Method of preparation Prepare as directed under Injections, with Arbekacin Sulfate.

Description Arbekacin Sulfate Injection occurs as a clear and colorless liquid.

Identification To 0.2 mL of Arbekacin Sulfate Injection add 1 mL of water, and use this solution as the sample solution. Separately, dissolve 10 mg of Arbekacin Sulfate Reference Standard in 1 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia solution (28), methanol, chloroform and ethanol (95) (7:6:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 80°C for 10 minutes: the principal spot with the sample solution and the spot with the standard solution show a purple-brown color and the same R_f value.

Osmotic pressure ratio <2.47> 0.8 – 1.2 (for the preparation intended for intramuscular use).

pH <2.54> 6.0 – 8.0

Bacterial endotoxins <4.01> Less than 0.50 EU/mg (potency).

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to the Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) **Test organism, Culture medium and Standard solutions:** Proceed as directed in the Assay under Arbekacin Sul-

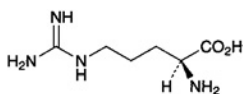
fate.

(ii) Sample solutions—Take exactly a volume of Arbekacin Sulfate Injection, equivalent to about 20 mg (potency), and add water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

L-Arginine

L-アルギニン



$C_6H_{14}N_4O_2$: 174.20

(2S)-2-Amino-5-guanidinopentanoic acid

[74-79-3]

L-Arginine, when dried, contains not less than 98.5% and not more than 101.0% of $C_6H_{14}N_4O_2$.

Description L-Arginine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of previously dried L-Arginine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +26.9 – +27.9° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of L-Arginine in 10 mL of water is between 10.5 and 12.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of L-Arginine in 10 mL of water is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Arginine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Arginine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02> Perform the test with 0.25 g of L-Arginine, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 2.0 g of L-Arginine in 30 mL of water, add 1 drop of phenolphthalein TS, neutralize with dilute hydrochloric acid, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution

(not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Arginine according to Method 1, and perform the test using Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.10 g of L-Arginine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol and ammonia solution (28) (7:3) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 80°C for 10 minutes: the spot other than the principal spot with the sample solution is not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

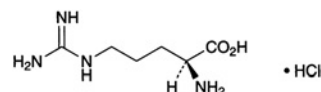
Assay Weigh accurately about 80 mg of L-Arginine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.710 mg of $C_6H_{14}N_4O_2$

Containers and storage Containers—Tight containers.

L-Arginine Hydrochloride

L-アルギニン塩酸塩



$C_6H_{14}N_4O_2 \cdot HCl$: 210.66

(2S)-2-Amino-5-guanidinopentanoic acid monohydrochloride [1119-34-2]

L-Arginine Hydrochloride, when dried, contains not less than 98.5% of $C_6H_{14}N_4O_2 \cdot HCl$.

Description L-Arginine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and very slightly soluble in ethanol (95).

Identification (1) Determine the infrared absorption spectrum of L-Arginine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of L-Arginine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +21.5 – +23.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the pH of this solution is between 4.7 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Arginine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.6 g of L-Arginine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium <1.02>—Perform the test with 0.25 g of L-Arginine Hydrochloride, using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of L-Arginine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Arginine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of L-Arginine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), water, 1-butanol and ammonia water (28) (2:1:1:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Arginine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 10.53 mg of C₆H₁₄N₄O₂HCl

Containers and storage Containers—Tight containers.

L-Arginine Hydrochloride Injection

L-アルギニン塩酸塩注射液

L-Arginine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of L-arginine hydrochloride (C₆H₁₄N₄O₂·HCl: 210.66).

Method of preparation

L-Arginine Hydrochloride	100 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description L-Arginine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 100) add 1 mL of ninhydrin TS, and heat for 3 minutes: a blue-purple color develops.

(2) To 5 mL of a solution of L-Arginine Hydrochloride Injection (1 in 10) add 2 mL of sodium hydroxide TS and 1 to 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 1000), allow to stand for 5 minutes, and add 1 to 2 drops of sodium hypochlorite TS: a red-orange color develops.

pH <2.54> 5.0 – 6.0

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Perform the test with L-Arginine Hydrochloride Injection stored in a container in a volume exceeding 10 mL: it meets the requirement.

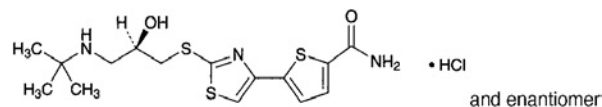
Assay Pipet 20 mL of L-Arginine Hydrochloride Injection, add 7.5 mol/L hydrochloric acid TS to make exactly 100 mL, and determine the optical rotation α_D as directed under Optical Rotation Determination <2.49> at 20 ± 1°C in a 100-mm cell.

Amount (mg) of L-arginine hydrochloride (C₆H₁₄N₄O₂·HCl)
= $\alpha_D \times 4444$

Containers and storage Containers—Hermetic containers.

Arotinolol Hydrochloride

アロチノロール塩酸塩



C₁₅H₂₁N₃O₂S₃·HCl: 408.00

5-{2-[(2RS)-3-(1,1-Dimethylethyl)amino-2-hydroxypropylsulfanyl]-1,3-thiazol-4-yl}thiophene-2-carboxamide monohydrochloride [68377-91-3]

Arotinolol Hydrochloride, when dried, contains not less than 99.0% of $C_{15}H_{21}N_3O_2S_3 \cdot HCl$.

Description Arotinolol Hydrochloride occurs as a white to light yellow crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in methanol and in water, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

A solution of Arotinolol Hydrochloride in methanol (1 in 125) does not show optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Arotinolol Hydrochloride in methanol (1 in 75,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Arotinolol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Arotinolol Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Arotinolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Arotinolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (30:10:10:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Arotinolol Hydrochloride, previously dried, dissolve in dimethylsulfoxide to make exactly 25 mL. Pipet 5 mL of this solution, add 100 mL of water and 5 mL of sodium hydroxide TS, and extract with three 50-mL portions of dichloromethane. Filter each dichloromethane extract through a pledget of absorbent cotton with anhydrous sodium sulfate on it. Evaporate combined filtrate to dryness in vacuum. Dissolve the residue in 70 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS

= 20.40 mg of $C_{15}H_{21}N_3O_2S_3 \cdot HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Arsenical Paste

亜ヒ酸パスタ

Arsenical Paste contains not less than 36.0% and not more than 44.0% of arsenic (III) trioxide (As_2O_3 : 197.84).

Method of preparation

Arsenic Trioxide, finely powdered	40 g
Procaine Hydrochloride, finely powdered	10 g
Hydrophilic Ointment	30 g
Clove Oil	a suitable quantity
Medicinal Carbon	a suitable quantity

To make 100 g

Mix Arsenic Trioxide and Procaine Hydrochloride with Hydrophilic Ointment, and add Clove Oil to make a suitably viscous liquid, followed by Medicinal Carbon for coloring.

Description Arsenical Paste is grayish black and has the odor of clove oil.

Identification (1) Place 0.1 g of Arsenical Paste in a small flask, add 5 mL of fuming nitric acid and 5 mL of sulfuric acid, and heat over a flame until the reacting liquid becomes colorless and white fumes begin to evolve. After cooling, add the reacting liquid to 20 mL of water cautiously, and add 10 mL of hydrogen sulfide TS while warming: a yellow precipitate is produced (arsenic (III) trioxide).

(2) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether, 5 mL of dilute hydrochloric acid and 20 mL of water, separate the water layer, and filter: 5 mL of the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines (procaine hydrochloride).

(3) Shake thoroughly 0.5 g of Arsenical Paste with 25 mL of diethyl ether and 25 mL of water, separate the water layer, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and standard solution exhibit the same R_f value.

Assay Weigh accurately about 0.3 g of Arsenical Paste into a 150-mL Kjeldahl flask, add 5 mL of fuming nitric acid and 10 mL of sulfuric acid, and shake thoroughly. Heat cautiously the mixture, gently at first, and then continue strong heating, until red fumes of nitrogen oxide are sparingly evolved. After cooling, add 5 mL of fuming nitric acid, heat again until red fumes of nitrogen oxide are no longer evolved and

the reacting liquid becomes clear, and cool. Add 30 mL of a saturated solution of ammonium oxalate monohydrate, heat again until white fumes of sulfuric acid are evolved, and continue the heating for 10 minutes. Decompose completely oxalic acid, cool, transfer cautiously the colorless reacting liquid to a glass-stoppered flask, containing 40 mL of water. Wash thoroughly the Kjeldahl flask with 60 mL of water, add the washings to the content of the glass-stoppered flask, and cool. Dissolve 3 g of potassium iodide in this solution, allow to stand in a dark place at room temperature for 45 minutes, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 5 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 4.946 mg of As_2O_3

Containers and storage Containers—Tight containers.

Arsenic Trioxide

Arsenous Acid

三酸化ヒ素

As_2O_3 : 197.84

Arsenic Trioxide, when dried, contains not less than 99.5% of As_2O_3 .

Description Arsenic Trioxide occurs as a white powder.

It is odorless. It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification Dissolve 0.2 g of Arsenic Trioxide in 40 mL of water by heating on a water bath: the solution responds to the Qualitative Tests <1.09> for arsenite.

Purity Clarity of solution—To 1.0 g of Arsenic Trioxide add 10 mL of ammonia TS, and heat gently: the solution is clear.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.15 g of Arsenic Trioxide, previously dried, dissolve in 20 mL of a solution of sodium hydroxide (1 in 25), by warming, if necessary. Add 40 mL of water and 2 drops of methyl orange TS, then add dilute hydrochloric acid until the color of the solution becomes light red. Add 2 g of sodium hydrogen carbonate and 50 mL of water to this solution, and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 3 mL of starch TS).

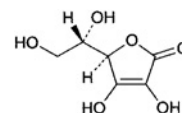
Each mL of 0.05 mol/L iodine VS = 4.946 mg of As_2O_3

Containers and storage Containers—Tight containers.

Ascorbic Acid

Vitamin C

アスコルビン酸



$\text{C}_6\text{H}_8\text{O}_6$: 176.12

L-threo-Hex-2-enono-1,4-lactone [50-81-7]

Ascorbic Acid, when dried, contains not less than 99.0% of L-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$).

Description Ascorbic Acid occurs as white crystals or a white, crystalline powder. It is odorless, and has an acid taste.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 190°C (with decomposition).

Identification (1) To 5 mL each of a solution of Ascorbic Acid (1 in 50) add 1 drop of potassium permanganate TS or 1 to 2 drops of 2,6-dichloroindophenol sodium TS: the color of the solution is discharged immediately in each case.

(2) Dissolve 0.1 g of Ascorbic Acid in 100 mL of a solution of metaphosphoric acid (1 in 50). To 5 mL of the solution add iodine TS until the color of the solution becomes light yellow. Then add 1 drop of a solution of copper (II) sulfate pentahydrate (1 in 1000) and 1 drop of pyrrole, and warm the mixture at 50°C for 5 minutes: a blue color develops.

Optical rotation <2.49> $[\alpha]_D^{20}$: + 20.5 – + 21.5° (2.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Ascorbic Acid in 20 mL of water: the pH of this solution is between 2.2 and 2.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 of Ascorbic Acid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Perform the test with 1.0 g of Ascorbic Acid according to Method 1. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.20% (1 g, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ascorbic Acid, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 8.806 mg of L-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ascorbic Acid Injection

Vitamin C Injection

アスコルビン酸注射液

Ascorbic Acid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 115% of the labeled amount of L-ascorbic acid ($C_6H_8O_6$; 176.12).

Method of preparation Prepare as directed under Injections, with the sodium salt of Ascorbic Acid.

Description Ascorbic Acid Injection occurs as a clear, colorless liquid.

Identification (1) Measure a volume of Ascorbic Acid Injection, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, and add water to make 25 mL. Proceed with 5 mL each of the solution as directed in the Identification (1) under Ascorbic Acid.

(2) Measure a volume of Ascorbic Acid Injection, equivalent to 5 mg of Ascorbic Acid according to the labeled amount. Add a solution of metaphosphoric acid (1 in 50) to make 5 mL, and proceed with this solution as directed in the Identification (2) under Ascorbic Acid.

(3) Ascorbic Acid Injection responds to the Qualitative Tests (1) for sodium salt.

pH <2.54> 5.6 – 7.4

Extractable volume <6.05> It meets requirement.

Assay Measure exactly a volume of Ascorbic Acid Injection, equivalent to about 0.1 g of L-ascorbic acid ($C_6H_8O_6$), previously diluted with metaphosphoric acid-acetic acid TS, if necessary, and add metaphosphoric acid-acetic acid TS to make exactly 200 mL. Measure exactly 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate <2.50> with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2, 6-dichlorophenol-indophenol sodium TS for titration
= A mg of L-ascorbic acid ($C_6H_8O_6$)

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation—Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.5 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

Standardization—Weigh accurately about 50 mg of Ascorbic Acid Reference Standard, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL. Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate <2.50> with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 se-

conds. Perform a blank determination, and make any necessary correction. Calculate the quantity (A mg) of L-ascorbic acid ($C_6H_8O_6$) equivalent to 1 mL of this test solution.

Containers and storage Containers—Hermetic containers.
Storage—Under nitrogen atmosphere.

Ascorbic Acid Powder

Vitamin C Powder

アスコルビン酸散

Ascorbic Acid Powder contains not less than 95% and not more than 120% of the labeled amount of L-ascorbic acid ($C_6H_8O_6$; 176.12).

Method of preparation Prepare as directed under Powders, with Ascorbic Acid.

Identification (1) Weigh a portion of Ascorbic Acid Powder, equivalent to 0.5 g of Ascorbic Acid according to the labeled amount, add 30 mL of water, shake for 1 minute, and filter. Proceed with 5 mL each of the filtrate as directed in the Identification (1) under Ascorbic Acid.

(2) Weigh a portion of Ascorbic Acid Powder, equivalent to about 0.01 g of Ascorbic Acid according to the labeled amount, add 10 mL of a solution of metaphosphoric acid (1 in 50), shake for 1 minute, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (2) under Ascorbic Acid.

Purity Rancidity—Ascorbic Acid Powder is free from any unpleasant or rancid odor and taste.

Assay Weigh accurately a portion of Ascorbic Acid Powder, equivalent to about 0.1 g of L-ascorbic acid ($C_6H_8O_6$) according to the labeled amount, extract with several successive portions of metaphosphoric acid-acetic acid TS, combine the extracts, and filter. Wash the residue with metaphosphoric acid-acetic acid TS. Combine the filtrates and washings, and add metaphosphoric acid-acetic acid to make exactly 200 mL. Pipet 2 mL of the solution, and shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS. Titrate <2.50> with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction.

Each mL of 2,6-dichlorophenol-indophenol sodium TS for titration
= A mg of $C_6H_8O_6$

A is decided by the following standardization of 2,6-dichloroindophenol sodium TS for titration.

2,6-Dichlorophenol-indophenol sodium TS for titration:

Preparation—Dissolve 42 mg of sodium hydrogen carbonate in 50 mL of water, add 0.05 g of 2,6-dichloroindophenol sodium dihydrate and water to make 200 mL, and filter. Prepare before use.

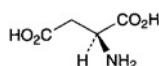
Standardization—Weigh accurately about 50 mg of Ascorbic Acid Reference Standard, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in metaphosphoric acid-acetic acid TS to make exactly 100 mL.

Pipet 2 mL of this solution, shake with 8 mL of metaphosphoric acid-acetic acid TS and 2 mL of hydrogen peroxide TS, and titrate <2.50> with 2,6-dichloroindophenol sodium TS for titration until a light red color persists for 5 seconds. Perform a blank determination, and make any necessary correction. Calculate the quantity (*A* mg) of L-ascorbic acid (C₆H₈O₆) equivalent to 1 mL of this test solution.

Containers and storage Containers—Tight containers.

L-Aspartic Acid

L-アスパラギン酸



C₄H₇NO₄: 133.10
(2*S*)-2-Aminobutanedioic acid
[56-84-8]

L-Aspartic Acid, when dried, contains not less than 98.5% and not more than 101.0% of C₄H₇NO₄.

Description L-Aspartic Acid occurs as white, crystals or crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid and in 0.2 mol/L sodium hydroxide TS.

Identification Determine the infrared absorption spectrum of L-Aspartic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +24.0 – +26.0° (2 g, after drying, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 0.4 g of L-Aspartic Acid in 100 mL of water by warming, and allow to cool: between 2.5 and 3.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Aspartic Acid in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Aspartic Acid in 6 mL of dilute nitric acid and 20 mL of water, add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Aspartic Acid in 5 mL of dilute hydrochloric acid and 30 mL of water, add water to make 45 mL, and add 5 mL of barium chloride TS. Perform the test with this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS, add 5 mL of dilute hydrochloric acid and water to make 45 mL, and add 5 mL of barium chloride (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Aspartic Acid. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Aspartic Acid according to Method 4, and perform the test. Pre-

pare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 1.0 g of L-Aspartic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 0.20 g of L-Aspartic Acid in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in a mixture of methanol and acetic acid (100) (97:3) (1 in 100), and heat at 80°C for 10 minutes: the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of L-Aspartic Acid, previously dried, dissolve in 50 mL of water by warming. After cooling, titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

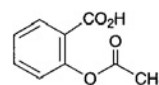
Each mL of 0.1 mol/L sodium hydroxide VS
= 13.31 mg of C₄H₇NO₄

Containers and storage Containers—Tight containers.

Aspirin

Acetylsalicylic Acid

アスピリン



C₉H₈O₄: 180.16
2-Acetoxybenzoic acid [50-78-2]

Aspirin, when dried, contains not less than 99.5% of C₉H₈O₄.

Description Aspirin occurs as white crystals, granules or powder. It is odorless, and has a slight acid taste.

It is freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

In moist air, it gradually hydrolyzes to salicylic acid and acetic acid.

Melting point: about 136°C (bath fluid is heated at 130°C previously).

Identification (1) Boil 0.1 g of Aspirin in 5 mL of water for 5 to 6 minutes, cool, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color is produced.

(2) Boil 0.5 g of Aspirin in 10 mL of sodium carbonate TS for 5 minutes, and add 10 mL of dilute sulfuric acid: the odor of acetic acid is perceptible, and a white precipitate is produced. Filter the precipitate, add 3 mL of ethanol (95) and 3 mL of sulfuric acid to the filtrate, and heat: the odor of ethyl acetate is perceptible.

Purity (1) Clarity of solution—Dissolve 0.5 g of Aspirin in 10 mL of warm sodium carbonate TS: the solution is clear.

(2) Salicylic acid—Dissolve 2.5 g of Aspirin in 25 mL of ethanol (95), and add 1.0 mL of this solution to a solution which is prepared by transferring 1 mL of a freshly prepared dilute ammonium iron (III) sulfate TS to a Nessler tube and diluting with water to 50 mL. Allow to stand for 30 seconds: the solution has no more color than the following control solution.

Control solution: Dissolve 0.100 g of salicylic acid in water, and add 1 mL of acetic acid (100) and water to make 1000 mL. Add 1.0 mL of this solution to a solution which is prepared by transferring 1 mL of freshly prepared dilute ammonium iron (III) sulfate TS and 1 mL of ethanol (95) to a Nessler tube and diluting with water to 50 mL. Allow to stand for 30 seconds.

(3) Chloride <1.03>—Boil 1.8 g of Aspirin in 75 mL of water for 5 minutes, cool, add water to make 75 mL, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(4) Sulfate <1.14>—To 25 mL of the filtrate obtained in (3) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040%).

(5) Heavy metals <1.07>—Dissolve 2.5 g of Aspirin in 30 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.5 mL of Standard Lead Solution, 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Readily carbonizable substances <1.15>—Weigh 0.5 g of Aspirin, and perform the test. The solution has no more color than Matching Fluid Q.

Loss on drying <2.41> Not more than 0.5% (3 g, silica gel, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Aspirin, previously dried, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and boil gently for 10 minutes under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate <2.50> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L sodium hydroxide VS
= 45.04 mg of C₉H₈O₄

Containers and storage Containers—Well-closed contain-

ers.

Aspirin Tablets

Acetylsalicylic Acid Tablets

アスピリン錠

Aspirin Tablets contain not less than 95% and not more than 105% of the labeled amount of aspirin (C₉H₈O₄: 180.16).

Method of preparation Prepare as directed under Tablets, with Aspirin.

Identification (1) Weigh a quantity of powdered Aspirin Tablets, equivalent to 0.1 g of Aspirin according to the labeled amount, add 10 mL of water, and boil for 5 to 6 minutes. After cooling, filter, and add 1 to 2 drops of iron (III) chloride TS to the filtrate: a red-violet color develops.

(2) Weigh a portion of powdered Aspirin Tablets, equivalent to 0.5 g of Aspirin according to the labeled amount, extract with two 10-mL portions of warm ethanol (95), and filter the combined extracts. Evaporate the filtrate to dryness, and boil the residue with 10 mL of sodium carbonate TS for 5 minutes. Proceed as directed in the Identification (2) under Aspirin.

Purity Salicylic acid—Take a portion of the powdered Aspirin Tablets, equivalent to 1.0 g of Aspirin according to the labeled amount, shake with 15 mL of ethanol (95) for 5 minutes, filter, discard the first 5 mL of the filtrate, and add 1.0 mL of the subsequent filtrate to a solution which is prepared by transferring 1 mL of freshly prepared dilute ammonium iron (III) sulfate TS to a Nessler tube and diluting with water to make 50 mL. Proceed as directed in the Purity (2) under Aspirin.

Assay Weigh accurately and powder not less than 20 Aspirin Tablets. Weigh accurately a portion of the powder, equivalent to about 1.5 g of aspirin (C₉H₈O₄), add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, and proceed as directed in the Assay under Aspirin.

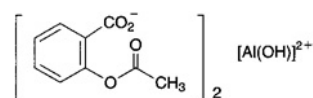
Each mL of 0.5 mol/L sodium hydroxide VS
= 45.04 mg of aspirin (C₉H₈O₄)

Containers and storage Containers—Well-closed containers.

Aspirin Aluminum

Aluminum Acetylsalicylate

アスピリンアルミニウム



C₁₈H₁₅AlO₉: 402.29

Bis(2-acetoxybenzoato)hydroxoaluminium [23413-80-1]

Aspirin Aluminum contains not less than 83.0% and

not more than 90.0% of aspirin ($C_9H_8O_4$: 180.16), and not less than 6.0% and not more than 7.0% of aluminum (Al: 26.98), calculated on the anhydrous basis.

Description Aspirin Aluminum occurs as a white, crystalline powder. It is odorless or has a slight, acetic odor.

It is practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether.

It dissolves, with decomposition, in sodium hydroxide TS and in sodium carbonate TS.

Identification (1) Dissolve 0.1 g of Aspirin Aluminum in 10 mL of sodium hydroxide TS by heating, if necessary. Neutralize 2 mL of the solution with hydrochloric acid, and add 1 to 2 drops of iron (III) chloride TS: a red-purple color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 279 nm.

(3) Place 2 g of Aspirin Aluminum in a platinum crucible, and ignite until charred. To the residue add 1 g of anhydrous sodium carbonate, and ignite for 20 minutes. After cooling, to the residue add 15 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests <1.09> for aluminum salt.

Purity (1) Salicylate—Using A_{T2} and A_{S2} obtained in the Assay (1), calculate the amount of salicylate [as salicylic acid ($C_7H_6O_3$: 138.12)] by the following equation: salicylate content is not more than 7.5%, calculated on the anhydrous basis.

$$\begin{aligned} &\text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3\text{)} \\ &= W_s \times (A_{T2}/A_{S2}) \times (1/4) \end{aligned}$$

W_s : Amount (mg) of salicylic acid for assay

(2) Heavy metals <1.07>—Place 2.0 g of Aspirin Aluminum in a porcelain crucible, cover the crucible loosely, and ignite at a low temperature until charred. After cooling, add 2 mL of nitric acid and 1 mL of sulfuric acid to the content of the crucible, heat gently the crucible until white fumes are evolved, and continue the heating until white fumes are no longer evolved, then ignite between 500°C and 600°C until the carbon is incinerated. When the incineration is not completed, add 2 mL of nitric acid and 1 mL of sulfuric acid, and heat gently in the same manner, then ignite between 500°C and 600°C to incinerate completely. After cooling, add 2 mL of hydrochloric acid, and proceed as directed in Method 2, and perform the test. Prepare the control solution by using the same quantities of the same reagents as directed for the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 10 ppm).

(3) Arsenic <1.11>—Dissolve 1.0 g of Aspirin Aluminum in 15 mL of sodium hydroxide TS. To this solution add 1 drop of phenolphthalein TS, and with stirring, add dropwise hydrochloric acid until the red color of the solution disappears. Then add 2 mL of hydrochloric acid, cool with occasional shaking for 10 minutes, and filter with a glass filter (G3). Wash the residue with two 5 mL portions of 1 mol/L hydrochloric acid TS, and combine the filtrate and the washings. Use this solution as the test solution, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 4.0% (0.15 g, direct titration).

Assay (1) Aspirin—Weigh accurately about 0.1 g of Aspirin Aluminum, add 40 mL of sodium fluoride TS, and shake for 5 minutes. Allow the solution to stand for 10 minutes with frequent shaking. Extract the solution with six 20-mL portions of chloroform. Combine all chloroform extracts, and add chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.09 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 5 mL of this solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution (1). Then weigh accurately about 0.09 g of aspirin for assay, previously dried in a desiccator (silica gel) for 5 hours, and dissolve in chloroform to make exactly 200 mL. Measure exactly 10 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>. Determine the absorbances, A_{T1} and A_{S1} , of the sample solution and standard solution (1) at 278 nm, and absorbances, A_{T2} and A_{S2} , of these solution, at 308 nm, respectively. Then determine the absorbance A_{S3} of the standard solution (2) at 278 nm.

$$\begin{aligned} &\text{Amount (mg) of aspirin (C}_9\text{H}_8\text{O}_4\text{)} \\ &= W_s \times \left[\frac{A_{T1} - \frac{A_{T2} \times A_{S1}}{A_{S2}}}{A_{S3}} \right] \end{aligned}$$

W_s : Amount (mg) of aspirin for assay

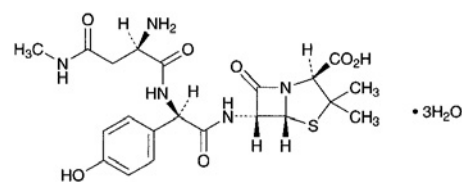
(2) Aluminum—Weigh accurately about 0.4 g of Aspirin Aluminum, and dissolve in 10 mL of sodium hydroxide TS. Add dropwise 1 mol/L hydrochloric acid TS to adjust the solution to a pH of about 1, add 20 mL of acetic acid-ammonium acetate buffer solution, pH 3.0, and 0.5 mL of Cu-PAN TS, and heat. While boiling, titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to yellow and persists for 1 minute. Perform a blank determination, and make any necessary correction.

$$\begin{aligned} &\text{Each mL of 0.05 mol/L disodium dihydrogen} \\ &\text{ethylenediamine tetraacetate VS} \\ &= 1.349 \text{ mg of Al} \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Aspoxicillin Hydrate

アスポキシリン水和物



$C_{21}H_{27}N_5O_7S \cdot 3H_2O$: 547.58
(2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(2*R*)-2-Amino-3-methylcarbamoylpropanoylamino]-

2-(4-hydroxyphenyl)acetamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate [63358-49-6, anhydride]

Aspxocillin Hydrate contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Aspxocillin Hydrate is expressed as mass (potency) of aspxocillin ($\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_7\text{S}$: 493.53).

Description Aspxocillin Hydrate occurs as a white, crystals or crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in water, and practically insoluble in acetonitrile, in methanol and in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aspxocillin Hydrate (1 in 4000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Aspxocillin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Aspxocillin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of Aspxocillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +170 – +185° (0.2 g calculated on the anhydrous bases, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Aspxocillin Hydrate in 50 mL of water: the pH of the solution is between 4.2 and 5.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Aspxocillin Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Aspxocillin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Aspxocillin Hydrate according to Method 5, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.05 g of Aspxocillin Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of each peak other than aspxocillin from the sample solution is not more than 3/10 of the peak area of aspxocillin from the standard solution, and the total of peak areas other than aspxocillin from the sample solution is not more than the peak area of aspxocillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of aspxocillin.

System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add the mobile phase to make exactly 10 mL. Confirm that the peak area of aspxocillin obtained from 10 μL of this solution is equivalent to 15 to 25% of that of aspxocillin obtained from 10 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aspxocillin is not more than 5%.

Water <2.48> Not less than 9.5% and not more than 13.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Aspxocillin Hydrate and Aspxocillin Reference Standard, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of water, add exactly 10 mL of the internal standard solution, 6.5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of aspxocillin to that of the internal standard of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of aspxocillin (C}_{21}\text{H}_{27}\text{N}_5\text{O}_7\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Aspxocillin Reference Standard

Internal standard solution—A solution of *N*-(3-hydroxyphenyl)acetamide (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 130 mL of acetonitrile add potassium dihydrogenphosphate TS, pH 3.0 to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of aspxocillin is about 3 minutes.

System suitability—

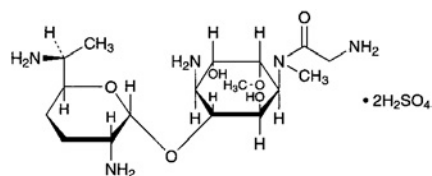
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, aspxocillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of aspxocillin to that of the internal standard is not more than 0.8%.

Containers and storage Containers—Tight containers.

Astromicin Sulfate

アストロマイシン硫酸塩



$C_{17}H_{35}N_5O_6 \cdot 2H_2SO_4$: 601.65

2,6-Diamino-2,3,4,6,7-pentadeoxy- β -L-lyxo-heptopyranosyl-(1 \rightarrow 3)-4-amino-1-(2-aminoacetyl-N-methylamino)-1,4-dideoxy-6-O-methyl-L-chiro-inositol disulfate
[72275-67-3]

Astromicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora olivasterospora*.

It contains not less than 610 μ g (potency) and not more than 680 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Astromicin Sulfate is expressed as mass (potency) of astromicin ($C_{17}H_{35}N_5O_6$: 405.49).

Description Astromicin Sulfate occurs as a white to light yellowish white, powder or masses.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 10 mg each of Astromicin Sulfate and Astromicin Sulfate Reference Standard in 10 mL of water. To 5 mL each of these solutions add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of astromicin obtained from the sample solution is the same with that from the standard solution.

Operating conditions—

Detector, column, column temperature, reaction coil, temperature of reaction coil, mobile phase, reaction reagent, reaction temperature, flow rate of mobile phase, and flow rate of reaction reagent: Proceed as directed in the operating conditions in the Purity (3).

(2) To 2 mL of a solution of Astromicin Sulfate (1 in 100) add 2 to 3 drops of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation <2.49> $[\alpha]_D^{20}$: +90 – +110° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Astromicin Sulfate in 10 mL of water is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Astromicin Sulfate in 10 mL of water: the solution is clear

and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Astromicin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Astromicin Sulfate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the related substance III, having the relative retention time of about 0.1, and the related substance I, having the relative retention time of about 1.2 with respect to the peak of astromicin, from the sample solution are not more than the peak area of astromicin from the standard solution, the peak area of the related substance II, having the related retention time of about 0.8, is not more than 2.0 times the peak area of astromicin from the standard solution, and the total area of the peaks other than astromicin from the sample solution is not more than 3.5 times the peak area of astromicin from the standard solution.

Operating conditions—

Detector: A fluorophotometer (excitation wavelength: 340 nm; detection wavelength: 430 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A stainless steel tube 0.25 mm in inside diameter and 150 cm in length.

Temperature of reaction coil: 50°C

Mobile phase: To 800 mL of a solution of anhydrous sodium sulfate (71 in 2000) add 25 mL of a solution of sodium 1-heptanesulfonate (1 in 1000) and 1 mL of acetic acid (100), and add water to make 1000 mL.

Reaction reagent: Dissolve 11.2 g of potassium hydroxide, 0.458 g of polyoxyethylene (23) lauryl ether, 0.300 g of *o*-phthalaldehyde and 1 mL of 2-mercaptoethanol in 400 mL of a solution of boric acid (31 in 1000), and add water to make 500 mL.

Reaction temperature: 50°C

Flow rate of mobile phase: 0.7 mL per minute

Flow rate of reaction reagent: 0.2 mL per minute

Time span of measurement: About 2 times as long as the retention time of astromicin.

System suitability—

Test for required detectability: To 5 mL of the sample solution add water to make 100 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution for system suitability test, and add water to make exactly 100 mL. Confirm that the peak area of astromicin obtained from 10 μ L of this solution is equivalent to 1.5 to 2.5% of that from 10 μ L of the solution for system suitability test.

System performance: To 100 mL of water add 5 mL of the sample solution and 2 mL of a solution of L-valine (1 in 5000). When the procedure is run with 10 μ L of this solution under the above operating conditions, L-valine and astromi-

cin are eluted in this order with the resolution between these peaks being not less than 1.5, and when the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the symmetry factor of the peak of astromicin is not more than 2.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of astromicin is not more than 2.0%.

Water <2.48> Not more than 8.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

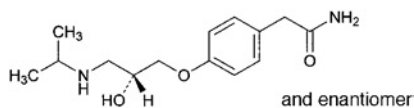
(iii) Standard solutions—Weigh accurately an amount of Astromicin Sulfate Reference Standard, equivalent to about 25 mg (potency), dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5–15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Astromicin Sulfate, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Atenolol

アテノロール



$\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$; 266.34

2-(4-[(2*R,S*)-2-Hydroxy-3-

[(1-methylethyl)amino]propyloxy]phenyl)acetamide
[29122-68-7]

Atenolol, when dried, contains not less than 99.0% and not more than 101.0% of $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$.

Description Atenolol occurs as a white to pale yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (99.5), and slightly soluble in water.

A solution of Atenolol in methanol (1 in 25) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Atenolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Atenolol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting Point <2.60> 152–156°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Atenolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Atenolol in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than atenolol is not larger than 1/2 times the peak area of atenolol obtained with the standard solution, and the total area of the peaks other than atenolol is not larger than the peak area of atenolol with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 226 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust to pH 3.0 with phosphoric acid. To 40 volume of this solution add 9 volume of methanol and 1 volume of tetrahydrofuran. Dissolve 1 g of sodium 1-octanesulfonate and 0.4 g of tetrabutylammonium hydrogensulfate in 1000 mL of this solution.

Flow rate: Adjust the flow rate so that the retention time of atenolol is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of atenolol.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of atenolol obtained with 10 μL of this solution is equivalent to 14 to 26% of that obtained with 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry fac-

tor of the peak of atenolol are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of atenolol is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

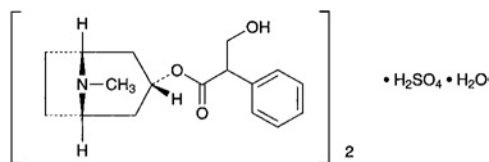
Assay Weigh accurately about 0.3 g of Atenolol, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.63 mg of $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$

Containers and storage Containers—Tight containers.

Atropine Sulfate Hydrate

アトロピン硫酸塩水和物



$(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$: 694.83
(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [(2*RS*)-3-hydroxy-2-phenyl]propanoate hemisulfate hemihydrate [5908-99-6]

Atropine Sulfate Hydrate, when dried, contains not less than 98.0% of atropine sulfate [$(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$: 676.82].

Description Atropine Sulfate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: 188 – 194°C (with decomposition). Introduce a capillary tube charged with dried sample into a bath previously heated to 180°C, and continue to heat at a rate of rise of about 3°C per minute.

It is affected by light.

Identification (1) To 1 mg of Atropine Sulfate Hydrate add 3 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) To 2 mL of a solution of Atropine Sulfate Hydrate (1 in 50) add 4 to 5 drops of hydrogen tetrachloroaurate (III) TS: a lusterless, yellowish white precipitate is formed.

(3) To 5 mL of a solution of Atropine Sulfate Hydrate (1 in 25) add 2 mL of ammonia TS, and allow to stand for 2 to 3 minutes. Collect the precipitate, wash with water, and dry in

a desiccator (in vacuum, silica gel) for 4 hours: it melts <2.60> between 115°C and 118°C.

(4) A solution of Atropine Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Atropine Sulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 1.0 g of Atropine Sulfate Hydrate in 20 mL of water, and add 0.30 mL of 0.02 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Related substances—Dissolve 0.25 g of Atropine Sulfate Hydrate in 1 mL of diluted hydrochloric acid (1 in 10), add water to make 15 mL, and use this solution as the sample solution.

(i) To 5 mL of the sample solution add 2 to 3 drops of hydrogen hexachloroplatinate (IV) TS: no precipitate is formed.

(ii) To 5 mL of the sample solution add 2 mL of ammonia TS, and shake vigorously: the turbidity of the solution is not greater than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL. To this solution add 1 mL of silver nitrate TS, and allow 7 mL of the mixture to stand for 5 minutes.

(4) Hyoscyamine—Weigh accurately about 1 g of Atropine Sulfate Hydrate, previously dried, and dissolve in water to make exactly 10 mL: the specific optical rotation $[\alpha]_D^{20}$ <2.49> of this solution in a 100-mm cell is between -0.60° and $+0.10^\circ$.

(5) Readily carbonizable substances <1.15>—Take 0.20 g of Atropine Sulfate Hydrate, and perform the test: the solution has no more color than Matching Fluid A.

Loss on drying <2.41> Not more than 4.0% (0.5 g, in vacuum, phosphorus (V) oxide, 110°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 0.25 g of Atropine Sulfate Hydrate, previously dried and accurately weighed, in 30 mL of acetic acid (100). If necessary, dissolve it by warming, and cool. Titrate <2.50> with 0.05 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 33.84 mg of atropine sulfate [$(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$]

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Atropine Sulfate Injection

アトロピン硫酸塩注射液

Atropine Sulfate Injection is an aqueous solution for injection.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of atropine sulfate hydrate [$(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$: 694.83].

Method of preparation Prepare as directed under Injec-

tions, with Atropine Sulfate Hydrate.

Description Atropine Sulfate Injection is a clear, colorless liquid.

pH: 4.0 – 6.0

Identification (1) Evaporate a volume of Atropine Sulfate Injection, equivalent to 1 mg of Atropine Sulfate Hydrate according to the labeled amount, on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Atropine Sulfate Hydrate.

(2) Evaporate an exactly measured volume of Atropine Sulfate Injection, equivalent to 5 mg of Atropine Sulfate Hydrate according to the labeled amount, on a water bath to dryness. After cooling, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. If insoluble substance remains, crush it, allow to stand, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Atropine Sulfate Reference Standard in 2 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: the spots obtained from the sample solution and the standard solution show an orange color and the same *R_f* value.

(3) Atropine Sulfate Injection responds to the Qualitative Tests <1.09> for sulfate.

Bacterial endotoxins <4.01> Less than 75 EU/mg.

Extractable volume <6.05> It meets the requirements.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test: it meets the requirement.

Assay To an exactly measured volume of Atropine Sulfate Injection, equivalent to about 5 mg of atropine sulfate Hydrate [(C₁₇H₂₃NO₃)₂·H₂SO₄·H₂O], add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate Reference Standard, separately determine its loss on drying <2.41> in the same conditions as for Atropine Sulfate Hydrate, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 3 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q_T* and *Q_S*, of the peak area of atropine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of atropine sulfate Hydrate} \\ &[(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}] \\ &= W_S \times (Q_T/Q_S) \times (1/5) \times 1.0266 \end{aligned}$$

W_S: Amount (mg) of Atropine Reference Standard, calculated based on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.4 g of sodium lauryl sulfate add 500 mL of diluted phosphoric acid (1 in 1000) to dissolve, and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 16 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

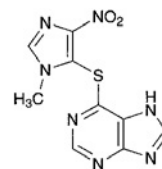
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of atropine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Azathioprine

アザチオプリン



C₉H₇N₇O₂S: 277.26

6-(1-Methyl-4-nitro-1*H*-imidazol-5-ylthio)purine
[446-86-6]

Azathioprine, when dried, contains not less than 98.5% of C₉H₇N₇O₂S.

Description Azathioprine is light yellow crystals or crystalline powder. It is odorless.

It is sparingly soluble in *N,N*-dimethylformamide and in pyridine, very slightly soluble in water and in ethanol (99.5), and practically insoluble in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

It is gradually colored by light.

Melting point: about 240°C (with decomposition).

Identification (1) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 5 mL of this solution add 1 mL of dilute hydrochloric acid and 0.01 g of zinc powder, and allow to stand for 5 minutes: a yellow color is produced.

Filter this solution: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines, and a red color is produced.

(2) Dissolve 0.01 g of Azathioprine in 50 mL of water by warming. To 1 mL of this solution add 0.5 mL of phosphotungstic acid TS and 0.5 mL of dilute hydrochloric acid: a white precipitate is formed.

(3) Prepare the test solution by proceeding with 0.03 g of Azathioprine according to the Oxygen Flask Combustion Method <1.06>, using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (1) for sulfate.

(4) Dissolve 0.01 g of Azathioprine in 2 mol/L hydrochloric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Azathioprine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Azathioprine in 50 mL of *N,N*-dimethylformamide: the solution is clear and shows a light yellow color.

(2) Acidity or alkalinity—Add 100 mL of water to 2.0 g of Azathioprine, shake well for 15 minutes, centrifuge for 5 minutes at 10,000 revolutions per minute, and filter. Discard the first 20 mL of the filtrate, add 2 drops of methyl red TS to 40 mL of the subsequent filtrate, and use this solution as the sample solution

(i) Add 0.10 mL of 0.02 mol/L hydrochloric acid VS to 20 mL of the sample solution: a red color develops.

(ii) Add 0.10 mL of 0.02 mol/L sodium hydroxide VS to 20 mL of the sample solution: a yellow color develops.

(3) Sulfate <1.14>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Azathioprine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Azathioprine, according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 10 mg of Azathioprine in 80 mL of the mobile phase by warming, cool, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of azathioprine from the sample solution is not larger than 1/2 of the peak area of azathioprine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 296 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust to pH 2.5 of a solution of 0.05 mol/L potassium dihydrogenphosphate TS (1 in 2) with diluted phosphoric acid (3 in 2000). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of azathioprine is about 8 minutes.

Time span of measurement: About three times as long as the retention time of azathioprine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of azathioprine obtained from 20 μ L of this solution is equivalent to 8 to 12% of that of azathioprine obtained from 20 μ L of the standard solution.

System performance: Dissolve 10 mg of Azathioprine in 80 mL of water by warming, cool, and add water to make 100 mL. To 2 mL of this solution add 2 mL of a solution, separately prepared by dissolving 0.06 g of benzoic acid in 3 mL of methanol and diluting with water to make 10 mL, and add the mobile phase to make 25 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, azathioprine and benzoic acid are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of azathioprine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Azathioprine, previously dried, add 80 mL of *N,N*-dimethylformamide, and warm to dissolve. After cooling, titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue-green (indicator: 1 mL of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 27.73 mg of C₉H₇O₇S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Azathioprine Tablets

アザチオプリン錠

Azathioprine Tablets contain not less than 95.0%

and not more than 105.0% of the labeled amount of azithioprine ($C_9H_7N_7O_2S$: 277.26).

Method of preparation Prepare as directed under Tablets, with Azathioprine.

Identification (1) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.01 g of Azathioprine according to the labeled amount. Add 50 mL of water, shake well while warming, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Azathioprine.

(2) Proceed with 1 mL of the filtrate obtained in (1) as directed in the Identification (2) under Azathioprine.

(3) Determine the absorption spectrum of the sample solution in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm.

(4) Weigh a quantity of powdered Azathioprine Tablets, equivalent to 0.1 g of Azathioprine to the labeled amount. Add 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.1 g of Azathioprine Reference Standard in 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, a solution of ammonia solution (28) in methanol (1 in 10), *n*-butyl formate and 1,2-dichloroethane (15:10:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *R_f* value.

Assay Weigh accurately and powder not less than 20 Azathioprine Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of azathioprine ($C_9H_7N_7O_2S$), add 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake well, add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 3 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Azathioprine Reference Standard, previously dried at 105°C for 5 hours, dissolve in 20 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Measure exactly 3 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

$$\begin{aligned} & \text{Amount (mg) of azathioprine (C}_9\text{H}_7\text{N}_7\text{O}_2\text{S)} \\ & = W_S \times (A_T/A_S) \end{aligned}$$

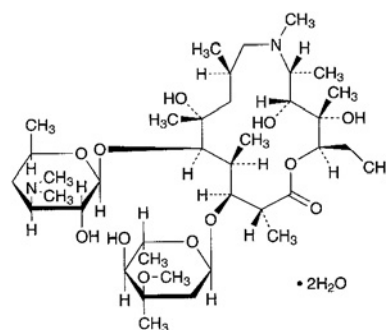
W_S : Amount (mg) of Azathioprine Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Azithromycin Hydrate

アジスロマイシン水和物



$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$: 785.02

(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,11*R*,12*R*,13*S*,14*R*)-5-(3,4,6-Trideoxy-3-dimethylamino- β -D-xylohexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl- α -L-ribo-hexopyranosyloxy)-10-aza-6,12,13-trihydroxy-2,4,6,8,10,11,13-heptamethylhexadecan-14-olide dihydrate [11772-70-0]

Azithromycin Hydrate is the derivative of erythromycin.

It contains not less than 945 μ g (potency) and not more than 1030 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Azithromycin Hydrate is expressed as mass (potency) of azithromycin ($C_{38}H_{72}N_2O_{12}$: 748.98).

Description Azithromycin Hydrate occurs as a white crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Azithromycin Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Azithromycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-45 - -49^\circ$ (0.4 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Azithromycin Hydrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately.

(3) Residual solvent—Being specified separately.

Water <2.48> Not less than 4.0% and not more than 5.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Azithromycin Hydrate and Azithromycin Reference Standard, equivalent to about 50 mg (potency), dissolve each in an adequate amount of a mixture of acetonitrile and water (3:2), add

exactly 2 mL of the internal standard solution and the mixture of acetonitrile and water (3:2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of azithromycin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of azithromycin } (\text{C}_{38}\text{H}_{72}\text{N}_2\text{O}_{12}) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Azithromycin Reference Standard

Internal standard solution—A solution of 4,4'-bis(diethylamino)benzophenone in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.97 g of dipotassium hydrogen phosphate in about 750 mL of water, adjust the pH to 11.0 with potassium hydroxide TS, and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of azithromycin is about 10 minutes.

System suitability—

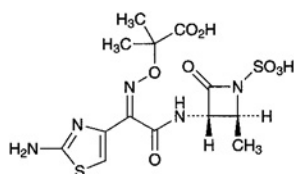
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, azithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of azithromycin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Aztreonam

アズトレオナム



$\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}_8\text{S}_2$: 435.43

2-[(Z)-(2-Aminothiazol-4-yl)-[(2S,3S)-2-methyl-4-oxo-1-sulfoazetidin-3-ylcarbamoyl]methyleneaminoxy]-2-methyl-1-propanoic acid [78110-38-0]

Aztreonam contains not less than 920 μ g (potency)

and not more than 1030 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Aztreonam is expressed as mass (potency) of aztreonam ($\text{C}_{13}\text{H}_{17}\text{N}_5\text{O}_8\text{S}_2$).

Description Aztreonam occurs as a white to yellowish white crystalline powder.

It is freely soluble in dimethylsulfoxide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Aztreonam (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Aztreonam Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Aztreonam in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using a light hydrogen substance existing in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy as an internal reference compound and 2.50 ppm for its chemical shift, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H): it exhibits a multiple signal at around δ 1.5 ppm, and a single signal at around δ 7.0 ppm. The ratio of integrated intensity of each signal is 9:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-26 - -32^\circ$ (0.25 g calculated on the anhydrous bases, water, 50 mL, 100 mm).

pH <2.54> Dissolve 0.05 g of Aztreonam in 10 mL of water: the pH of this solution is between 2.2 and 2.8.

Purity (1) Clarity and color of solution—Dissolve 0.1 g of Aztreonam in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Aztreonam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Aztreonam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.04 g of Aztreonam in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of each peak is not more than the peak area of aztreonam from the standard solution, and the total area of peaks other than aztreonam from the sample solution is not more than 2.5 times of the peak area of aztreonam from the standard solution.

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Time span of measurement: About 4 times as long as the retention time of aztreonam beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 5 mL of the standard solution, add water to make exactly 10 mL, and use this solution as the solution for the test for required detection. Pipet 1 mL of the solution, and add water to make exactly 10 mL. Confirm that the peak area of aztreonam obtained from 25 μ L of this solution is equivalent to 7 to 13% of that obtained from 25 μ L of the solution for the test for required detection.

System performance: When the procedure is run under the above operating conditions with 25 μ L of the standard solution obtained in the Assay, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of aztreonam is not more than 2.0%.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Aztreonam and Aztreonam Reference Standard, equivalent to about 20 mg (potency), dissolve each in 70 mL of water, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 25 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of aztreonam to that of the internal standard of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of aztreonam (C}_{13}\text{H}_{17}\text{N}_5\text{O}_8\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Aztreonam Reference Standard

Internal standard solution—A solution of 4-aminobenzoic acid (1 in 6250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium hydrogensulfate in 300 mL of water, adjust to pH 3.0 with 0.5 mol/L disodium hydrogenphosphate TS, and add water to make 1000 mL. To 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of aztreonam is about 8 minutes.

System suitability—

System performance: When the procedure is run with 25 μ L of the standard solution under the above operating conditions, the internal standard and aztreonam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating

conditions, the relative standard deviation of the ratios of the peak area of aztreonam to that of the internal standard is not more than 1.5%.

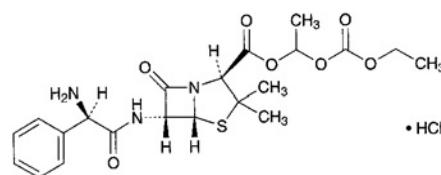
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Bacampicillin Hydrochloride

Ampicillin Ethoxycarbonyloxyethyl Hydrochloride

バカンピシリン塩酸塩



$\text{C}_{21}\text{H}_{27}\text{N}_3\text{O}_7\text{S} \cdot \text{HCl}$: 501.98

1-Ethoxycarbonyloxyethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [37661-08-8]

Bacampicillin Hydrochloride is a hydrochloride of ampicillin ethoxycarbonyloxyethyl ester.

It contains not less than 626 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Bacampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$: 349.40).

Description Bacampicillin Hydrochloride occurs as a white to pale yellow crystalline powder. It has a characteristic odor.

It is freely soluble in methanol and in ethanol (95), and soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Bacampicillin Hydrochloride in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Bacampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bacampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Bacampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bacampicillin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +140 – +170° (0.1 g calculated on the anhydrous basis, ethanol (95), 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bacampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bacampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Bacampicillin Hydrochloride, transfer into a 100-mL separator, add exactly 15 mL of ice-cold water to dissolve, add and mix with exactly 10 mL of ice-cold 0.05 mol/L phosphate buffer solution, pH 7.0, then add 25 mL of ice-cold chloroform, shake, and abandon the chloroform layer. Repeat the procedure twice with two 25-mL portions of ice-cold chloroform. Centrifuge the water layer, filter the supernatant, and use the filtrate as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 20 mg, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0 and water to make exactly 25 mL, and use this solution as the standard solution. To exactly 10 mL each of the sample solution and standard solution add exactly 2 mL of sodium hydroxide TS, allow to stand for exactly 15 minutes, add exactly 2 mL of 1 mol/L hydrochloric acid TS, exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6, and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 20 minutes without exposure to light. Titrate <2.50> these solutions with 0.01 mol/L sodium thiosulfate VS until the color of the solution changes to colorless. Separately, to exactly 10 mL each of the sample solution and the standard solution add exactly 10 mL of 0.3 mol/L potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, and perform a blank determination with the same manner. Determine the consumed amounts (mL) of 0.005 mol/L iodine VS, V_T and V_S , of the sample solution and the standard solution: the amount of ampicillin is not more than 1.0%.

$$\begin{aligned} &\text{Amount (mg) of ampicillin (C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S)} \\ &= W_S \times (V_T/V_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Ampicillin Reference Standard

Water <2.48> Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 1.5% (1 g).

Assay Weigh accurately an amount of Bacampicillin Hydrochloride and Bacampicillin Hydrochloride Reference Standard, equivalent to about 40 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of bacampicillin of these solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of ampicillin (C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S)} \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Bacampicillin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle

diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of diluted 2 mol/L sodium dihydrogen phosphate TS (1 in 100), add diluted 0.05 mol/L disodium hydrogen phosphate TS (2 in 5) to adjust the pH to 6.8. To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bacampicillin is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of bacampicillin are not less than 10,000 and not more than 2, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of bacampicillin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Bacitracin

バシトラシン

[1405-87-4]

Bacitracin is a mixture of peptide substances having antibacterial activity including bacitracin A as the main component produced by the growth of *Bacillus subtilis* or *Bacillus licheniformis*.

It contains not less than 60 Units per mg. The potency of Bacitracin is expressed as unit calculated from the amount of bacitracin A (C₆₆H₁₀₃N₁₇O₁₆S: 1422.69). One unit of Bacitracin is equivalent to 23.8 μ g of bacitracin A (C₆₆H₁₀₃N₁₇O₁₆S).

Description Bacitracin occurs as a white to light brown powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) To 3 mL of a solution of Bacitracin (1 in 100) add 3 mL of 4-dimethylaminobenzaldehyde TS, shake until red-rosy to red-purple color appears, then add several drops of a solution of sodium nitrite (1 in 100), and shake: a green to dark green color is produced.

(2) Dissolve 60 mg each of Bacitracin and Bacitracin Reference Standard in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatograph. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (30:15:10:6:5) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 110 °C for 5 minutes: the spots obtained from the sample solution and standard solution show the same R_f value.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bacitracin according to Method 2, and perform the test. Pre-

pare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.15 g of Bacitracin in 0.05 mol/L sulfuric acid TS to make 100 mL. To 2 mL of this solution add 0.05 mol/L sulfuric acid TS to make 10 mL, and determine the absorbances of this solution, A_1 and A_2 , at 252 nm and 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: A_2/A_1 is not more than 0.20.

Loss on drying <2.41> Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 10240.

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Bacitracin Reference Standard, equivalent to about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

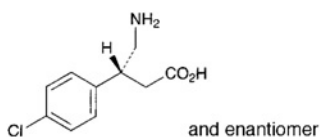
(iv) Sample solutions—Weigh accurately an amount of Bacitracin, equivalent about 400 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Baclofen

バクロフェン



$C_{10}H_{12}ClNO_2$: 213.66

(3*RS*)-4-Amino-3-(4-chlorophenyl)butanoic acid

[1134-47-0]

Baclofen contains not less than 98.5% of $C_{10}H_{12}ClNO_2$, calculated on the anhydrous basis.

Description Baclofen occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification (1) To 5 mL of a solution of Baclofen (1 in 1000) add 1 mL of ninhydrin TS, and heat on a water bath for 3 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Baclofen in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Baclofen Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Baclofen as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Baclofen in 50 mL of acetic acid (100), and add water to make 100 mL. To 10 mL of this solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.21%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Baclofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Baclofen according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Baclofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1.0 mL and 1.5 mL of the sample solution, to each add the mobile phase to make exactly 100 mL, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with exactly 25 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak height of these solutions: each height of the peaks other than the peak of baclofen from the sample solution is not larger than the peak height of baclofen from the standard solution (1), and the total height of these peaks is not larger than the peak height of baclofen from the standard solution (2).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 900) (3:2).

Flow rate: Adjust the flow rate so that the retention time of baclofen is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of baclofen beginning after the solvent peak. **System suitability**—

Test for required detection: Adjust the sensitivity so that the peak height of baclofen obtained from 25 μ L of the standard solution (1) is between 5 and 10 mm.

System performance: Dissolve 0.40 g of Baclofen and 5 mg

of methyl parahydroxybenzoate in 200 mL of the mobile phase. To 10 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 25 μ L of this solution under the above operating conditions, baclofen and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak heights of baclofen is not more than 3.0%.

Water <2.48> Not more than 1.0% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.5 g of Baclofen, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.37 mg of $C_{10}H_{12}ClNO_2$

Containers and storage Containers—Well-closed containers.

Baclofen Tablets

バクロフェン錠

Baclofen Tablets contain not less than 93% and not more than 107% of the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$; 213.66).

Method of preparation Prepare as directed under Tablets, with Baclofen.

Identification (1) To a portion of powdered Baclofen Tablets, equivalent to 0.01 g of Baclofen according to the labeled amount, add 10 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and proceed as directed in the Identification (1) under Baclofen.

(2) To a portion of powdered Baclofen Tablets, equivalent to 25 mg of Baclofen according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 257 nm and 261 nm, between 264 nm and 268 nm, and between 272 nm and 276 nm.

(3) To a portion of powdered Baclofen Tablets, equivalent to 0.01 g of Baclofen according to the labeled amount, add 2 mL of a mixture of methanol and acetic acid (100) (4:1), shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.01 g of Baclofen Reference Standard in 2 mL of a mixture of methanol and acetic acid (100) (4:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-

butanol, water and acetic acid (100) (4:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R_f* value.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Baclofen Tablets at 50 revolutions per minute according to the Paddle method using 500 mL of water as the dissolution medium. Take 20 mL or more of the dissolved solution 45 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent, add water to make exactly *V'* mL so that each mL contains about 10 μ g of baclofen ($C_{10}H_{12}ClNO_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baclofen Reference Standard (separately determine the water content <2.48> in the same manner as Baclofen), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, *A_T* and *A_S*, of the sample solution and the standard solution at 220 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate (%) of Baclofen Tablets in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of baclofen ($C_{10}H_{12}ClNO_2$)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 50$

W_S: Amount (mg) of Baclofen Reference Standard.

C: Labeled amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Baclofen Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of baclofen ($C_{10}H_{12}ClNO_2$), add 130 mL of 0.1 mol/L hydrochloric acid TS, shake for 10 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 200 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Baclofen Reference Standard (separately determine the water content <2.48> in the same manner as Baclofen), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, and add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 2 drops of phenolphthalein TS, neutralize with dilute sodium hydroxide TS, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and the standard solution, to each add 4 mL of ninhydrin-stannous chloride TS, shake, heat on a water bath for 20 minutes, and shake at once vigorously for 2 minutes. After cooling, to each solution add a mixture of water and 1-propanol (1:1) to make exactly 25 mL. Determine the absorbances, *A_T* and *A_S*, of these solutions at 570 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a blank prepared with 2 mL of water in the same manner.

Amount (mg) of baclofen ($C_{10}H_{12}ClNO_2$)

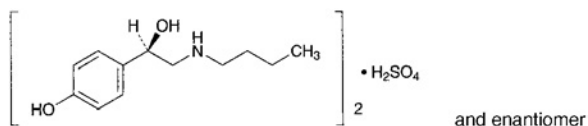
$$= W_s \times (A_T/A_S) \times (1/5)$$

W_s : Amount (mg) of Baclofen Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Bamethan Sulfate

バメタン硫酸塩



$(C_{12}H_{19}NO_2)_2 \cdot H_2SO_4$: 516.65
(1*RS*)-2-Butylamino-1-(4-hydroxyphenyl)ethanol hemisulfate [5716-20-1]

Bamethan Sulfate, when dried, contains not less than 99.0% of $(C_{12}H_{19}NO_2)_2 \cdot H_2SO_4$.

Description Bamethan Sulfate occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 169°C (with decomposition).

Identification (1) To 1 mL of a solution of Bamethan Sulfate (1 in 1000) add 5 mL of a solution of 4-nitrobenzenediazonium fluoroborate (1 in 2000) and 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.2: an orange-red color develops.

(2) Determine the absorption spectrum of a solution of Bamethan Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bamethan Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1618, 1597, 1518, 1118 and 833 cm^{-1} .

(4) A solution of Bamethan Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Bamethan Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Bamethan Sulfate in 20 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of Matching Fluid O add diluted hydrochloric acid (1 in 40) to make 200 mL.

(2) Chloride <1.03>—Perform the test with 3.5 g of Bamethan Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bamethan Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bamethan Sulfate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Bamethan Sulfate in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (7:2) in a developing vessel saturated with ammonia vapor to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry for 15 minutes, spray Dragendorff's TS for spraying again, then, after 1 minute, spray evenly a solution of sodium nitrite (1 in 20), and immediately put a glass plate on the plate. Examine the plate after 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

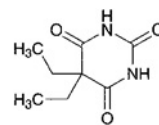
Assay Weigh accurately about 0.75 g of Bamethan Sulfate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.67 mg of $(C_{12}H_{19}NO_2)_2 \cdot H_2SO_4$

Containers and storage Containers—Tight containers.

Barbitol

バルビタール



$C_8H_{12}N_2O_3$: 184.19
5,5-Diethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione
[57-44-3]

Barbitol, when dried, contains not less than 99.0% of $C_8H_{12}N_2O_3$.

Description Barbitol occurs as colorless or white crystals or a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone and in pyridine, soluble in ethanol (95), sparingly soluble in diethyl ether, and slightly soluble in water and in chloroform.

It dissolves in sodium hydroxide TS and in ammonia TS.

The pH of its saturated solution is between 5.0 and 6.0.

Identification (1) Boil 0.2 g of Barbitol with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red

litmus paper to blue.

(2) Dissolve 0.05 g of Barbitol in 5 mL of diluted pyridine (1 in 10), add 0.3 mL of copper (II) sulfate TS, shake, and allow to stand for 5 minutes: a red-purple precipitate is formed. Shake the mixture with 5 mL of chloroform: a red-purple color develops in the chloroform layer. Separately, dissolve 0.05 g of Barbitol in 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7, and 5 mL of diluted pyridine (1 in 10). Add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS to the solution: a red-purple precipitate is produced in the aqueous layer. The red-purple precipitate is not dissolved in the chloroform by shaking.

(3) To 0.4 g of Barbitol add 0.1 g of anhydrous sodium carbonate and 4 mL of water, shake, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat the mixture on a water bath under a reflux condenser for 30 minutes, and allow to stand for 1 hour. Collect the separated crystals, wash with 7 mL of sodium hydroxide TS and a small amount of water, recrystallize from a mixture of ethanol (95) and chloroform (1:1), and dry at 105°C for 30 minutes: the crystals melt <2.60> between 192°C and 196°C.

Melting point <2.60> 189 – 192°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Barbitol in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.30 g of Barbitol in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Dissolve 0.40 g of Barbitol in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Barbitol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Barbitol. The solution is not more colored than Matching Fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Barbitol, previously dried, and dissolve in 5 mL of ethanol (95) and 50 mL of chloroform. Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 18.42 mg of C₈H₁₂N₂O₃

Containers and storage Containers—Well-closed contain-

ers.

Barium Sulfate

硫酸バリウム

BaSO₄: 233.39

Description Barium Sulfate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It does not dissolve in hydrochloric acid, in nitric acid and in sodium hydroxide TS.

Identification (1) Mix 0.5 g of Barium Sulfate with 2 g each of anhydrous sodium carbonate and potassium carbonate in a crucible, heat the mixture until fusion is complete, treat the cooled mass with hot water, and filter. The filtrate, acidified with hydrochloric acid, responds to the Qualitative Tests <1.09> for sulfate.

(2) Wash the hot water-insoluble residue obtained in (1) with water, dissolve in 2 mL of acetic acid (31), and filter, if necessary: the solution responds to the Qualitative Tests <1.09> for barium salt.

Purity (1) Acidity or alkalinity—Agitate 1.0 g of Barium Sulfate with 20 mL of water for 5 minutes: the solution is neutral.

(2) Phosphate—Boil 1.0 g of Barium Sulfate with 3 mL of nitric acid and 5 mL of water for 5 minutes, cool, and add water to restore the original volume. Filter through a filter paper, previously washed with dilute nitric acid, to the filtrate add an equal volume of hexaammonium heptamolybdate TS, and allow to stand between 50°C and 60°C for 1 hour: no yellow precipitate is produced.

(3) Sulfide—Place 10 g of Barium Sulfate in a 250-mL conical flask, add 10 mL of dilute hydrochloric acid and water to make 100 mL, and boil for 10 minutes: the gas evolved does not darken moistened lead (II) acetate paper.

(4) Heavy metals <1.07>—Boil 5.0 g of Barium Sulfate with 2.5 mL of acetic acid (100) and 50 mL of water for 10 minutes, cool, add 0.5 mL of ammonia TS and water to make 100 mL, and filter. Perform the test with a 50-mL portion of this filtrate. Prepare the control solution with 2.5 mL of Standard Lead Solution, 1.25 mL of acetic acid (100), 0.25 mL of ammonia TS and water to make 50 mL (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 2.0 g of Barium Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(6) Hydrochloric acid-soluble substances and soluble barium salts—Cool the solution obtained in (3), add water to make 100 mL, and filter. Evaporate 50 mL of the filtrate on a water bath to dryness, add 2 drops of hydrochloric acid and 10 mL of warm water, filter through filter paper for assay, and wash with 10 mL of warm water. Evaporate the combined filtrate and washings on a water bath to dryness, and dry the residue at 105°C for 1 hour: the residue weighs not more than 15 mg. Shake the residue, if any, with 10 mL of water, and filter. To the filtrate add 0.5 mL of dilute sulfuric acid, and allow to stand for 30 minutes: no turbidity is

produced.

Containers and storage Containers—Well-closed containers.

Freeze-dried BCG Vaccine (for Percutaneous Use)

乾燥 BCG ワクチン

Freeze-dried BCG Vaccine (for Percutaneous Use) is a preparation for injection which is dissolved before use.

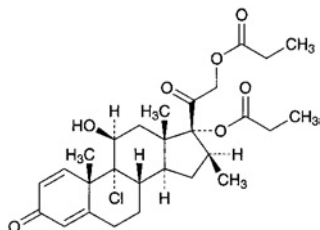
It contains live bacteria derived from a culture of the bacillus of Calmette and Guérin.

It conforms to the requirements of Freeze-dried BCG Vaccine (for Percutaneous Use) in the Minimum Requirements for Biological Products.

Description Freeze-dried BCG Vaccine (for Percutaneous Use) becomes a white to light yellow, turbid liquid on addition of solvent.

Beclometasone Dipropionate

ベクロメタゾンプロピオン酸エステル



$C_{28}H_{37}ClO_7$: 521.04

9-Chloro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5534-09-8]

Beclometasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{28}H_{37}ClO_7$.

Description Beclometasone Dipropionate occurs as a white to pale yellow powder. It is odorless.

It is freely soluble in chloroform, soluble in methanol, sparingly soluble in ethanol (95) and in 1,4-dioxane, slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 208°C (with decomposition).

Identification (1) Dissolve 2 mg of Beclometasone Dipropionate in 2 mL of sulfuric acid: initially a yellowish color develops, and gradually changes through orange to dark red-brown. To this solution add carefully 10 mL of water: the color changes to bluish green, and a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Beclometasone Dipropionate in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red to red-brown precipitate is formed.

(3) Prepare the test solution with 0.02 g of Beclometasone Dipropionate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 1 mL of sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for chloride.

(4) Determine the infrared absorption spectrum of Beclometasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Beclometasone Dipropionate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Beclometasone Dipropionate and Beclometasone Dipropionate Reference Standard in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +88 – +94° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Beclometasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Beclometasone Dipropionate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (475:25:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Beclometasone Dipropionate and Beclometasone Dipropionate Reference Standard, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of beclometasone dipropionate to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{28}H_{37}ClO_7 \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Beclometasone Dipropionate Reference Standard

Internal standard solution—A solution of testosterone propionate in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of beclometasone dipropionate is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, beclometasone dipropionate and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of beclometasone dipropionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Beef Tallow

Sevum Bovinum

牛脂

Beef Tallow is a purified fat obtained by wet steam rendering from the fresh fatty tissues of *Bos taurus* Linné var. *domesticus* Gmelin (*Bovidae*).

Description Beef Tallow occurs as a white, uniform mass. It has a characteristic odor and a mild taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It is breakable at a low temperature, but softens above 30°C.

Melting point: 42 – 50°C

Acid value <1.13> Not more than 2.0.

Saponification value <1.13> 193 – 200

Iodine value <1.13> 33–50 (When the sample is insoluble in 20 mL of cyclohexane, dissolve it by shaking a glass-stoppered flask in warm water. Then, if insoluble, increase the volume of solvent.)

Purity (1) Moisture and coloration—Beef Tallow (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Beef Tallow add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Beef Tallow add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser,

and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the mixture does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of an ethanolic solution of silver nitrate (1 in 50).

Containers and storage Containers—Well-closed containers.

White Beeswax

Cera Alba

サラシミツロウ

White Beeswax is bleached Yellow Beeswax.

Description White Beeswax occurs as white to yellowish white masses. It has a characteristic odor. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

It is slightly soluble in diethyl ether, and practically insoluble in water and in ethanol (99.5).

Acid value <1.13> 5 – 9 or 17 – 22 Weigh accurately about 6 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value <1.13> 80 – 100 Weigh accurately about 3 g of White Beeswax, place in a glass-stoppered 250-mL flask, and add exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS and 50 mL of ethanol (95), heat for 4 hours on a water bath under a reflux condenser, and proceed as directed in the Saponification value.

Melting point <1.13> 60 – 67°C.

Purity Paraffin, fat, Japan wax or resin—Melt White Beeswax at the lowest possible temperature, drip the liquid into a vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Yellow Beeswax

Cera Flava

ミツロウ

Yellow Beeswax is the purified wax obtained from honeycombs such as those of *Apis indica*

Radoszkowski or *Apis mellifera* Linné (*Apidae*).

Description Yellow Beeswax occurs as light yellow to brownish yellow masses. It has a characteristic odor, which is not rancid. It is comparatively brittle when cooled, and the fractured surface is granular, and non-crystalline.

Acid value <1.13> 5 – 9 or 17 – 22 Weigh accurately about 6 g of Yellow Beeswax, place in a glass-stoppered 250-mL flask, and add 50 mL of ethanol (99.5). Warm the mixture to dissolve the wax, add 1 mL of phenolphthalein TS, and proceed as directed in the Acid value. Perform a blank determination using solvent which is not previously neutralized, and make any necessary correction.

Saponification value <1.13> 80 – 100 Weigh accurately about 3 g of Yellow Beeswax, place in a 250-mL glass-stoppered flask, and add 25 mL of 0.5 mol/L potassium hydroxide-ethanol and 50 mL of ethanol (95), insert a reflux condenser, heat for 4 hours on a water bath, and proceed as directed in the Saponification value.

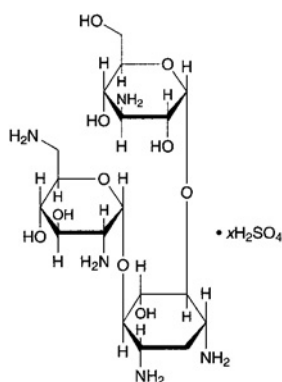
Melting point <1.13> 60 – 67°C.

Purity Paraffin, fat, Japan wax or resin—Melt Yellow Beeswax at the lowest possible temperature, drip the liquid into a glass vessel containing ethanol (95) to form granules, and allow them to stand in air for 24 hours. Drop the granules into two mixtures of ethanol (95) and water, one adjusted so as to have a specific gravity of 0.95 and the other 0.97: the granules sink or are suspended in the mixture with the specific gravity of 0.95, and float or are suspended in the other mixture.

Containers and storage Containers—Well-closed containers.

Bekanamycin Sulfate

ベカナマイシン硫酸塩



$C_{18}H_{37}N_5O_{10} \cdot xH_2SO_4$

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-2-deoxy-D-streptomine sulfate [70550-99-1]

Bekanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of the mutant of *Streptomyces kanamyceticus*.

It contains not less than 680 μ g (potency) and not

more than 770 μ g (potency) per mg, calculated on the dried basis. The potency of Bekanamycin Sulfate is expressed as mass (potency) of bekanamycin ($C_{18}H_{37}N_5O_{10}$: 483.51).

Description Bekanamycin Sulfate occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 20 mg of Bekanamycin Sulfate in 2 mL of 1/15 mol/L phosphate buffer solution, pH 5.6, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 30 mg each of Bekanamycin Sulfate and Bekanamycin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R_f* value.

(3) To a solution of Bekanamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +102 – +116° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.50 g of Bekanamycin Sulfate in 10 mL of water is between 6.0 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Bekanamycin Sulfate in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Bekanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Bekanamycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 60 mg of Bekanamycin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer having pH <2.54> 7.8 to 8.0 after sterilization.

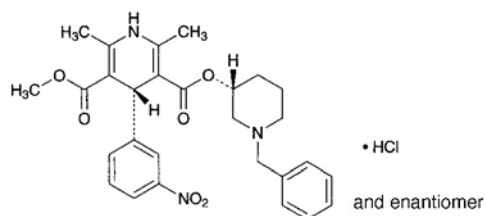
(iii) Standard solutions—Weigh accurately an amount of Bekanamycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 µg (potency) and 2.5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Bekanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 10 µg (potency) and 2.5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Benidipine Hydrochloride

ベニジピン塩酸塩



$C_{28}H_{31}N_3O_6 \cdot HCl$: 542.02

3-[(3*RS*)-1-Benzylpiperidin-3-yl] 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride [91599-74-5]

Benidipine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{28}H_{31}N_3O_6 \cdot HCl$.

Description Benidipine Hydrochloride occurs as a yellow crystalline powder.

It is very soluble in formic acid, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Benidipine Hydrochloride in methanol (1 in 100) shows no optical rotation.

Melting point: about 200°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Benidipine Hydrochloride in methanol (1 in

100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benidipine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Benidipine Hydrochloride (1 in 10) add 5 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. The filtrate, which is acidified with dilute nitric acid, responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Benidipine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Benidipine Hydrochloride in 100 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of bisbenzylpiperidyl ester having the relative retention time of about 0.35 with respect to benidipine, dehydro derivative having the relative retention time of about 0.75 and other related substances are not larger than 1/2 times the peak area of benidipine with the standard solution, and the total area of the peaks other than benidipine is not larger than the peak area of benidipine with the standard solution. For this calculation, use the peak areas of bisbenzylpiperidyl ester and dehydro derivative after multiplying by their response factors, 1.6, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 µm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and tetrahydrofuran (65:27:8).

Flow rate: Adjust the flow rate so that the retention time of benidipine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of benidipine beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mixture of water and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of benidipine obtained with 10 µL of this solution is equivalent to 18 to 32% of that with 10 µL of the standard solution.

System performance: Dissolve 6 mg of Benidipine Hydrochloride and 5 mg of benzoin in 200 mL of the mixture of water and methanol (1:1). When the procedure is run with 10 μ L of this solution under the above operating conditions, benzoin and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 3.5%.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Benidipine Hydrochloride, previously dried, dissolve in 10 mL of formic acid, add 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 54.20 mg of $C_{28}H_{31}N_3O_6 \cdot HCl$

Containers and storage Containers—Tight containers.

Benidipine Hydrochloride Tablets

ベニジピン塩酸塩錠

Benidipine Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of benidipine hydrochloride ($C_{28}H_{31}N_3O_6 \cdot HCl$; 542.02).

Method of preparation Prepare as directed under Tablets, with Benidipine Hydrochloride.

Identification Shake well a quantity of powdered Benidipine Hydrochloride Tablets, equivalent to 10 mg of Benidipine Hydrochloride according to the labeled amount, with 100 mL of methanol, and centrifuge. To 10 mL of the supernatant liquid add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm, and between 350 nm and 360 nm.

Purity Dehydro derivative—Powder Benidipine Hydrochloride Tablets in an agate mortar. To an amount of the powder, equivalent to 20 mg of Benidipine Hydrochloride, add about 80 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake well, and add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Filter through a membrane filter with pore size of 0.45 μ m, and use the filtrate as the sample solution. Separately, dissolve 20 mg of benidipine hydrochloride for assay in the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL. Pipet 1 mL of this solution, add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution

as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of dehydro derivative having the relative retention time of about 0.75 with respect to benidipine is not larger than 1/2 times the peak area of benidipine with the standard solution. For this calculation, use the peak area of dehydro derivative after multiplying by the relative response factor, 1.6.

Operating conditions—

Perform as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of benidipine obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: Dissolve 6 mg of benidipine hydrochloride and 5 mg of benzoin in 200 mL of a mixture of water and methanol (1:1). When the procedure is run with 10 μ L of this solution under the above operating conditions, benzoin and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Benidipine Hydrochloride Tablets add 40 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake to disintegrate, and add a suitable amount of the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly V mL of a solution, containing 40 μ g of benidipine hydrochloride ($C_{28}H_{31}N_3O_6 \cdot HCl$) per mL. Centrifuge the solution, pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of benidipine hydrochloride ($C_{28}H_{31}N_3O_6 \cdot HCl$)
= $W_S \times (Q_T/Q_S) \times (V/1000)$

W_S : Amount (mg) of benidipine hydrochloride for assay

Internal standard solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Benidipine Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method using the sinker, using 900 mL of 1st fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test for 2-mg and 4-mg tablets and 45 minutes after starting the test for 8-mg tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μ m. Discard the first 10 mL of the filtrate pipet the subsequent V mL, and add 1st fluid for dissolution test to make exactly V' mL so that each mL contains about 2.2 μ g of benidipine hydrochloride (C_{28}

H₃₁N₃O₆.HCl) according to the labeled amount. Pipet 5 mL of this solution, add exactly 5 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of benidipine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of 1st fluid for dissolution test, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of benidipine: the dissolution rates for 2-mg or 4-mg tablet in 30 minutes and for 8-mg tablet in 45 minutes are not less than 80% and not less than 85%, respectively.

Dissolution rate (%) of benidipine hydrochloride (C₂₈H₃₁N₃O₆.HCl) with respect to the labeled amount

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 9$$

W_S: Amount (mg) of benidipine hydrochloride for assay
 C: Labeled amount (mg) of benidipine hydrochloride (C₂₈H₃₁N₃O₆.HCl) in 1 tablet.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of benidipine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of benidipine are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benidipine is not more than 1.5%.

Assay Weigh accurately the mass of not less than 20 Benidipine Hydrochloride Tablets, and powder using an agate mortar. Weigh accurately a part of the powder, equivalent to about 8 mg of benidipine hydrochloride (C₂₈H₃₁N₃O₆.HCl), add about 150 mL of a mixture of diluted phosphoric acid (1 in 500) and methanol (1:1), shake, then add the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly 200 mL, and centrifuge. Pipet 20 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of benidipine hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make exactly

100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S, of the peak area of benidipine to that of the internal standard.

Amount (mg) of benidipine hydrochloride (C₂₈H₃₁N₃O₆.HCl)

$$= W_S \times (Q_T/Q_S) \times (1/5)$$

W_S: Amount (mg) of benidipine hydrochloride for assay

Internal standard solution—A solution of benzoin in a mixture of water and methanol (1:1) (13 in 200,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 237 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0, methanol and tetrahydrofuran (65:27:8).

Flow rate: Adjust the flow rate so that the retention time of benidipine is about 20 minutes.

System suitability—

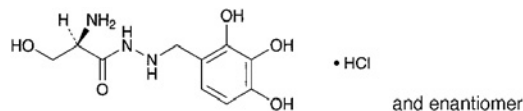
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and benidipine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of benidipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Benserazide Hydrochloride

ベンセラジド塩酸塩



C₁₀H₁₅N₃O₅.HCl: 293.70
 (2*RS*)-2-Amino-3-hydroxy-*N'*-(2,3,4-trihydroxybenzyl)propanoylhydrazide monohydrochloride [14919-77-8]

Benserazide Hydrochloride contains not less than 98.0% of C₁₀H₁₅N₃O₅.HCl, calculated on the anhydrous basis.

Description Benserazide Hydrochloride occurs as a white to

grayish white, crystalline powder.

It is freely soluble in water and in formic acid, soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Benserazide Hydrochloride (1 in 100) is between 4.0 and 5.0.

It is hygroscopic.

It is gradually colored by light.

A solution of Benserazide Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Benserazide Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benserazide Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Benserazide Hydrochloride (1 in 30) add silver nitrate TS: a white precipitate is formed. To a portion of this precipitate add dilute nitric acid: the precipitation does not dissolve.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Benserazide Hydrochloride in 10 mL of water, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of this solution at 430 nm is not more than 0.10.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Benserazide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.25 g of Benserazide Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add methanol to make exactly 200 mL, and use these solutions as the standard solution (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (1) and (2) on a plate of cellulose for thin-layer chromatography. Develop the plate with a solution of formic acid in sodium chloride TS (1 in 1000) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium carbonate TS, air-dry, and then spray evenly Folin's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2), and the number of the spots which intense more than the spot from the standard solution (1) are not more than 2.

Water <2.48> Not more than 2.5% (0.5 g, direct titration). Use a solution of salicylic acid in methanol for Karl Fischer method (3 in 20) instead of methanol for Karl Fischer method.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Benserazide Hydrochloride, dissolve in 5 mL of formic acid, add 50 mL

of acetic acid (100), and titrate <2.50> immediately with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.37 mg of $C_{10}H_{15}N_3O_5 \cdot HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Bentonite

ベントナイト

Bentonite is a natural, colloidal, hydrated aluminum silicate.

Description Bentonite occurs as a very fine, white to light yellow-brown powder. It is odorless. It has a slightly earthy taste.

It is practically insoluble in water and in diethyl ether.

It swells in water.

Identification (1) Add 0.5 g of Bentonite to 3 mL of diluted sulfuric acid (1 in 3), and heat until white fumes are evolved. Cool, add 20 mL of water, and filter. To 5 mL of the filtrate add 3 mL of ammonia TS: a white, gelatinous precipitate is produced, which turns red on the addition of 5 drops of alizarin red S TS.

(2) Wash the residue obtained in (1) with water, add 2 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash again with water: the residue is blue in color.

pH <2.54> To 1.0 g of Bentonite add 50 mL of water, and shake: the pH of the suspension is between 9.0 and 10.5.

Purity (1) Heavy metals <1.07>—To 1.5 g of Bentonite add 80 mL of water and 5 mL of hydrochloric acid, and boil gently for 20 minutes with thorough stirring. Cool, centrifuge, collect the supernatant liquid, wash the residue with two 10-mL portions of water, and centrifuge each. Combine the supernatant liquid and the washings, and add dropwise ammonia solution (28). When a precipitate is produced, add dropwise dilute hydrochloric acid with vigorous stirring, and dissolve. To the solution add 0.45 g of hydroxylammonium chloride, and heat. Cool, and add 0.45 g of sodium acetate trihydrate, 6 mL of dilute acetic acid and water to make 150 mL. Pipet 50 mL of the solution, and perform the test using this solution as the test solution. Prepare the control solution as follows: mix 2.5 mL of Standard Lead Solution, 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 50 ppm).

(2) Arsenic <1.11>—To 1.0 g of Bentonite add 5 mL of dilute hydrochloric acid, and gently heat to boil while stirring well. Cool immediately, and centrifuge. To the residue add 5 mL of dilute hydrochloric acid, shake well, and centrifuge. To the residue add 10 mL of water, and perform the same operations. Combine all the extracts, and heat on a water bath to concentrate to 5 mL. Perform the test with this solution as the test solution (not more than 2 ppm).

(3) Foreign matter—Place 2.0 g of Bentonite in a mortar, add 20 mL of water to swell, disperse evenly with a pestle, and dilute with water to 100 mL. Pour the suspension

through a No. 200 (74 μm) sieve, and wash the sieve thoroughly with water. No grit is felt when the fingers are rubbed over the wire mesh of the sieve.

Loss on drying <2.41> 5.0 – 10.0% (2 g, 105°C, 2 hours).

Gel formation Mix 6.0 g of Bentonite with 0.30 g of magnesium oxide. Add the mixture, in several portions, to 200 mL of water contained in a glass-stoppered 500-mL cylinder. Agitate for 1 hour, transfer 100 mL of the suspension to a 100-mL graduated cylinder, and allow to stand for 24 hours: not more than 2 mL of supernatant appears on the surface.

Swelling power To 100 mL of water in a glass-stoppered 100-mL cylinder add 2.0 g of Bentonite in ten portions, allowing each portion to settle before adding the next, and allow to stand for 24 hours: the apparent volume of the sediment at the bottom is not less than 20 mL.

Containers and storage Containers—Well-closed containers.

Benzalkonium Chloride

ベンザルコニウム塩化物

Benzalkonium Chloride is represented by the formula $[\text{C}_6\text{H}_5\text{CH}_2\text{N}(\text{CH}_3)_2\text{R}]\text{Cl}$, in which R extends from C_8H_{17} to $\text{C}_{18}\text{H}_{37}$, with $\text{C}_{12}\text{H}_{25}$ and $\text{C}_{14}\text{H}_{29}$ comprising the major portion.

It contains not less than 95.0% and not more than 105.0% of benzalkonium chloride (as $\text{C}_{22}\text{H}_{40}\text{ClN}$: 354.01), calculated on the anhydrous basis.

Description Benzalkonium Chloride occurs as a white to yellowish white powder, colorless to light yellow, gelatinous pieces, or jelly-like fluid or mass. It has a characteristic odor.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Benzalkonium Chloride foams strongly when shaken.

Identification (1) Dissolve 0.2 g of Benzalkonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzalkonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid

and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Benzalkonium Chloride in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Petroleum ether-soluble substances—To 3.0 g of Benzalkonium Chloride add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether. Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.

Water <2.48> Not more than 15.0%.

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.15 g of Benzalkonium Chloride, and dissolve in 75 mL of water. Adjust the pH between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate <2.50> with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of $\text{C}_{22}\text{H}_{40}\text{ClN}$

Containers and storage Containers—Tight containers.

Benzalkonium Chloride Solution

ベンザルコニウム塩化物液

Benzalkonium Chloride Solution is an aqueous solution containing not more than 50.0 w/v% of benzalkonium chloride.

It contains not less than 93% and not more than 107% of the labeled amount of benzalkonium chloride ($\text{C}_{22}\text{H}_{40}\text{ClN}$: 354.01).

Method of preparation Dissolve Benzalkonium Chloride in Water or Purified Water. It is also prepared by diluting Concentrated Benzalkonium Chloride Solution 50 with Water or Purified Water.

Description Benzalkonium Chloride Solution is a clear, colorless to light yellow liquid, having a characteristic odor.

It foams strongly on shaking.

Identification (1) Evaporate a volume of Benzalkonium Chloride Solution, equivalent to 0.2 g of Benzalkonium Chloride according to the labeled amount, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzalkonium Chloride.

(2) To a volume of Benzalkonium Chloride Solution, equivalent to 0.01 g of Benzalkonium Chloride according to the labeled amount, add water to make 10 mL. Proceed with 2 mL of this solution as directed in the Identification (2) under Benzalkonium Chloride.

(3) To a volume of Benzalkonium Chloride Solution, equivalent to 1 g of Benzalkonium Chloride according to the labeled amount, add water or concentrate on a water bath, if necessary, to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid VS to make 200 mL, and proceed as directed in the Identification (3) under Benzalkonium Chloride.

(4) To a volume of Benzalkonium Chloride Solution, equivalent to 0.1 g of Benzalkonium Chloride according to the labeled amount, add water or concentrate on a water bath, if necessary, to make 10 mL. Proceed with 1 mL of this solution as directed in the Identification (4) under Benzalkonium Chloride.

Assay Pipet a volume of Benzalkonium Chloride Solution, equivalent to about 0.15 g of benzalkonium chloride ($C_{22}H_{40}ClN$), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzalkonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 7.080 mg of benzalkonium chloride ($C_{22}H_{40}ClN$)

Containers and storage Containers—Tight containers.

Benzalkonium Chloride Concentrated Solution 50

濃ベンザルコニウム塩化物液 50

Benzalkonium Chloride Concentrated Solution 50 is an aqueous solution, presented as $[C_6H_5CH_2N(CH_3)_2R]Cl$, where R ranges from C_8H_{17} to $C_{18}H_{37}$, and mainly consisting of $C_{12}H_{25}$ and $C_{14}H_{29}$.

It contains more than 50.0 w/v% and not more than 55.0 w/v% of benzalkonium chloride ($C_{22}H_{40}ClN$; 354.01).

Description Benzalkonium Chloride Concentrated Solution 50 is a colorless to light yellow liquid or jelly-like fluid, and has a characteristic odor.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution prepared by adding water to it vigorously foams when shaken.

Identification (1) Dissolve 0.4 g of Benzalkonium Chloride Concentrated Solution 50 in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines. The color of the solution is red.

(2) To 2 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 500) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise, with stirring, a solution of sodium lauryl sulfate (1 in 1000): the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzalkonium Chloride Concentrated Solution 50 in 0.1 mol

/L hydrochloric acid TS (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzalkonium Chloride Concentrated Solution 50 (1 in 50) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on the addition of dilute nitric acid, but dissolves on the addition of ammonia TS.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Benzalkonium Chloride Concentrated Solution 50 in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Petroleum ether-soluble substances—To 6.0 g of Benzalkonium Chloride Concentrated Solution 50 add water to make 50 mL, then add 50 mL of ethanol (99.5) and 5 mL of 0.5 mol/L sodium hydroxide TS, and extract with three 50-mL portions of petroleum ether. Combine the petroleum ether extracts, and wash with three 50-mL portions of dilute ethanol. After shaking well with 10 g of anhydrous sodium sulfate, filter through a dry filter paper, and wash the filter paper with two 10-mL portions of petroleum ether. Evaporate the petroleum ether on a water bath by heating, and dry the residue at 105°C for 1 hour: the residue is not more than 1.0%.

Residue on ignition <2.44> Not more than 0.2% (1 g).

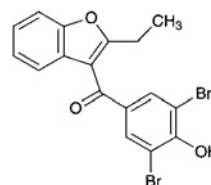
Assay Weigh accurately about 0.3 g of Benzalkonium Chloride Concentrated Solution 50, and dissolve in 75 mL of water. Adjust the pH to between 2.6 and 3.4 by adding dropwise diluted dilute hydrochloric acid (1 in 2), add 1 drop of methyl orange TS, and titrate <2.50> with 0.02 mol/L sodium tetraphenylboron VS until the color of the solution becomes red.

Each mL of 0.02 mol/L sodium tetraphenylborate VS
= 7.080 mg of benzalkonium chloride ($C_{22}H_{40}ClN$)

Containers and storage Containers—Tight containers.

Benzbromarone

ベンズブロマロン



$C_{17}H_{12}Br_2O_3$: 424.08
3,5-Dibromo-4-hydroxyphenyl 2-ethylbenzo[b]furan-3-yl ketone [3562-84-3]

Benzbromarone, when dried, contains not less than 98.5% and not more than 101.0% of $C_{17}H_{12}Br_2O_3$.

Description Benzbromarone occurs as a white to light yellow, crystalline powder.

It is very soluble in *N,N*-dimethylformamide, freely soluble in acetone, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Benzbromarone in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzbromarone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 149 – 153°C

Purity (1) Sulfate <1.14>—Dissolve 1.0 g of Benzbromarone in 40 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 40 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.019%).

(2) Soluble halides—Dissolve 0.5 g of Benzbromarone in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Proceed with this solution as directed under Chloride Limit Test <1.03>. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Benzbromarone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Iron <1.10>—Prepare the test solution with 1.0 g of Benzbromarone according to Method 3, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Benzbromarone in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 4-methyl-2-pentanone, ethanol (99.5) and acetic acid (100) (100:20:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 50°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Benzbromarone, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

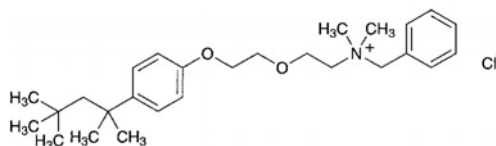
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS

= 42.41 mg of $C_{17}H_{12}Br_2O_3$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Benzethonium Chloride

ベンゼトニウム塩化物



$C_{27}H_{42}ClNO_2$: 448.08

N-Benzyl-*N,N*-dimethyl-2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethylammonium chloride [I21-54-0]

Benzethonium Chloride, when dried, contains not less than 97.0% of $C_{27}H_{42}ClNO_2$.

Description Benzethonium Chloride occurs as colorless or white crystals. It is odorless.

It is very soluble in ethanol (95), freely soluble in water, and practically insoluble in diethyl ether.

A solution of Benzethonium Chloride foams strongly when shaken.

Identification (1) Dissolve 0.2 g of Benzethonium Chloride in 1 mL of sulfuric acid, add 0.1 g of sodium nitrate, and heat for 5 minutes on a water bath. After cooling, add 10 mL of water and 0.5 g of zinc powder, heat for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines, developing a red color.

(2) To 2 mL of a solution of Benzethonium Chloride (1 in 1000) add a mixture of 0.2 mL of a solution of bromophenol blue (1 in 2000) and 0.5 mL of sodium hydroxide TS: a blue color develops. Add 4 mL of chloroform to this solution, and shake vigorously: the blue color shifts to the chloroform layer. Collect the chloroform layer, and add dropwise a solution of sodium lauryl sulfate (1 in 1000) with stirring: the chloroform layer turns colorless.

(3) Determine the absorption spectrum of a solution of Benzethonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 1 mL of a solution of Benzethonium Chloride (1 in 100) add 2 mL of ethanol (95), 0.5 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. This precipitate does not dissolve on addition of dilute nitric acid, but dissolves on addition of ammonia TS.

Melting point <2.60> 158 – 164°C (after drying).

Purity Ammonium—Dissolve 0.10 g of Benzethonium Chloride in 5 mL of water, and boil with 3 mL of sodium hydroxide TS: the evolving gas does not change moistened red litmus paper to blue.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Benzethonium Chloride, previously dried, dissolve in 75 mL of water, add diluted dilute hydrochloric acid (1 in 2) dropwise to adjust the pH to 2.6–3.4, then add 1 drop of methyl orange TS, and titrate <2.50> with 0.02 mol/L tetraphenylboron VS until the solution develops a red.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of $C_{27}H_{42}ClNO_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzethonium Chloride Solution

ベンゼトニウム塩化物液

Benzethonium Chloride Solution contains not less than 93% and not more than 107% of the labeled amount of benzethonium chloride ($C_{27}H_{42}ClNO_2$; 448.08).

Method of preparation Dissolve Benzethonium Chloride in Water or Purified Water.

Description Benzethonium Chloride Solution is a clear, colorless liquid. It is odorless.

It foams strongly when shaken.

Identification (1) Evaporate a volume of Benzethonium Chloride Solution, equivalent to 0.2 g of Benzethonium Chloride according to the labeled amount, on a water bath to dryness, and proceed with the residue as directed in the Identification (1) under Benzethonium Chloride.

(2) To a volume of Benzethonium Chloride Solution, equivalent to 0.01 g of Benzethonium Chloride according to the labeled amount, add water to make 10 mL, proceed with 2 mL of this solution as directed in the Identification (2) under Benzethonium Chloride.

(3) To a volume of Benzethonium Chloride Solution, equivalent to 1 g of Benzethonium Chloride according to the labeled amount, and add water or concentrate on a water bath to make 10 mL. To 1 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 262 nm and 264 nm, between 268 nm and 270 nm, and between 274 nm and 276 nm.

(4) To a volume of Benzethonium Chloride Solution, equivalent to 0.1 g of Benzethonium Chloride according to the labeled amount, add water, or concentrate on a water bath, if necessary, to make 10 mL, and proceed with 1 mL of this solution as directed in the Identification (4) under Benzethonium Chloride.

Purity (1) Nitrite—Add 1.0 mL of Benzethonium Chloride Solution to a mixture of 1 mL of a solution of glycine (1 in 10) and 0.5 mL of acetic acid (31): no gas is evolved.

(2) Oxidizing substances—To 5 mL of Benzethonium

Chloride Solution add 0.5 mL of potassium iodide TS and 2 to 3 drops of dilute hydrochloric acid: no yellow color is produced.

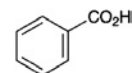
Assay Pipet a volume of Benzethonium Chloride Solution, equivalent to about 0.2 g of benzethonium chloride ($C_{27}H_{42}ClNO_2$), dilute with water to make 75 mL, if necessary, and proceed as directed in the Assay under Benzethonium Chloride.

Each mL of 0.02 mol/L sodium tetraphenylboron VS
= 8.962 mg of benzethonium chloride ($C_{27}H_{42}ClNO_2$)

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzoic Acid

安息香酸



$C_7H_6O_2$: 122.12
Benzoic acid [65-85-0]

Benzoic Acid, when dried, contains not less than 99.5% of $C_7H_6O_2$.

Description Benzoic Acid occurs as white crystals or crystalline powder. It is odorless, or has a faint, benzaldehyde-like odor.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, soluble in hot water, and slightly soluble in water.

Identification Dissolve 1 g of Benzoic Acid in 8 mL of sodium hydroxide TS, and add water to make 100 mL. This solution responds to the Qualitative Tests <1.09> (2) for benzoate.

Melting point <2.60> 121 – 124°C

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Benzoic Acid in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of acetone and water to make 50 mL (not more than 20 ppm).

(2) Chlorinated compounds—Take 0.5 g of Benzoic Acid and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, dissolve in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL, and add 0.5 mL of silver nitrate TS: this solution has not more turbid than the following control solution.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, and add 0.5 mL of silver nitrate TS.

(3) Potassium permanganate-reducing substances—Add 0.02 mol/L potassium permanganate VS dropwise to a boiling mixture of 100 mL of water and 1.5 mL of sulfuric acid, until a red color persists for 30 seconds. Dissolve 1.0 g of

Benzoic Acid in this boiling solution, and add 0.50 mL of 0.02 mol/L potassium permanganate VS: a red color persists for at least 15 seconds.

(4) Phthalic acid—To 0.10 g of Benzoic Acid add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C. After evaporating the water, heat the residue for 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Measure exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Benzoic Acid. The solution is not more colored than Matching Fluid Q.

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (1 g).

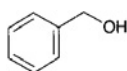
Assay Weigh accurately about 0.5 g of Benzoic Acid, previously dried, dissolve in 25 mL of neutralized ethanol and 25 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.21 mg of C₇H₆O₂

Containers and storage Containers—Well-closed containers.

Benzyl Alcohol

ベンジルアルコール



C₇H₈O: 108.14

Benzyl alcohol [100-51-6]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Benzyl Alcohol contains not less than 98.0% and not more than 100.5% of C₇H₈O.

◆The label states, where applicable, that it is suitable for use in the manufacture of injection forms.◆

◆**Description** Benzyl Alcohol is a clear, colorless oily liquid.

It is miscible with ethanol (95), with fatty oils and with essential oils.

It is soluble in water.

Specific gravity d_{20}^{20} : 1.043 – 1.049◆

◆**Identification** Determine the infrared absorption spectrum of Benzyl Alcohol as directed in the liquid film method

under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Refractive index <2.45> n_D^{20} : 1.538 – 1.541

Purity (1) Clarity and color of solution—Dissolve 2.0 mL of Benzyl Alcohol in 60 mL of water: the solution is clear and colorless.

(2) Acidity—To 10 mL of Benzyl Alcohol add 10 mL of neutralized ethanol, 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Benzaldehyde and other related substances—Use Benzyl Alcohol as the sample solution. Separately, weigh exactly 0.750 g of benzaldehyde and 0.500 g of cyclohexylmethanol, and add Benzyl Alcohol to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 2 mL of ethylbenzene internal standard solution and exactly 3 mL of dicyclohexyl internal standard solution, then add Benzyl Alcohol to make exactly 20 mL, and use this solution as the standard solution (1). Perform the test with 0.1 μL each of the sample solution and standard solution (1) as directed under Gas Chromatography <2.02> according to the following conditions: no peaks of ethylbenzene and dicyclohexyl appear on the chromatogram obtained with the sample solution. When 0.1 μL of the standard solution (1) is injected, adjust the sensitivity of the detector so that the peak height of ethylbenzene is not more than 30% of the full scale of the recorder. The peak area of benzaldehyde obtained with the sample solution is not more than the difference between the peak areas of benzaldehyde of the sample solution and the standard solution (1) (0.15%), and the peak area of cyclohexylmethanol with the sample solution is not more than the difference between the peak areas of cyclohexylmethanol of the sample solution and the standard solution (1) (0.10%). The total area of the peaks having smaller retention time than benzyl alcohol and other than benzaldehyde and cyclohexylmethanol obtained with the sample solution is not more than 4 times the peak area of ethylbenzene with the standard solution (1) (0.04%). The total area of the peaks having larger retention time than benzyl alcohol obtained with the sample solution is not more than the peak area of dicyclohexyl with the standard solution (1) (0.3%). For these calculations the peak areas less than 1/100 times the peak area of ethylbenzene with the standard solution (1) are excluded.

Benzyl Alcohol labeled that it is suitable for use in the manufacture of injection forms meets the following requirements.

Use Benzyl Alcohol as the sample solution. Separately, weigh exactly 0.250 g of benzaldehyde and 0.500 g of cyclohexylmethanol, and add Benzyl Alcohol to make exactly 25 mL. Pipet 1 mL of this solution, add exactly 2 mL of the ethylbenzene internal standard solution and exactly 2 mL of the dicyclohexyl internal standard solution, then add Benzyl Alcohol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with 0.1 μL each of the sample solution and standard solution (2) as directed under Gas Chromatography <2.02> according to the following conditions: no peaks of ethylbenzene and dicyclohexyl appear on the chromatogram obtained with the sample solution. When 0.1 μL of the standard solution (2) is injected, adjust the sensitivity of the detector so that the peak height of ethylbenzene is not more than 30% of the full scale of the recorder. The peak area of benzaldehyde of obtained with the

sample solution is not more than the difference between the peak areas of benzaldehyde of the sample solution and the standard solution (2) (0.05%), and the peak area of cyclohexylmethanol with the sample solution is not more than the difference between the peak areas of cyclohexylmethanol of the sample solution and the standard solution (2) (0.10%). The total area of the peaks having smaller retention time than benzyl alcohol and other than benzaldehyde and cyclohexylmethanol obtained with the sample solution is not more than 2 times the peak area of ethylbenzene with the standard solution (2) (0.02%). The total area of the peaks having larger retention time than benzyl alcohol obtained with the sample solution is not more than the peak area of dicyclohexyl with the standard solution (2) (0.2%). For these calculation the peak areas less than 1/100 times the peak area of ethylbenzene with the standard solution (2) are excluded.

Ethylbenzene internal standard solution: Dissolve exactly 0.100 g of ethylbenzene in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, and add Benzyl Alcohol to make exactly 20 mL.

Dicyclohexyl internal standard solution: Dissolve exactly 2.000 g of dicyclohexyl in Benzyl Alcohol to make exactly 10 mL. Pipet 2 mL of this solution, and add Benzyl Alcohol to make exactly 20 mL.

Operating conditions—

Detector: A hydrogen flame – ionization detector

Column: A fused silica column 0.32 mm in inside diameter and 30 m in length, coated inside with polyethylene glycol 20 M for gas chromatography in 0.5 μ m thickness.

Column temperature: Raise the temperature at a rate of 5°C per minutes from 50°C to 220°C, and maintain at 220°C for 35 minutes.

Temperature of injection port: A constant temperature of about 200°C.

Temperature of detector: A constant temperature of about 310°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of benzyl alcohol is between 24 and 28 minutes.

Split ratio: Splitless

System suitability—

System performance: When the procedure is run with the standard solution (1) under the above operating conditions, the relative retention times of ethylbenzene, dicyclohexyl, benzaldehyde and cyclohexylmethanol with respect to benzyl alcohol are about 0.28, about 0.59, about 0.68 and about 0.71, respectively, and the resolution between the peaks of benzaldehyde and cyclohexylmethanol is not less than 3.0. In the case of Benzyl Alcohol labeled to use for injection, proceed with the standard solution (2) instead of the standard solution (1).

(4) **Peroxide value—**Dissolve 5 g of Benzyl Alcohol in 30 mL of a mixture of acetic acid (100) and chloroform (3:2) in a 250-mL glass-stoppered conical flask. Add 0.5 mL of potassium iodide saturated solution, shake exactly for 1 minute, add 30 mL of water, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 10 mL of starch TS near the end point where the solution is a pale yellow color. Perform a blank determination in the same manner. Calculate the amount of peroxide by the following formula: not more than 5.

$$\text{Amount (mEq/kg) of peroxide} = \{10 \times (V_1 - V_0)\} / W$$

V_1 : Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the test

V_0 : Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed in the blank

W : Amount (g) of the sample

(5) **Residue on evaporation—**Perform the test after conformation that the sample meets the requirement of the peroxide value. Transfer 10.0 g of Benzyl Alcohol to a porcelain or quartz crucible or platinum dish, previously weighed accurately, and heat on a hot-plate at not exceeding 200°C, taking care to avoid boiling, to evaporate to dryness. Dry the residue on the hot-plate for 1 hour, and allow to cool in a desiccator: not more than 5 mg.

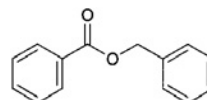
Assay Weigh accurately about 0.9 g of Benzyl Alcohol, add exactly 15.0 mL of a mixture of pyridine and acetic anhydride (7:1), and heat on a water bath under a reflux condenser for 30 minutes. Cool, add 25 mL of water, and titrate <2.50> the excess acetic acid with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 108.1 \text{ mg of } C_7H_8O \end{aligned}$$

♦ **Containers and storage** Containers—Tight containers. Storage—Light-resistant. ♦

Benzyl Benzoate

安息香酸ベンジル



$C_{14}H_{12}O_2$: 212.24

Benzyl benzoate [120-51-4]

Benzyl Benzoate contains not less than 99.0% of $C_{14}H_{12}O_2$.

Description Benzyl Benzoate is a colorless, clear, viscous liquid. It has a faint, aromatic odor and a pungent, burning taste.

It is miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

Congealing point: about 17°C

Specific gravity d_{20}^{20} : about 1.123

Boiling point: about 323°C

Identification (1) Heat gently 1 mL of Benzyl Benzoate with 5 mL of sodium carbonate TS and 2 mL of potassium permanganate TS: the odor of benzaldehyde is perceptible.

(2) Warm the titrated mixture obtained in the Assay on a water bath to remove ethanol, and add 0.5 mL of iron (III) chloride TS: a light yellow-red precipitate is produced, which turns white on the addition of dilute hydrochloric acid.

Refractive index <2.45> n_D^{20} : 1.568 – 1.570

Purity Acidity—Dissolve 5.0 mL of Benzyl Benzoate in 25 mL of neutralized ethanol, and add 0.50 mL of 0.1 mol/L so-

dium hydroxide VS: a red color develops.

Residue on ignition <2.44> Not more than 0.05% (2 g).

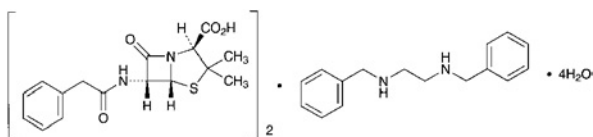
Assay Weigh accurately about 2 g of Benzyl Benzoate, add exactly 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and boil gently for 1 hour under a reflux condenser with a carbon dioxide-absorbing tube (soda lime). Cool, and titrate <2.50> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS = 106.1 mg of $C_{14}H_{12}O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Benzylpenicillin Benzathine Hydrate

ベンジルペニシリンベンザチン水和物



$(C_{16}H_{18}N_2O_4S)_2 \cdot C_{16}H_{20}N_2 \cdot 4H_2O$: 981.18
(2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid hemi(*N,N'*-dibenzylethylenediamine) dihydrate [41372-02-5]

Benzylpenicillin Benzathine Hydrate is the *N,N'*-dibenzylethylenediamine salt of a penicillin compound having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1213 Units and not more than 1333 Units per mg, calculated on the anhydrous basis. The potency of Benzylpenicillin Benzathine Hydrate is expressed as unit calculated from the amount of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$: 356.37). 1 Unit of Benzylpenicillin Benzathine Hydrate is equivalent to 0.6 μ g of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$). It contains not less than 24.0% and not more than 27.0% of *N,N'*-dibenzylethylenediamine ($C_{16}H_{20}N_2$: 240.34), calculated on the anhydrous basis.

Description Benzylpenicillin Benzathine Hydrate occurs as a white crystalline powder.

It is slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Benzylpenicillin Benzathine Hydrate in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Benzathine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference

Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +217 – +233° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Benzylpenicillin Benzathine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 70 mg of Benzylpenicillin Benzathine Hydrate in 25 mL of methanol, add a solution prepared by dissolving 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 2.4 with respect to benzylpenicillin is not more than 2 times the total area of the peaks of benzylpenicillin and *N,N'*-dibenzylethylenediamine obtained from the standard solution, and the area of the peak other than benzylpenicillin, *N,N'*-dibenzylethylenediamine and the peak having the relative retention time of about 2.4 is not more than the total area of the peaks of benzylpenicillin and *N,N'*-dibenzylethylenediamine obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (6:3:1).

Mobile phase B: A mixture of methanol, water and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (6:3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	75	25
10 – 20	75 → 0	25 → 100
20 – 55	0	100

Flow rate: 1.0 mL/min

Time span of measurement: About 3 times as long as the retention time of benzylpenicillin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase A to make exactly 20 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μ L of this solution is equivalent to 3.5 to 6.5% of that from the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, *N,N'*-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of benzylpenicillin is not more than 2.0%.

Water <2.48> 5.0 – 8.0% (1 g, volumetric titration, direct titration).

Assay (1) Benzylpenicillin—Weigh accurately an amount of Benzylpenicillin Benzathine Hydrate, equivalent to about 85,000 Units, dissolve in 25 mL of methanol, and add a solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Benzylpenicillin Potassium Reference Standard, equivalent to about 85,000 Units, and about 25 mg of *N,N'*-dibenzylethylenediamine diacetate, dissolve in 25 mL of methanol, and add the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of the solution containing 1.02 g of disodium hydrogen phosphate and 6.80 g of potassium dihydrogen phosphate in 1000 mL of water and methanol (1:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of benzylpenicillin.

Amount (unit) of benzylpenicillin sodium ($C_{16}H_{17}N_2NaO_4S$)
 $= W_S \times (A_T/A_S)$

W_S : Amount (unit) of Benzylpenicillin Potassium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, methanol and 0.25 mol/L potassium dihydrogen phosphate TS, pH 3.5 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 18 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating

conditions, *N,N'*-dibenzylethylenediamine and benzylpenicillin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of *N,N'*-dibenzylethylenediamine and benzylpenicillin are not more than 2.0%, respectively.

(2) *N,N'*-Dibenzylethylenediamine—Determine the areas, A_T and A_S , of the peak corresponding to *N,N'*-dibenzylethylenediamine on the chromatograms obtained in (1) with the sample solution and standard solution.

Amount (%) of *N,N'*-dibenzylethylenediamine ($C_{16}H_{20}N_2$)
 $= (W_S/W_T) \times (A_T/A_S) \times 100 \times 0.667$

W_S : Amount (mg) of *N,N'*-dibenzylethylenediamine diacetate

W_T : Amount (mg) of the sample

0.667: Conversion factor for the molecular mass of *N,N'*-dibenzylethylenediamine diacetate ($C_{16}H_{20}N_2 \cdot 2CH_3COOH$) to that of *N,N'*-dibenzylethylenediamine (benzathine, $C_{16}H_{20}N_2$)

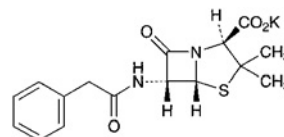
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Benzylpenicillin Potassium

Penicillin G Potassium

ベンジルペニシリンカリウム



$C_{16}H_{17}KN_2O_4S$: 372.48

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(phenylacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [113-98-4]

Benzylpenicillin Potassium is the potassium salt of a penicillin substance having antibacterial activity produced by the growth of *Penicillium* species.

It contains not less than 1430 units and not more than 1630 units per mg, calculated on the dried basis. The potency of Benzylpenicillin Potassium is expressed as mass unit of benzylpenicillin potassium ($C_{16}H_{17}KN_2O_4S$). One unit of Benzylpenicillin Potassium is equivalent to 0.57 μ g of benzylpenicillin potassium.

Description Benzylpenicillin Potassium occurs as white, crystals or crystalline powder.

It is very soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Benzylpenicillin Potassium (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Benzylpenicillin Potassium Refer-

ence Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Benzylpenicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Benzylpenicillin Potassium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Benzylpenicillin Potassium responds to the Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +270 – +300° (1.0 g calculated on the dried basis, water, 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Benzylpenicillin Potassium in 100 mL of water is between 5.0 and 7.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1 g of Benzylpenicillin Potassium in 10 mL of water is clear, and colorless or light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution by incinerating 1.0 g of Benzylpenicillin Potassium according to Method 4, and perform the test. In the incineration, use a crucible of porcelain, and after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) add 1 mL of hydrogen peroxide (30), then burn the ethanol (not more than 2 ppm).

(4) Related substances—Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than benzylpenicillin obtained from the sample solution is not more than the peak area of benzylpenicillin from the standard solution, and the total area of the peaks other than benzylpenicillin from the sample solution is not more than 3 times the peak area of benzylpenicillin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (33 in 5000) and acetonitrile (19:6), adjusted the pH to 8.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of benzylpenicillin is about 7.5 minutes.

Time span of measurement: About 5 times as long as the

retention time of benzylpenicillin.

System suitability—

Test for required detection: Pipet 10 mL of the standard solution, and add water to make exactly 100 mL. Confirm that the peak area of benzylpenicillin obtained from 20 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L of the standard solution.

System performance: Dissolve 40 mg of Benzylpenicillin Potassium in 20 mL of water. Separately, dissolve 10 mg of methyl parahydroxybenzoate in 20 mL of acetonitrile. To 1 mL of this solution add water to make 20 mL. Mix 1 mL each of these solutions, and add water to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, benzylpenicillin and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of benzylpenicillin is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (3 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer.

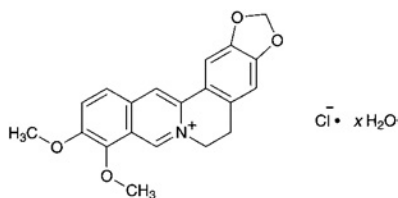
(iii) Standard solutions—Weigh accurately an amount of Benzylpenicillin Potassium Reference Standard, equivalent to about 40,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 2 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Benzylpenicillin Potassium, equivalent to about 40,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 2 units and 0.5 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Berberine Chloride Hydrate

ベルベリン塩化物水和物



$C_{20}H_{18}ClNO_4 \cdot xH_2O$

9,10-Dimethoxy-5,6-dihydro[1,3]dioxolo[4,5-g]isoquino[3,2-a]isoquinolin-7-ium chloride hydrate [633-65-8, anhydride]

Berberine Chloride Hydrate contains not less than 95.0% and not more than 102.0% of berberine chloride ($C_{20}H_{18}ClNO_4$; 371.81), calculated on the anhydrous basis.

Description Berberine Chloride Hydrate occurs as yellow crystals or crystalline powder. It is odorless or has a faint, characteristic odor. It has a very bitter taste.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Berberine Chloride Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Berberine Chloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Berberine Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Berberine Chloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Berberine Chloride Hydrate in 20 mL of water by warming, add 0.5 mL of nitric acid, cool, and filter after allowing to stand for 10 minutes. To 3 mL of the filtrate add 1 mL of silver nitrate TS, and collect the produced precipitate: the precipitate does not dissolve in dilute nitric acid, but it dissolves in an excess amount of ammonia TS.

Purity (1) Acidity—Shake thoroughly 0.10 g of Berberine Chloride Hydrate with 30 mL of water, and filter. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the yellow color changes to an orange to red color.

(2) Sulfate <1.14>—Shake 1.0 g of Berberine Chloride Hydrate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric

acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Berberine Chloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Related substances—Dissolve 10 mg of Berberine Chloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak of the areas other than berberine of the sample solution is not larger than the peak area of berberine of the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of berberine beginning after the solvent peak.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of berberine obtained from 10 μ L of the standard solution is about 10% of the full scale.

Water <2.48> 8 – 12% (0.1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately 10 mg of Berberine Chloride Hydrate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (separately, determine the water content <2.48> in the same manner as Berberine Chloride Hydrate), and dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S of berberine in each solution.

$$\begin{aligned} \text{Amount (mg) of berberine chloride (C}_{20}\text{H}_{18}\text{ClNO}_4) \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Berberine Chloride Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of monobasic potassium phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve each 1 mg of berberine

chloride and palmatin chloride in the mobile phase to make 10 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of palmatin and berberine in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Berberine Tannate

タンニン酸ベルベリン

Berberine Tannate is a compound of berberine and tannic acid.

It contains not less than 27.0% and not more than 33.0% of berberine ($C_{20}H_{19}NO_5$; 353.37), calculated on the anhydrous basis.

Description Berberine Tannate occurs as a yellow to light yellow-brown powder. It is odorless or has a faint, characteristic odor, and is tasteless.

It is practically insoluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification (1) To 0.1 g of Berberine Tannate add 10 mL of ethanol (95), and heat in a water bath for 3 minutes with shaking. Cool, filter, and to 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced, and on allowing to stand, a bluish black precipitate is formed.

(2) Dissolve 0.01 g of Berberine Tannate in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS, and add water to make 200 mL. To 8 mL of the solution add water to make 25 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Berberine Tannate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Berberine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Acidity—To 0.10 g of Berberine Tannate add 30 mL of water, and filter after shaking well. To the filtrate add 2 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L sodium hydroxide VS: the color of the solution changes from yellow to orange to red.

(2) Chloride <1.03>—Shake 1.0 g of Berberine Tannate with 38 mL of water and 12 mL of dilute nitric acid for 5 minutes, and filter. Discard the first 5 mL of the filtrate, to 25 mL of the subsequent filtrate add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.01 mol/L hydrochloric acid VS by adding 6 mL of dilute nitric acid, 10

to 15 drops of bromophenol blue TS and water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Shake 1.0 g of Berberine Tannate with 48 mL of water and 2 mL of dilute hydrochloric acid for 1 minute, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid, 5 to 10 drops of bromophenol blue TS and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Berberine Tannate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Related substances—Dissolve 10 mg of Berberine Tannate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 4 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total of the peak areas other than berberine of the sample solution is not larger than the peak area of berberine of the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of berberine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of berberine obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 3.0%.

Water <2.48> Not more than 6.0% (0.7 g, direct titration).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Weigh accurately about 30 mg of Berberine Tannate, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (separately, determine the water content <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of berberine in each solution.

$$\begin{aligned} &\text{Amount (mg) of berberine (C}_{20}\text{H}_{19}\text{NO}_5) \\ &= W_S \times (A_T/A_S) \times 0.9504 \end{aligned}$$

W_5 : Amount (mg) of Berberine Chloride Reference Standard, calculated on the dehydrated basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of berberine chloride and palmatin chloride in the mobile phase to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, palmatin and berberine are eluted in this order with the resolution between these peaks being not less than 1.5.

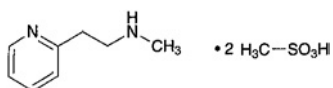
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betahistine Mesilate

ベタヒスチンメシル酸塩



$C_8H_{12}N_2 \cdot 2CH_4O_3S$: 328.41

N-Methyl-2-pyridin-2-ylethylamine dimethanesulfonate [5638-76-6, Betahistine]

Betahistine Mesilate, when dried, contains not less than 98.0% and not more than 101.0% of $C_8H_{12}N_2 \cdot 2CH_4O_3S$.

Description Betahistine Mesilate occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Betahistine Mesilate in 0.1 mol/L hydrochloric acid (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Betahistine Mesilate, previously dried, as directed in the potassi-

um bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 30 mg of Betahistine Mesilate add 0.1 g of sodium nitrate and 0.1 g of anhydrous sodium carbonate, mix well, and heat gradually. After cooling, dissolve the residue in 2 mL of dilute hydrochloric acid and 10 mL of water, filter if necessary, and to the filtrate add 1 mL of barium chloride TS: a white precipitate is formed.

Melting point <2.60> 110 – 114°C (after drying).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Betahistine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Betahistine Mesilate in 10 mL of a mixture of water and acetonitrile (63:37), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than betahistine with the sample solution is not larger than 1/10 times the peak area of betahistine with the standard solution, and the total area of the peaks other than the peak of betahistine with the sample solution is not larger than 1/2 times the peak area of betahistine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 5 mL of diethyl amine and 20 mL of acetic acid (100) add water to make 1000 mL. Dissolve 2.3 g of sodium lauryl sulfate in 630 mL of this solution, and add 370 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of betahistine is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of betahistine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of betahistine obtained with 20 μ L of this solution is equivalent to 7 to 13% of that with 20 μ L of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with

20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 70°C, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Betahistine Mesilate, previously dried, dissolve in 1 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.42 mg of $\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$

Containers and storage Containers—Tight containers.

Betahistine Mesilate Tablets

ベタヒスチンメシル酸塩錠

Betahistine Mesilate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$; 328.41).

Method of preparation Prepare as directed under Tablets, with Betahistine Mesilate.

Identification To 5 mL of the sample solution obtained in the Assay add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 259 nm and 263 nm.

Purity Related substances—Powder not less than 20 Betahistine Mesilate Tablets. To a portion of the powder, equivalent to about 50 mg of Betahistine Mesilate, add 10 mL of a mixture of water and acetonitrile (63:37), agitate for 10 minutes with the aid of ultrasonic waves, centrifuge, and use the supernatant liquid as the sample solution. Pipet 1 mL of this solution, add the mixture of water and acetonitrile (63:37) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.9 with respect to betahistine, is not more than 3/5 times the peak area of betahistine obtained from the standard solution, and the total area of the peaks other than betahistine is not more than the peak area of betahistine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the Assay.

Time span of measurement: About 8 times as long as the retention time of betahistine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mixture of water and acetonitrile (63:37) to make exactly 50 mL. Confirm that the peak area of

betahistine obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: Dissolve 10 mg of betahistine mesilate and 10 mg of 2-vinylpyridine in 50 mL of the mixture of water and acetonitrile (63:37). To 2 mL of this solution add the mixture of water and acetonitrile (63:37) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 2-vinylpyridine and betahistine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Betahistine Mesilate Tablets add exactly V mL of 0.1 mol/L hydrochloric acid TS so that each mL contains about 0.4 mg of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$), agitate for about 10 minutes with the aid of ultrasonic waves to disintegrate the tablet, then centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay

Amount (mg) of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$)
= $W_S \times (A_T/A_S) \times (V/250)$

W_S : Amount (mg) of betahistine mesilate for assay

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Betahistine Mesilate Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm . Discard the first 10 mL of the filtrate, pipet V' mL of the subsequent filtrate, add water to make exactly V'' mL so that each mL contains about 6.7 μg of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of betahistine. The dissolution rate in 15 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$)

= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S : Amount (mg) of betahistine mesilate for assay

C : Labeled amount (mg) of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$) in 1 tablet

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Betahistine Mesilate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of betahistine mesilate ($\text{C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S}$), add 40 mL of 0.1 mol/L hydrochloric acid TS, agitate for 10 minutes with the aid of ultrasonic waves, and add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.1 g of betahistine mesilate for assay, previously dried under reduced pressure with phosphorous (V) oxide at 70°C for 24 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, according to the following conditions, and determine the peak areas, A_T and A_S , of betahistine.

$$\begin{aligned} \text{Amount (mg) of betahistine mesilate (C}_8\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_4\text{O}_3\text{S)} \\ = W_S \times (A_T/A_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of betahistine mesilate for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 261 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 5 mL of diethylamine and 20 mL of acetic acid (100) add water to make 1000 mL. In 630 mL of this solution dissolve 2.3 g of sodium lauryl sulfate, and add 370 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of betahistine is about 5 minutes.

System suitability—

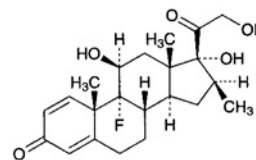
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betahistine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betahistine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone

ベタメタゾン



$\text{C}_{22}\text{H}_{29}\text{FO}_5$: 392.46

9-Fluoro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione [378-44-9]

Betamethasone, when dried, contains not less than 96.0% and not more than 103.0% of $\text{C}_{22}\text{H}_{29}\text{FO}_5$.

Description Betamethasone occurs as a white to pale yellowish white, crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water.

Melting point: about 240°C (with decomposition).

Identification (1) Proceed 10 mg of Betamethasone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid, and prepare the test solution: the test solution so obtained responds to the Qualitative Tests <1.09> for fluoride.

(2) Dissolve 1.0 mg of Betamethasone in 10 mL of ethanol (95). Mix 2.0 mL of the solution with 10 mL of phenylhydrazinium hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using as the blank the solution prepared with 2.0 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Betamethasone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Betamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Betamethasone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Betamethasone and Betamethasone Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +118 – +126° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Betamethasone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 10 mg of Betamethasone in 5 mL of a mixture of chloroform and methanol (9:1),

and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g, platinum crucible).

Assay Dissolve about 20 mg each of Betamethasone and Betamethasone Reference Standard, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of betamethasone to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of } C_{22}H_{29}FO_5 \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Betamethasone Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (2 in 3500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

System suitability—

System performance: When proceed the test with 10 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Tablets

ベタメタゾン錠

Betamethasone Tablets contain not less than 90.0% and not more than 107.0% of the labeled amount of betamethasone ($C_{22}H_{29}FO_5$; 392.46).

Method of preparation Prepare as directed under Tablets, with Betamethasone.

Identification Pulverize Betamethasone Tablets. To a portion of the powder, equivalent to 2 mg of Betamethasone according to the labeled amount, add 20 mL of methanol, shake for 5 minutes, and filter. Evaporate the filtrate on a water bath to dryness, dissolve the residue after cooling in 2 mL of methanol, filter if necessary, and use this as the sample solution. Separately, dissolve 2 mg of Betamethasone Reference Standard in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the principal spot obtained with the sample solution and the spot with the standard solution show the same Rf value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Betamethasone Tablets add V mL of water so that each mL contains about 50 μg of betamethasone ($C_{22}H_{29}FO_5$) according to the labeled amount, add exactly an amount of the internal standard solution equivalent to 2 mL per 50 μg of betamethasone, shake vigorously for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of betamethasone to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of betamethasone } (C_{22}H_{29}FO_5) \\ &= W_S \times (Q_T/Q_S) \times (V/400) \end{aligned}$$

W_S : Amount (mg) of Betamethasone Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 40,000)

Operating conditions—

Proceed as directed in the Assay

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating condi-

tions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Dissolution <6.10> Perform the test according the following method: it meets the requirement.

Perform the test with 1 tablet of Betamethasone Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add water to make exactly V' mL so that each mL contains about 0.56 μg of betamethasone ($\text{C}_{22}\text{H}_{29}\text{FO}_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Betamethasone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of betamethasone. The dissolution rate in 30 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of betamethasone ($\text{C}_{22}\text{H}_{29}\text{FO}_5$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/5)$$

W_S : Amount (mg) of Betamethasone Reference Standard

C : Labeled amount (mg) of betamethasone ($\text{C}_{22}\text{H}_{29}\text{FO}_5$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of betamethasone is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Be-

tamethasone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of betamethasone ($\text{C}_{22}\text{H}_{29}\text{FO}_5$), add 25 mL of water, then add exactly 50 mL of the internal standard solution, and shake vigorously for 10 minutes. Filter through a membrane filter with pore size of not more than 0.5 μm , discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of Betamethasone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution and 5 mL of water, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of betamethasone to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of betamethasone (C}_{22}\text{H}_{29}\text{FO}_5) \\ &= W_S \times (Q_T/Q_S) \times (1/4) \end{aligned}$$

W_S : Amount (mg) of Betamethasone Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of betamethasone is about 4 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, betamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

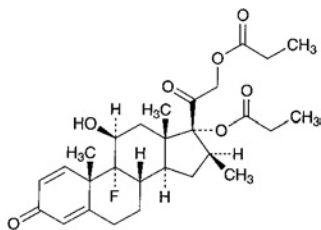
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Dipropionate

ベタメタゾンジプロピオン酸エステル



$C_{28}H_{37}FO_7$: 504.59

9-Fluoro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17,21-dipropionate [5593-20-4]

Betamethasone Dipropionate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{28}H_{37}FO_7$, and not less than 3.4% and not more than 4.1% of fluorine (F:19.00).

Description Betamethasone Dipropionate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water and in hexane.

It is affected gradually by light.

Identification (1) To 1 mL of a solution of Betamethasone Dipropionate in methanol (1 in 10,000) add 4 mL of isoniazid TS, and heat on a water bath for 2 minutes: a yellow color develops.

(2) Proceed with 0.01 g of Betamethasone Dipropionate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution so obtained responds to the Qualitative Tests <1.09> for fluoride.

(3) Determine the absorption spectrum of a solution of Betamethasone Dipropionate in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Betamethasone Dipropionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 176 – 180°C

Optical rotation <2.49> $[\alpha]_D^{20}$: +63 – +70° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Fluoride—To 0.10 g of Betamethasone Dipropionate add 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), shake for 10 minutes, and filter through a membrane filter (0.4- μ m pore size). Place 5.0 mL of the filtrate in a 20-mL volumetric flask, and add 10 mL of

a mixture of alizalin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), add water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, place 1.0 mL of Standard Fluorine Solution in a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), then 10 mL of a mixture of alizalin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), proceed in the same manner as the preparation of the sample solution, and use this solution as the standard solution. Place 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a 20-mL volumetric flask, and proceed in the same manner as the preparation of the sample solution. Using this solution as the blank, determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of the sample solution is not greater than that of the standard solution (not more than 0.012%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Betamethasone Dipropionate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.010 g of Betamethasone Dipropionate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g, platinum crucible).

Assay (1) Betamethasone dipropionate—Weigh accurately about 15 mg of Betamethasone Dipropionate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 239 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{28}H_{37}FO_7 \\ = (A/312) \times 10,000 \end{aligned}$$

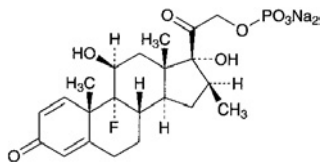
(2) Fluorine—Weigh accurately about 10 mg of Betamethasone Dipropionate, previously dried, and proceed as directed in the procedure of determination for fluorine under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Sodium Phosphate

ベタメタゾンリン酸エステルナトリウム



$C_{22}H_{28}FNa_2O_8P$: 516.40

Disodium 9-fluoro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 21-phosphate [151-73-5]

Betamethasone Sodium Phosphate contains not less than 97.0% and not more than 103.0% of $C_{22}H_{28}FNa_2O_8P$, calculated on the anhydrous basis.

Description Betamethasone Sodium Phosphate occurs as white to pale yellowish white, crystalline powder or masses. It is odorless.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Melting point: about 213°C (with decomposition).

Identification (1) Dissolve 2 mg of Betamethasone Sodium Phosphate in 2 mL of sulfuric acid: a brown color develops, and gradually changes to blackish brown.

(2) Prepare the test solution with 0.01 g of Betamethasone Sodium Phosphate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(3) Take 0.04 g of Betamethasone Sodium Phosphate in a platinum crucible, and carbonize by heating. After cooling, add 5 drops of nitric acid, and incinerate by heating. To the residue add 10 mL of diluted nitric acid (1 in 50), and boil for several minutes. After cooling, neutralize the solution with ammonia TS, filter if necessary, and use this solution as the sample solution. The sample solution responds to the Qualitative Tests <1.09> for sodium salt and for phosphate.

(4) Determine the infrared absorption spectrum of Betamethasone Sodium Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Betamethasone Sodium Phosphate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +99 – +105° (0.1 g, calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Betamethasone Sodium Phosphate in 20 mL of water: the pH of this solution is between 7.5 and 9.0.

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Betamethasone Sodium Phosphate in 10 mL of water: the

solution is clear and colorless.

(2) Free phosphoric acid—Weigh accurately about 20 mg of Betamethasone Sodium Phosphate, dissolve in 20 mL of water, and use this solution as the sample solution. Separately, pipet 4 mL of Standard Phosphoric Acid Solution, add 20 mL of water, and use this solution as the standard solution. To each of the sample solution and the standard solution add exactly 7 mL of dilute sulfuric acid, exactly 2 mL of hexaammonium heptamolybdate-sulfuric acid TS and exactly 2 mL of *p*-methylaminophenol sulfate TS, shake well, and allow to stand at 20 ± 1°C for 15 minutes. To each add water to make exactly 50 mL, and allow to stand at 20 ± 1°C for 15 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 20 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S , of each solution from the sample solution and standard solution at 730 nm: the amount of free phosphoric acid is not more than 0.5 %.

$$\begin{aligned} \text{Amount (\%)} \text{ of free phosphoric acid (H}_3\text{PO}_4) \\ = (A_T/A_S) \times (1/W) \times 10.32 \end{aligned}$$

W : Amount (mg) of Betamethasone Sodium Phosphate, calculated on the anhydrous basis.

(3) Betamethasone—Dissolve 20 mg of Betamethasone Sodium Phosphate in exactly 2 mL of methanol, and use this solution as the sample solution. Separately, dissolve 20 mg of Betamethasone Reference Standard in exactly 10 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a freshly prepared mixture of 1-butanol, water and acetic anhydride (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Water <2.48> Not more than 10.0% (0.2 g, back titration).

Assay Weigh accurately about 20 mg each of Betamethasone Sodium Phosphate and Betamethasone Sodium Phosphate Reference Standard (determine its water content <2.48> before using in the same manner as Betamethasone Sodium Phosphate), and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, and exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of betamethasone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{22}H_{28}FNa_2O_8P = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Betamethasone Sodium Phosphate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of butyl parahydrox-

ybenzoate in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of tetra-*n*-butylammonium bromide, 3.2 g of disodium hydrogen phosphate dodecahydrate and 6.9 g of potassium dihydrogen phosphate in 1000 mL of water, and add 1500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of betamethasone phosphate is about 5 minutes.

System suitability—

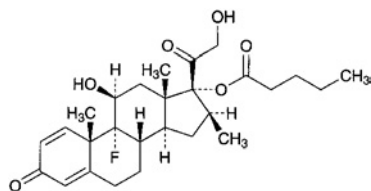
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, betamethasone phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of betamethasone phosphate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone Valerate

ベタメタゾン吉草酸エステル



$C_{27}H_{37}FO_6$: 476.58

9-Fluoro-11 β ,17,21-trihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17-pentanoate [2152-44-5]

Betamethasone Valerate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{27}H_{37}FO_6$.

Description Betamethasone Valerate occurs as a white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 190°C (with decomposition).

Identification (1) Proceed with 0.01 g of Betamethasone Valerate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution so obtained responds to the Qualitative Tests <1.09> for fluoride.

(2) Determine the infrared absorption spectrum of

Betamethasone Valerate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Betamethasone Valerate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +77 – +83° (after drying, 0.1 g, methanol, 20 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight. Dissolve 0.02 g of Betamethasone Valerate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g, platinum crucible).

Assay Dissolve about 10 mg each of Betamethasone Valerate and Betamethasone Valerate Reference Standard, previously dried and accurately weighed, in methanol to make exactly 100 mL. Pipet 10 mL each of these solutions, add 10 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of betamethasone valerate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{27}H_{37}FO_6 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Betamethasone Valerate Reference Standard

Internal standard solution—A solution of isoamyl benzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 10 minutes.

System suitability—

System performance: When the procedure is run with

10 μ L of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Betamethasone Valerate and Gentamicin Sulfate Cream

ベタメタゾン吉草酸エステル・ゲンタマイシン硫酸塩クリーム

Betamethasone Valerate and Gentamicin Sulfate Cream contains not less than 90.0% and not more than 110.0% of the labeled amount of betamethasone valerate ($C_{27}H_{37}FO_6$: 476.58) and not less than 90.0% and not more than 115.0% of the labeled amount of gentamicin $C_1(C_{21}H_{43}N_5O_7$: 477.60).

Method of preparation Prepare as directed under Cream, with Betamethasone Valerate and Gentamicin Sulfate.

Identification (1) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1.2 mg of Betamethasone Valerate according to the labeled amount, add 20 mL of methanol and 20 mL of hexane, shake vigorously for 10 minutes, and allow to stand. Take 15 mL of the lower layer, evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, mix, and use as the sample solution. Separately, dissolve about 18 mg of Betamethasone Valerate Reference Standard in 20 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with ethyl acetate to a distance of about 10 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate, and heat at 100°C: the principal spot with the sample solution and the spot with the standard solution are purple in color, and their R_f values are the same.

(2) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 2 mg (potency) of Gentamicin Sulfate according to the labeled amount, add 20 mL of ethyl acetate and 10 mL of water, shake vigorously for 10 minutes, and centrifuge. To 3 mL of the lower layer add 1 mL of dilute sodium hydroxide TS and 2 mL of ninhydrin TS, and heat in a water bath at 90–95°C for 10 minutes: a purple to dark purple color develops.

pH <2.54> To a quantity of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to 6 mg of Betamethasone Valerate according to the labeled amount, add 15 mL of water, and mix while warming on a water bath to make a milky liquid: the pH of the cooled liquid is between 4.0 and 6.0.

Purity Related substances—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg of Betamethasone Valerate according to the labeled amount, and add 10 mL of a mixture of methanol and water (7:3). Warm in a water bath at 60°C for 5 minutes, and shake vigorously for 20 minutes. Repeat this procedure 2 times. After cooling for 15 minutes with ice, centrifuge for 5 minutes, take away the bubbles from the upper surface, and filter the remaining liquid. Discard first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 150 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate these amounts by the area percentage method: the amount of the substance other than betamethasone valerate is not more than 3.5%, and the total amount of them is not more than 7.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of water, acetonitrile and methanol (12:7:1)

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.

Time span of measurement: About 2.5 times as long as the retention time of betamethasone valerate beginning after the solvent peak. The peaks of the compounding ingredients are not determined.

System suitability—

Test for required detectability: Dissolve 20 mg of Betamethasone Valerate in 100 mL of a mixture of methanol and water (7:3). To exactly 1 mL of this solution add the mixture of methanol and water (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 2.5 mL of the solution add the mixture of methanol and water (7:3) to make exactly 50 mL. Confirm that the peak area of betamethasone valerate obtained with 150 μ L of this solution is equivalent to 3.5 to 6.5% of that with 150 μ L of the solution for system suitability test.

System performance: When the procedure is run with 150 μ L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of betamethasone valerate are not less than 4000 and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 6 times with 150 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of betamethasone valerate is not more than 2.0%.

Assay (1) Betamethasone valerate—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg of betamethasone valerate ($C_{27}H_{37}FO_6$), add 10 mL of a mixture of methanol and water (7:3), and add exactly 10 mL of the internal standard solution. After warming in a water bath at 60°C for 5 minutes, shake vigorously for 20 minutes. Repeat this procedure twice,

cool with ice for 15 minutes, centrifuge for 5 minutes, then filter the supernatant liquid, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of betamethasone valerate to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of betamethasone valerate (C}_{27}\text{H}_{37}\text{FO}_6) \\ &= W_S \times (Q_T/Q_S) \times (1/25) \end{aligned}$$

W_S : Amount (mg) of Betamethasone Valerate Reference Standard

Internal standard solution—Dissolve 20 mg of beclomethasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) **Gentamicin sulfate**—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) **Test organism, agar media for base layer and seed layer, agar medium for transferring test organisms, and standard solutions**—Proceed as directed in the Assay under Gentamicin Sulfate.

(ii) **Sample solutions**—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Cream, equivalent to about 1 mg (potency) of Gentamicin Sulfate, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, previously warmed to about 85°C, and shake well to dissolve. After cooling, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 250 mL to make the high concentration sample solution, which contains 4 μ g (potency) per mL. Pipet a suitable amount of the high concentration sample solution, add 0.1 mol/L phosphate buffer so-

lution, pH 8.0 so that each mL contains 1 μ g (potency), and use this solution as the low concentration sample solution.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Betamethasone Valerate and Gentamicin Sulfate Ointment

ベタメタゾン吉草酸エステル・ゲンタマイシン硫酸塩軟膏

Betamethasone Valerate and Gentamicin Sulfate Ointment contains not less than 95.0% and not more than 110.0% of the labeled amount of betamethasone valerate (C₂₇H₃₇FO₆: 476.58) and not less than 90.0% and not more than 115.0% of the labeled potency of gentamicin C₁ (C₂₁H₄₃N₅O₇: 477.60).

Method of preparation Prepare as directed under Ointment, with Betamethasone Valerate and Gentamicin Sulfate.

Identification (1) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 1.2 mg of Betamethasone Valerate according to the labeled amount, add 20 mL of methanol and 20 mL of hexane, and disperse the ointment with the aid of ultrasonic. Shake vigorously for 5 minutes, centrifuge for 5 minutes, cool for 15 minutes with ice, and take 15 mL of the lower layer. Evaporate the layer to dryness on a water bath under a current of nitrogen. To the residue add 1 mL of ethyl acetate, apply ultrasonic waves, filter, if necessary, and use the filtrate as the sample solution. Separately, dissolve 18 mg of Betamethasone Valerate Reference Standard in 20 mL of ethyl acetate, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with ethyl acetate to a distance of about 10 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate, and heat at 100°C: the principal spot from the sample solution and the spot from the standard solution are purple in color, and their R_f values are the same.

(2) To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 2 mg (potency) of Gentamicin Sulfate according to the labeled amount, add 20 mL of hexane and 10 mL of water, shake vigorously for 10 minutes, and centrifuge. To 3 mL of the lower layer add 1 mL of dilute sodium hydroxide TS and 2 mL of ninhydrin TS, and heat in a water bath at 90 – 95°C for 10 minutes: a red-brown color develops.

pH <2.54> To a quantity of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to 6 mg of Betamethasone Valerate according to the labeled amount, add 15 mL of water, and warm on a water bath to dissolve. After cooling, separate the water layer: the pH of the layer is between 4.0 and 7.0.

Assay (1) Betamethasone valerate—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to about 1 mg of betamethasone valerate (C₂₇H₃₇FO₆), add 10 mL of a mixture of methanol and

water (7:3), and add exactly 10 mL of the internal standard solution. After warming in a water bath at 75°C for 5 minutes, shake vigorously for 10 minutes. Repeat this procedure once more, cool with ice for 15 minutes, filter, discard the first 5 mL of filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Betamethasone Valerate Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, and add the mixture of methanol and water (7:3) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of betamethasone valerate to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of betamethasone valerate (C}_{27}\text{H}_{37}\text{FO}_6) \\ = W_S \times (Q_T/Q_S) \times (1/25) \end{aligned}$$

W_S : Amount (mg) of Betamethasone Valerate Reference Standard

Internal standard solution—Dissolve 20 mg of beclomethasone dipropionate in 10 mL of methanol, and add the mixture of methanol and water (7:3) to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 2.1 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3.5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (13:7).

Flow rate: Adjust the flow rate so that the retention time of betamethasone valerate is about 16 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating conditions, betamethasone valerate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of betamethasone valerate to that of the internal standard is not more than 1.0%.

(2) **Gentamicin sulfate**—Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) **Test organism, agar media for base layer and seed layer, agar medium for transferring test organisms, and standard solutions**—Proceed as directed in the Assay under Gentamicin Sulfate.

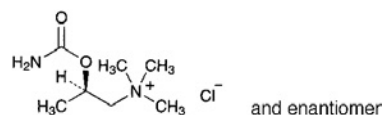
(ii) **Sample solutions**—Weigh accurately an amount of Betamethasone Valerate and Gentamicin Sulfate Ointment, equivalent to about 1 mg (potency) of Gentamicin Sulfate, transfer to a separator, add 50 mL of petroleum ether and exactly 100 mL of 0.1 mol/L phosphate buffer solution, pH 8.0, shake for 10 minutes, and allow to stand. Pipet a suitable amount of the water layer, add 0.1 mol/L phosphate buffer

solution, pH 8.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Bethanechol Chloride

ベタネコール塩化物



$C_7H_{17}ClN_2O_2$: 196.68
(2*RS*)-2-Carbamoyloxy-*N,N,N*-trimethylpropylaminium chloride [590-63-6]

Bethanechol Chloride, when dried, contains not less than 98.0% and not more than 101.0% of $C_7H_{17}ClN_2O_2$.

Description Bethanechol Chloride occurs as colorless or white crystals or a white, crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Bethanechol Chloride (1 in 10) shows no optical rotation.

It is hygroscopic.

Identification (1) To 2 mL of a solution of Bethanechol Chloride (1 in 40) add 0.1 mL of a solution of cobalt (II) chloride hexahydrate (1 in 100), then add 0.1 mL of potassium hexacyanoferrate (II) TS: A green color is produced, and almost entirely fades within 10 minutes.

(2) To 1 mL of a solution of Bethanechol Chloride (1 in 100) add 0.1 mL of iodine TS: a brown precipitate is produced, and the solution shows a greenish brown color.

(3) Determine the infrared absorption spectrum of Bethanechol Chloride as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bethanechol Chloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 217 – 221°C (after drying).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bethanechol Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) **Related substances**—Dissolve 1.0 g of Bethanechol Chloride in 2.5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of

about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate, and allow to stand for 30 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

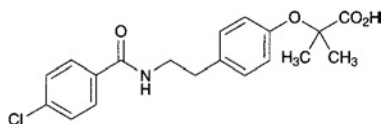
Assay Weigh accurately about 0.4 g of Bethanechol Chloride, previously dried, dissolve in 2 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.67 mg of C₇H₁₇ClN₂O₂

Containers and storage Containers—Tight containers.

Bezafibrate

ベザフィブラート



C₁₉H₂₀ClNO₄: 361.82

2-(4-{2-[(4-Chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid
[41859-67-0]

Bezafibrate, when dried, contains not less than 98.5% and not more than 101.0% of C₁₉H₂₀ClNO₄.

Description Bezafibrate occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Bezafibrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bezafibrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Bezafibrate as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 181 – 186°C

Purity (1) Chloride <1.03>—Dissolve 3.0 g of Bezafibrate in 15 mL of *N,N*-dimethylformamide, add water to make 60 mL, shake well, allow to stand for more than 12 hours, and filter. To 40 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this so-

lution as the test solution. Prepare the control solution as follows: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of *N,N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Bezafibrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Bezafibrate in 35 mL of methanol, add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 70 mL of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks having the relative retention times of about 0.65 and 1.86 with respect to bezafibrate obtained from the sample solution are not larger than 1/2 times the peak area of bezafibrate from the standard solution, the area of the peak other than those and other than bezafibrate from the sample solution is not larger than 1/5 times the peak area of bezafibrate from the standard solution, and the total area of the peaks other than the peak of bezafibrate from the sample solution is not larger than 3/4 times the peak area of bezafibrate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust the flow rate so that the retention time of bezafibrate is about 6 minutes.

Time span of measurement: About 2.5 times as long as the retention time of bezafibrate beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 50) (7:3) to make exactly 50 mL. Confirm that the peak area of bezafibrate obtained with 5 μL of this solution is equivalent to 7 to 13% of that with 5 μL of the standard solution.

System performance: Dissolve 20 mg of Bezafibrate and 10 mg of 4-chlorobenzoate in 70 mL of methanol, and add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, 4-chlorobenzoate and bezafibrate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bezafibrate is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Bezafibrate, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 36.18 mg of C₁₉H₂₀ClNO₄

Containers and storage Containers—Tight containers.

Bezafibrate Sustained Release Tablets

ベザフィブラート徐放錠

Bezafibrate Sustained Release Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of bezafibrate (C₁₉H₂₀ClNO₄; 361.82).

Method of preparation Prepare as directed under Tablets, with Bezafibrate.

Identification Mix well an amount of powdered Bezafibrate Sustained Release Tablets, equivalent to 0.1 g of Bezafibrate according to the labeled amount, with 100 mL of methanol, and filter. To 1 mL of the filtrate and add methanol to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 227 nm and 231 nm.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Bezafibrate Sustained Release Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 as the dissolution medium. Withdraw exactly 20 mL of the dissolution medium 1.5 hours, 2.5 hours and 8 hours after starting the test, and immediately fill up the dissolution medium each time with exactly 20 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 7.2, previously warmed to 37 ± 0.5°C. Filter these media through a membrane filter with a pore size not exceeding 0.45 μm. Discard the first 10 mL of the filtrate, pipet the subsequent *V* mL, add disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 to make exactly *V* mL so that each mL contains about 13 μg of bezafibrate (C₁₉H₂₀ClNO₄) according to the labeled amount, and use these solutions as the sample solutions. Separately, weigh accurately about 66 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add disodium hydrogen phosphate-citric acid buffer solution, pH 7.2 to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, *A*_{T(n)} (*n* = 1, 2, 3) and *A*_S, of the sample solutions and standard solution at 228 nm as directed under

Ultraviolet-visible Spectrophotometry <2.24>: the dissolution rates of a 100-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 35 – 65% and not less than 80%, respectively, and those of a 200-mg tablet in 1.5 hours, in 2.5 hours and in 8 hours are 15 – 45%, 30 – 60% and not less than 75 %, respectively.

Dissolution rate (%) in each case of *n* with respect to the labeled amount of bezafibrate (C₁₉H₂₀ClNO₄)

$$= W_S \times \left[\frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1} \left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45} \right) \right] \times \frac{V'}{V} \times \frac{1}{C} \times 18$$

*W*_S: Amount (mg) of bezafibrate for assay

C: Labeled amount (mg) of bezafibrate (C₁₉H₂₀ClNO₄) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Bezafibrate Sustained Release Tablets. Weigh accurately a portion of the powder, equivalent to about 20 mg of bezafibrate (C₁₉H₂₀ClNO₄), add 60 mL of methanol and exactly 10 mL of the internal standard solution, and shake for 20 minutes. Add diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of bezafibrate for assay, previously dried at 105°C for 3 hours, dissolve in 60 mL of methanol, add exactly 10 mL of the internal standard solution and diluted 0.5 mol/L ammonium acetate TS (1 in 50) to make 100 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q*_T and *Q*_S, of the peak area of bezafibrate to that of the internal standard.

Amount (mg) of bezafibrate (C₁₉H₂₀ClNO₄) = *W*_S × (*Q*_T/*Q*_S)

*W*_S: Amount (mg) of bezafibrate for assay

Internal standard solution—A solution of 4-nitrophenol in methanol (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (1 in 100) (9:4).

Flow rate: Adjust the flow rate so that the retention time of bezafibrate is about 6 minutes.

System suitability—

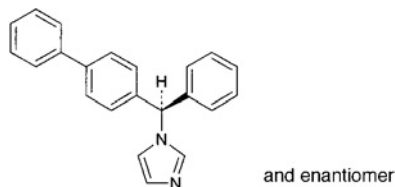
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and bezafibrate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bezafibrate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bifonazole

ビホナゾール



$C_{22}H_{18}N_2$: 310.39

1-[(*RS*)-(Biphenyl-4-yl)(phenyl)methyl]-1*H*-imidazole
[60628-96-8]

Bifonazole, when dried, contains not less than 98.5% of $C_{22}H_{18}N_2$.

Description Bifonazole occurs as a white to pale yellow powder. It is odorless and tasteless.

It is freely soluble in dichloromethane, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Bifonazole in methanol (1 in 100) does not show optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Bifonazole in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bifonazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 147 – 151°C

Purity (1) Chloride <1.03>—To 2.0 g of Bifonazole add 40 mL of water, warm for 5 minutes, and after cooling, filter. To 10 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) Sulfate <1.14>—To 10 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bifonazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bifonazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 25 mL and 5 mL of this solution, add methanol to make exactly 50

mL each, and use these solutions as the standard solutions (1) and (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (49:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot with *R_f* value of about 0.20 from the sample solution is not more intense than the spot from the standard solution (1). And the spots other than the spot mentioned above and the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

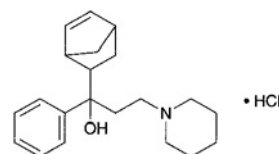
Assay Weigh accurately about 0.15 g of Bifonazole, previously dried, and dissolve in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution in a glass-stoppered conical flask, add 10 mL of water, 5 mL of dilute sulfuric acid and 25 mL of dichloromethane, and add 2 to 3 drops of a solution of methyl yellow in dichloromethane (1 in 500) as indicator, and titrate <2.50>, while shaking vigorously, with 0.01 mol/L sodium lauryl sulfate VS by a buret with 0.02-mL minimum graduation. The end point is reached when the color of the dichloromethane layer changes from yellow to orange-red after dropwise addition of 0.01 mol/L sodium lauryl sulfate VS, strong shaking, and standing for a while.

Each mL of 0.01 mol/L sodium lauryl sulfate VS
= 3.104 mg of $C_{22}H_{18}N_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Biperiden Hydrochloride

ビペリデン塩酸塩



$C_{21}H_{29}NO \cdot HCl$: 347.92

1-(Bicyclo[2.2.1]hept-5-en-2-yl)-1-phenyl-3-(piperidin-1-yl)propan-1-ol monohydrochloride [1235-82-1]

Biperiden Hydrochloride, when dried, contains not less than 99.0% of $C_{21}H_{29}NO \cdot HCl$.

Description Biperiden Hydrochloride occurs as a white to brownish and yellowish white, crystalline powder.

It is freely soluble in formic acid, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 270°C (with decomposition).

Identification (1) Dissolve 0.02 g of Biperiden Hydrochloride in 5 mL of phosphoric acid: a green color develops.

(2) Dissolve 0.01 g of Biperiden Hydrochloride in 5 mL of water by heating, cool, and add 5 to 6 drops of bromine TS: a yellow precipitate is formed.

(3) Determine the absorption spectrum of a solution of Biperiden Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Biperiden Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) Dissolve 0.02 g of Biperiden Hydrochloride in 10 mL of water by heating, and cool: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Acidity or alkalinity—To 1.0 g of Biperiden Hydrochloride add 50 mL of water, shake vigorously, filter, and to 20 mL of the filtrate add 1 drop of methyl red TS: no red to yellow color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Biperiden Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Biperiden Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Biperiden Hydrochloride in 20 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (80:15:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Biperiden Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

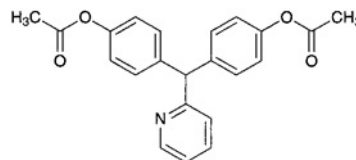
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 34.79 \text{ mg of } C_{21}H_{29}NO.HCl \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bisacodyl

ビスアコジル



$C_{22}H_{19}NO_4$: 361.39

4,4'-(Pyridin-2-ylmethylene)bis(phenyl acetate) [603-50-9]

Bisacodyl, when dried, contains not less than 98.5% of $C_{22}H_{19}NO_4$.

Description Bisacodyl occurs as a white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectrum of a solution of Bisacodyl in ethanol (95) (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Bisacodyl Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bisacodyl, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Bisacodyl Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 132 – 136°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Bisacodyl in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.012%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Bisacodyl in 2 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.35 mL of 0.005 mol/L sulfuric acid VS add 2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.017%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bisacodyl according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.20 g of Bisacodyl in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solu-

tion. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, chloroform and xylene (1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bisacodyl, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-yellow to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of $\text{C}_{22}\text{H}_{19}\text{NO}_4$

Containers and storage Containers—Well-closed containers.

Bisacodyl Suppositories

ビサコジル坐剤

Bisacodyl Suppositories contain not less than 90% and not more than 110% of the labeled amount of bisacodyl ($\text{C}_{22}\text{H}_{19}\text{NO}_4$; 361.39).

Method of preparation Prepare as directed under Suppositories, with Bisacodyl.

Identification (1) To a quantity of Bisacodyl Suppositories, equivalent to 6 mg of Bisacodyl according to the labeled amount, add 20 mL of ethanol (95), warm on a water bath for 10 minutes, shake vigorously for 10 minutes, and allow to stand in ice water for 1 hour. Centrifuge the solution, filter the supernatant liquid, and to 2 mL of the filtrate add ethanol (95) to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 6 mg of Bisacodyl Reference Standard in 20 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-butanone, chloroform and xylene (1:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R_f* value.

Assay Weigh accurately not less than 20 Bisacodyl Suppositories, make them fine fragments carefully, and mix

uniformly. Weigh accurately a portion of the fragments, equivalent to about 10 mg of bisacodyl ($\text{C}_{22}\text{H}_{19}\text{NO}_4$), add 40 mL of tetrahydrofuran, warm to 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and add the mobile phase to make 100 mL. Cool this solution in ice for 30 minutes, centrifuge, filter the supernatant liquid through a membrane filter with pore size of 0.5 μm , discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Bisacodyl Reference Standard, previously dried at 105°C for 2 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of bisacodyl to that of the internal standard, respectively.

Amount (mg) of bisacodyl ($\text{C}_{22}\text{H}_{19}\text{NO}_4$) = $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of Bisacodyl Reference Standard

Internal standard solution—A solution of ethyl parahydroxybenzoate in acetonitrile (3 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L citric acid TS, acetonitrile and methanol (2:1:1).

Flow rate: Adjust the flow rate so that the retention time of bisacodyl is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the internal standard and bisacodyl are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of bisacodyl to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bismuth Subgallate

Dermatol

次没食子酸ビスマス

Bismuth Subgallate, when dried, contains not less than 47.0% and not more than 51.0% of bismuth (Bi; 208.98).

Description Bismuth Subgallate occurs as a yellow powder.

It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid, in dilute nitric acid and in dilute sulfuric acid on warming. It dissolves in sodium hydroxide TS, forming a clear, yellow solution, which turns red immediately.

It is affected by light.

Identification (1) Ignite 0.5 g of Bismuth Subgallate: it chars at first, and leaves finally a yellow residue. The residue responds to the Qualitative Tests <1.09> for bismuth salt.

(2) To 0.5 g of Bismuth Subgallate add 25 mL of water and 20 mL of hydrogen sulfide TS, and shake well. Filter off the blackish brown precipitate, and add 1 drop of iron (III) chloride TS to the filtrate: a blue-black color is produced.

Purity (1) Clarity of solution—Dissolve 1.0 g of Bismuth Subgallate in 40 mL of diluted sodium hydroxide TS (1 in 8): the solution is clear.

(2) Sulfate—Ignite 3.0 g of Bismuth Subgallate in a porcelain crucible, and cautiously dissolve the residue in 2.5 mL of nitric acid by warming. Pour the solution into 100 mL of water, shake, and filter. Evaporate 50 mL of the filtrate on a water bath to 15 mL. Add water to make 20 mL, filter again, and use the filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Nitrate—To 0.5 g of Bismuth Subgallate add 5 mL of dilute sulfuric acid and 25 mL of iron (II) sulfate TS, shake well, and filter. Superimpose carefully 5 mL of the filtrate on sulfuric acid: no red-brown color develops at the zone of contact.

(4) Ammonium—Dissolve 1.0 g of Bismuth Subgallate in 5 mL of sodium hydroxide TS, and heat: the gas evolved does not change moistened red litmus paper to blue.

(5) Copper—To 5 mL of the sample solution obtained in (2) add 1 mL of ammonia TS, and filter: no blue color develops in the filtrate.

(6) Lead—Ignite 1.0 g of Bismuth Subgallate at about 500°C in a porcelain crucible, dissolve the residue in a smallest possible amount of nitric acid added dropwise, evaporate over a low flame to dryness, and cool. Add 5 mL of a solution of potassium hydroxide (1 in 6) to the residue, boil carefully for 2 minutes, cool, and centrifuge. Take the supernatant liquid in a test tube, add 10 drops of potassium chromate TS, and acidify the solution by adding acetic acid (100) dropwise: neither turbidity nor a yellow precipitate is produced.

(7) Silver—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(8) Alkaline earth metals and alkali metals—Boil 1.0 g of Bismuth Subgallate with 40 mL of diluted acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter the precipitate produced, and wash with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, and evaporate to dryness. Ignite as directed under Residue on Ignition <2.44>: the mass of the residue does not more than 5.0 mg.

(9) Arsenic <1.11>—Mix well 0.20 g of Bismuth Subgallate with 0.20 g of calcium hydroxide, and ignite the mixture.

Dissolve the residue in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 10 ppm).

(10) Gallic acid—To 1.0 g of Bismuth Subgallate add 20 mL of ethanol (95), shake for 1 minute, and filter. Evaporate the filtrate on a water bath to dryness: the mass of the residue does not more than 5.0 mg.

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Bismuth Subgallate, previously dried, ignite at about 500°C for 30 minutes, and cool. Dissolve the residue in 5 mL of diluted nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Measure exactly 30 mL of this solution, add 200 mL of water, and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 2 to 3 drops of xylenol orange TS).

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.180 mg of Bi

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bismuth Subnitrate

次硝酸ビスマス

Bismuth Subnitrate, when dried, contains not less than 71.5% and not more than 74.5% of bismuth (Bi: 208.98).

Description Bismuth Subnitrate occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It readily dissolves in hydrochloric acid and in nitric acid without effervescence.

It is slightly hygroscopic, and changes moistened blue litmus paper to red.

Identification Bismuth Subnitrate responds to the Qualitative Tests <1.09> for bismuth salt and nitrate.

Purity (1) Chloride <1.03>—Dissolve 0.7 g of Bismuth Subnitrate in 2 mL of water and 2 mL of nitric acid, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of nitric acid on a water bath to dryness, add 0.70 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Sulfate—Dissolve 3.0 g of Bismuth Subnitrate in 3.0 mL of warmed nitric acid, pour this solution into 100 mL of water, shake, and filter. Concentrate the filtrate on a water bath to 30 mL, filter, and use this filtrate as the sample solution. To 5 mL of the sample solution add 2 to 3 drops of barium nitrate TS: no turbidity is produced.

(3) Ammonium—Boil 0.10 g of bismuth Subnitrate with 5 mL of sodium hydroxide TS: the gas evolved does not

change moistened red litmus paper to blue.

(4) **Copper**—To 5 mL of the sample solution obtained in (2) add 2 mL of ammonia TS, and filter: no blue color develops.

(5) **Lead**—To 1.0 g of Bismuth Subnitrate add 5 mL of a solution of sodium hydroxide (1 in 6), boil carefully for 2 minutes, cool and centrifuge. Transfer the supernatant liquid to a test tube, add 10 drops of potassium chromate TS, and add dropwise acetic acid (31) to render the solution acid: no turbidity or yellow precipitate is produced.

(6) **Silver**—To 5 mL of the sample solution obtained in (2) add 0.5 mL of nitric acid and 2 to 3 drops of dilute hydrochloric acid: no turbidity is produced.

(7) **Alkaline earth metals and alkali metals**—Boil 2.0 g of Bismuth Subnitrate with 40 mL of diluted acetic acid (31) (1 in 2) for 2 minutes, cool, add water to make 40 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute hydrochloric acid, boil, immediately pass hydrogen sulfide thoroughly through the solution, filter, and wash the residue with water. Combine the filtrate and the washings, add 5 drops of sulfuric acid, evaporate to dryness, and ignite as directed under Residue on Ignition <2.44>: the residue does not exceed 5.0 mg

(8) **Arsenic** <1.11>—To 0.20 g of Bismuth Subnitrate add 2 mL of sulfuric acid, heat until white fumes evolve, dilute cautiously with water to 5 mL, use this solution as the test solution, and perform the test (not more than 10 ppm).

Loss on drying <2.41> Not more than 3.0% (2 g, 105°C, 2 hours).

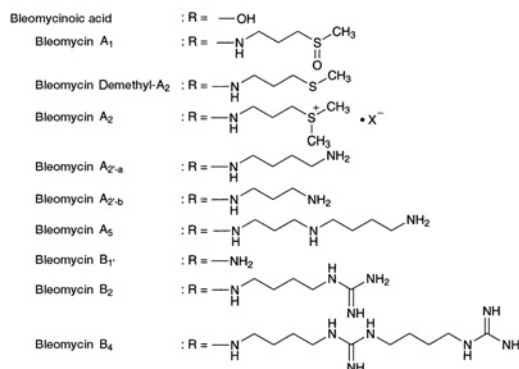
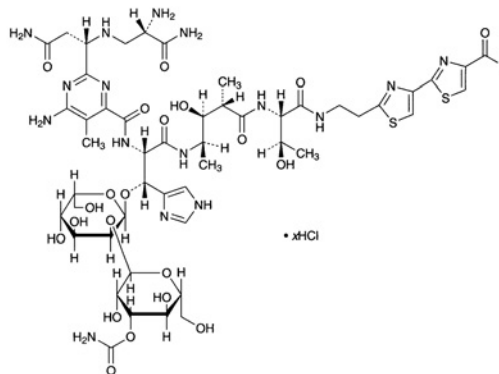
Assay Weigh accurately about 0.4 g of Bismuth Subnitrate, previously dried, dissolve in 5 mL of diluted nitric acid (2 in 5) by warming, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 200 mL of water and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to yellow (indicator: 5 drops of xylenol orange TS)

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.180 mg of Bi

Containers and storage Containers—Well-closed containers.

Bleomycin Hydrochloride

ブレオマイシン塩酸塩



Bleomycinoic Acid

1-Bleomycinoic acid hydrochloride

Bleomycin A₁

*N*¹-[3-(Methylsulfinyl)propyl]bleomycinamide hydrochloride

Bleomycin Demethyl-A₂
*N*¹-[3-(Methylsulfonyl)propyl]bleomycinamide hydrochloride

Bleomycin A₂

*N*¹-[3-(Dimethylsulfonio)propyl]bleomycinamide hydrochloride

Bleomycin A_{2'-a}

*N*¹-(4-Aminobutyl)bleomycinamide hydrochloride

Bleomycin A_{2'-b}

*N*¹-(3-Aminopropyl)bleomycinamide hydrochloride

Bleomycin A₅

*N*¹-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide hydrochloride

Bleomycin B_{1'}

Bleomycinamide hydrochloride

Bleomycin B₂

*N*¹-(4-Guanidinobutyl)bleomycinamide hydrochloride

Bleomycin B₄

*N*¹-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide hydrochloride

[11056-06-7, Bleomycin]

Bleomycin Hydrochloride is the hydrochloride of a

mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on the dried basis. The potency of Bleomycin Hydrochloride is expressed as mass (potency) of bleomycin A₂ (C₅₅H₈₄ClN₁₇O₂₁S₃; 1451.00).

Description Bleomycin Hydrochloride occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 4 mg of Bleomycin Hydrochloride add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Bleomycin Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Hydrochloride in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A₂ (the first principal peak) is between 55% and 70%, that of bleomycin B₂ (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A₂ and bleomycin B₂ is not less than 85%, the peak area of demethylbleomycin A₂ (a peak having the relative retention time of 1.5 – 2.5 to bleomycin A₂) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solu-

tion and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL per minute.

Time span of measurement: 20 minutes after elution of the peak of demethylbleomycin A₂ beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A₂ and bleomycin B₂ are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A₂ is not more than 2.0%.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Hydrochloride in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Hydrochloride in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: Copper hollow-cathode lamp

Wavelength: 324.8 nm

Loss on drying <2.41> Not more than 5.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iii) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the components, and sterilize. Adjust the pH after sterilization to 6.9 - 7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in the Preparation of cylinder-agar plate under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

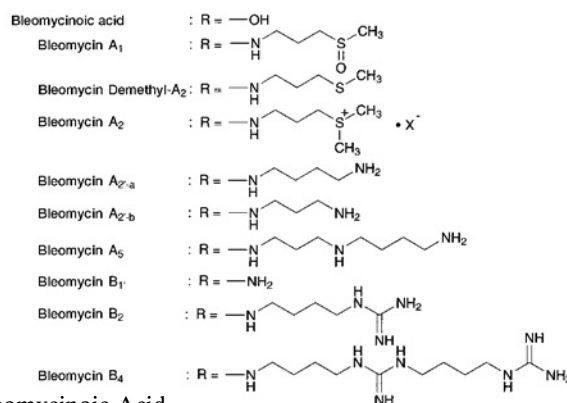
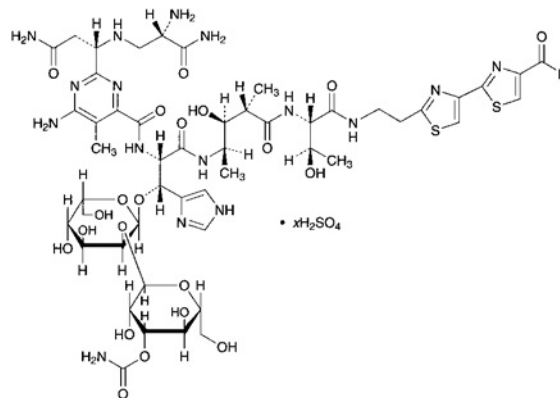
(vi) Standard solutions—Weigh accurately an amount of Bleomycin A₂ Hydrochloride Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Hydrochloride, equivalent to about 15 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 μg (potency) and 15 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Bleomycin Sulfate

ブレオマイシン硫酸塩



Bleomycinoic Acid

1-Bleomycinoic acid sulfate

Bleomycin A₁

*N*¹-[3-(Methylsulfinyl)propyl]bleomycinamide sulfate

Bleomycin Demethyl-A₂

*N*¹-[3-(Methylsulfonyl)propyl]bleomycinamide sulfate

Bleomycin A₂

*N*¹-[3-(Dimethylsulfonium)propyl]bleomycinamide sulfate

Bleomycin A_{27-a}

*N*¹-(4-Aminobutyl)bleomycinamide sulfate

Bleomycin A_{27-b}

*N*¹-(3-Aminopropyl)bleomycinamide sulfate

Bleomycin A₅

*N*¹-{3-[(4-Aminobutyl)amino]propyl}bleomycinamide sulfate

Bleomycin B₁'

Bleomycinamide sulfate

Bleomycin B₂

*N*¹-(4-Guanidinobutyl)bleomycinamide sulfate

Bleomycin B₄

*N*¹-{4-[3-(4-Guanidinobutyl)guanidino]butyl}-bleomycinamide sulfate

[9041-93-4, Bleomycin Sulfate]

Bleomycin Sulfate is the sulfate of a mixture of substances having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 1400 μg (potency) and not more than 2000 μg (potency) per mg, calculated on the dried basis. The potency of Bleomycin Sulfate is expressed as mass (potency) of bleomycin A₂ (C₅₅H₈₄ClN₁₇O₂₁S₃: 1451.00).

Description Bleomycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 4 mg of Bleomycin Sulfate add 5 μL of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bleomycin Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bleomycin Sulfate (1 in 200) responds to the Qualitative Tests <1.09> (1) and (2) for sulfate.

pH <2.54> The pH of a solution obtained by dissolving 10 mg of Bleomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Content ratio of the active principle Dissolve 10 mg of Bleomycin Sulfate in 20 mL of water, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of bleomycin A₂ (the first principal peak) is between 55% and 70%, that of bleomycin B₂ (the second principal peak) is between 25% and 32%, the total peak area of bleomycin A₂ and bleomycin B₂ is not less than 85%, the peak area of demethylbleomycin A₂ (a peak having the relative retention time of 1.5 – 2.5 against bleomycin A₂) is not more than 5.5%, and the total area of the rest peaks is not more than 9.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of the mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of the mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following

table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: About 1.2 mL/min

Time span of measurement: Twenty minutes after elution of the peak of demethylbleomycin A₂ beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, bleomycin A₂ and bleomycin B₂ are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of bleomycin A₂ is not more than 2.0%.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 80 mg of Bleomycin Sulfate in 4 mL of water is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Bleomycin Sulfate in 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to exactly 15 mL of Standard Copper Solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: Copper hollow-cathode lamp

Wavelength: 324.8 nm

Loss on drying <2.41> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Take the sample to be tested while avoiding moisture absorption.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC 607

(ii) Agar medium for seed, base layer and transferring the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9 – 7.1 with sodium hydroxide TS.

(iii) Liquid media for suspending the test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g

Water 1000 mL

Mix all the components and sterilize. Adjust the pH after sterilization to 6.9–7.1 with sodium hydroxide TS.

(iv) Preparation of seeded agar layer—Cultivate the test organism on the slant of the agar medium for transferring the test organism at 27°C for 40 to 48 hours, then inoculate the test organism thus obtained in 100 mL of the liquid media for suspending the test organism, cultivate with shaking at between 25°C and 27°C for 5 days, and use this as the suspension of test organism. Store the suspension of test organism at a temperature not exceeding 5°C, and use within 14 days. Add 0.5 mL of the suspension of test organism in 100 mL of the agar medium for seed previously kept at 48°C, mix thoroughly, and use as the seeded agar layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 7. Preparation of cylinder-agar plate under the Microbial Assay for Antibiotics, dispensing 5.0 mL of agar medium for base layer and 8.0 mL of the agar medium for seed into the Petri dish.

(vi) Standard solutions—Weigh accurately an amount of Bleomycin A₂ Hydrochloride Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at an ordinary temperature for 3 hours, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 µg (potency) and 15 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Bleomycin Sulfate, equivalent to about 15 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 30 µg (potency) and 15 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Boric Acid

ホウ酸

H₃BO₃: 61.83

Boric Acid, when dried, contains not less than 99.5% of H₃BO₃.

Description Boric Acid occurs as colorless or white crystals or crystalline powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in warm water, in hot ethanol (95) and in glycerin, soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Boric Acid (1 in 20) is between 3.5 and 4.1.

Identification A solution of Boric Acid (1 in 20) responds to the Qualitative Tests <1.09> for borate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Boric Acid in 25 mL of water or in 10 mL of hot ethanol (95): the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Boric Acid according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of Boric Acid according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 0.5% (2 g, silica gel, 5 hours).

Assay Weigh accurately about 1.5 g of Boric Acid, previously dried, add 15 g of D-sorbitol and 50 mL of water, and dissolve by warming. After cooling, titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 61.83 mg of H₃BO₃

Containers and storage Containers—Well-closed containers.

Freeze-dried Botulism Antitoxin, Equine

乾燥ボツリヌスウマ抗毒素

Freeze-dried Botulism Antitoxin, Equine, is a preparation for injection which is dissolved before use.

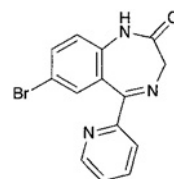
It contains botulism antitoxin type A, botulism antitoxin type B, botulism antitoxin type E and botulism antitoxin type F in immunoglobulin of horse origin. It may contain one, two or three of these four antitoxins.

It conforms to the requirements of Freeze-dried Botulism Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Botulism Antitoxin, Equine, becomes a colorless or yellow-brown, clear liquid or a slightly white-turbid liquid on the addition of solvent.

Bromazepam

ブロマゼパム



C₁₄H₁₀BrN₃O: 316.15
7-Bromo-5-(pyridin-2-yl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one [1812-30-2]

Bromazepam, when dried, contains not less than 99.0% and not more than 101.0% of $C_{14}H_{10}BrN_3O$.

Description Bromazepam occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, in ethanol (99.5) and in acetone, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Bromazepam in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bromazepam in a platinum crucible according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Bromazepam in 5 mL of a mixture of acetone and methanol (3:2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mixture of acetone and methanol (3:2) to make exactly 50 mL. Pipet 5 mL of this solution, add the mixture of acetone and methanol (3:2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and ethanol (99.5) (38:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution and the spot of the starting point are not more than 2, and not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

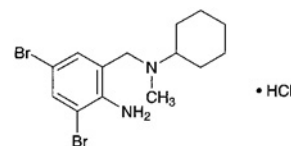
Assay Weigh accurately about 0.4 g of Bromazepam, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.62 mg of $C_{14}H_{10}BrN_3O$

Containers and storage Containers—Well-closed containers.

Bromhexine Hydrochloride

ブロムヘキシン塩酸塩



$C_{14}H_{20}Br_2N_2 \cdot HCl$: 412.59

2-Amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzylamine monohydrochloride [611-75-6]

Bromhexine Hydrochloride, when dried, contains not less than 98.5% of $C_{14}H_{20}Br_2N_2 \cdot HCl$.

Description Bromhexine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in formic acid, sparingly soluble in methanol, and slightly soluble in water and in ethanol (95).

The pH of its saturated solution is between 3.0 and 5.0.

Melting point: about 239°C (with decomposition).

Identification (1) Dissolve 3 mg of Bromhexine Hydrochloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Bromhexine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Infrared Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Add 20 mL of water to 1 g of Bromhexine Hydrochloride. After thorough shaking, add 3 mL of sodium hydroxide TS, and extract with four 20-mL portions of diethyl ether. Neutralize the water layer with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Bromhexine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Bromhexine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than bromhexine is not larger than the peak area of bromhexine of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of potassium dihydrogen phosphate in 900 mL of water, adjust the pH to 7.0 with 0.5 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 200 mL of this solution add 800 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of bromhexine is about 6 minutes.

Selection of column: To 0.05 g of bamethane sulfate add 0.5 mL of the sample solution, and add the mobile phase to make 10 mL. Proceed with 5 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of bamethane and bromhexine in this order with the resolution between these peaks being not less than 7.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bromhexine from 5 μ L of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 2 times as long as the retention time of bromhexine beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

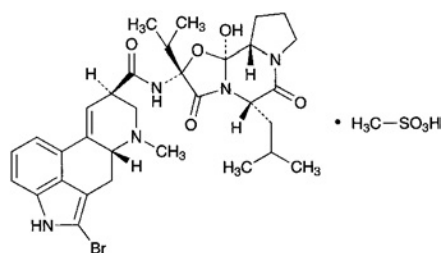
Assay Weigh accurately about 0.5 g of Bromhexine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and warm in a water bath at 50°C for 15 minutes. After cooling, titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.26 mg of C₁₄H₂₀Br₂N₂·HCl

Containers and storage Containers—Well-closed containers.
Storage—Light-resistant.

Bromocriptine Mesilate

ブロモクリプチンメシル酸塩



C₃₂H₄₀BrN₅O₅·CH₄O₃S: 750.70
(5'S)-2-Bromo-12'-hydroxy-2'-(1-methylethyl)-5'-

(2-methylpropyl)ergotaman-3',6',18-trione
monomethanesulfonate [22260-51-1]

Bromocriptine Mesilate contains not less than 98.0% of C₃₂H₄₀BrN₅O₅·CH₄O₃S, calculated on the dried basis.

Description Bromocriptine Mesilate occurs as a white to pale yellowish white or pale brownish white, crystalline powder. It is odorless, or has a faint characteristic odor.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in acetic anhydride, in dichloromethane and in chloroform, and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification (1) Dissolve 2 mg of Bromocriptine Mesilate in 1 mL of methanol, add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a purplish blue color develops.

(2) Determine the absorption spectrum of a solution of Bromocriptine Mesilate in methanol (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bromocriptine Mesilate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Bromocriptine Mesilate as directed under Flame Coloration Test <1.04> (2): a green color appears.

Optical rotation <2.49> [α]_D²⁰: +95 – +105° [0.1 g, calculated on the dried basis, a mixture of methanol and dichloromethane (1:1), 10 mL, 100 mm].

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of methanol: the solution is clear, and has no more color than the following control solution.

Control solution: To 2.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 1.0 mL of Copper (II) Sulfate Colorimetric Stock Solution add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Bromocriptine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bromocriptine Mesilate in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution

and standard solutions (1) and (2), as a band with 1 cm in width, on a plate of silica gel for thin-layer chromatography. Develop the plate immediately with a mixture of dichloromethane, 1,4-dioxane, ethanol (95) and ammonia solution (28) (1800:150:50:1) to a distance of about 10 cm, and dry the plate under reduced pressure for 30 minutes. Spray evenly Dragendorff's TS for spraying on the plate, then spray evenly hydrogen peroxide TS, cover the plate with a glass plate, and examine: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the spot other than the principal spot, which is more intense than the spot from the standard solution (2), is not more than one.

Loss on drying <2.41> Not more than 3.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 80°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Bromocriptine Mesilate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

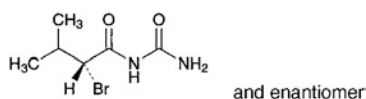
Each mL of 0.1 mol/L perchloric acid VS
= 75.07 mg of $C_{32}H_{40}BrN_5O_5 \cdot CH_4O_3S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding -18°C.

Bromovalerylurea

ブロモバレリル尿素



$C_6H_{11}BrN_2O_2$: 223.07

(*2RS*)-(2-Bromo-3-methylbutanoyl)urea [496-67-3]

Bromovalerylurea, when dried, contains not less than 98.0% of $C_6H_{11}BrN_2O_2$.

Description Bromovalerylurea occurs as colorless or white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sulfuric acid, in nitric acid and in hydrochloric acid, and precipitates are produced on the addition of water.

It dissolves in sodium hydroxide TS.

Identification (1) Boil 0.2 g of Bromovalerylurea with 5 mL of a solution of sodium hydroxide (1 in 10): the gas evolved changes moistened red litmus paper to blue. Boil this solution with an excess of dilute sulfuric acid: the odor of valeric acid is perceptible.

(2) To 0.1 g of Bromovalerylurea add 0.5 g of anhydrous sodium carbonate, and decompose thoroughly by gentle heating. Dissolve the residue in 5 mL of hot water, cool, acidify with acetic acid (31), and filter: the filtrate responds to the Qualitative Tests <1.09> (2) for bromide.

Melting point <2.60> 151 - 155°C

Purity (1) Acidity or alkalinity—To 1.5 g of Bromovalerylurea add 30 mL of water, shake for 5 minutes, and filter: the filtrate is neutral.

(2) Chloride <1.03>—Perform the test with a 10-mL portion of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate <1.14>—Perform the test with 10 mL of the filtrate obtained in (1). Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Bromovalerylurea according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Dissolve 0.5 g of Bromovalerylurea in 5 mL of sodium hydroxide TS, use this solution as the test solution, and perform the test (not more than 4 ppm).

(6) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Bromovalerylurea: the solution is not more colored than Matching Fluid A.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

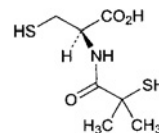
Assay Weigh accurately about 0.4 g of Bromovalerylurea, previously dried, in a 300-mL conical flask, add 40 mL of sodium hydroxide TS, and boil gently for 20 minutes under a reflux condenser. Cool, wash the lower part of the reflux condenser and the mouth of the flask with 30 mL of water, and combine the washings with the solution in the conical flask. Add 5 mL of nitric acid and exactly 30 mL of 0.1 mol/L silver nitrate VS, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 22.31 mg of $C_6H_{11}BrN_2O_2$

Containers and storage Containers—Well-closed containers.

Bucillamine

ブシラミン



$C_7H_{13}NO_3S_2$: 223.31

(*2R*)-2-(2-Methyl-2-sulfanylpropanoylamino)-3-sulfanylpropanoic acid
[65002-17-7]

Bucillamine, when dried, contains not less than 98.5% and not more than 101.0% of $C_7H_{13}NO_3S_2$.

Description Bucillamine occurs as white, crystals or crystal-

line powder.

It is freely soluble in methanol and in ethanol (95), and slightly soluble in water.

Identification (1) To 5 mL of a solution of Bucillamine (1 in 250) add 2 mL of sodium hydroxide TS and 2 drops of sodium pentacyanonitrosylferrate (III) TS: the solution reveals a red-purple color.

(2) Determine the infrared absorption spectrum of Bucillamine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +33.0 – +36.5° (after drying, 2 g, ethanol (95), 50 mL, 100 mm).

Melting point <2.60> 136 – 140°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bucillamine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bucillamine according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 60 mg of Bucillamine in 20 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mixture of water and methanol (1:1) to make exactly 200 mL, and use this solution as the standard solution. Immediately perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of related substances, having the relative retention time of about 2.3 and 3.1 with respect to the peak of bucillamine, obtained from the sample solution are not larger than 8/15 times and 2/5 times the peak area of bucillamine from the standard solution, respectively, and the area of the peak other than the peaks of bucillamine and two of the related substances mentioned above from the sample solution is not larger than 1/5 times the peak area of bucillamine from the standard solution. The total area of the peaks other than bucillamine from the sample solution is not larger than the peak area of bucillamine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.01 mol/L citric acid TS and methanol (1:1).

Flow rate: Adjust the flow rate so that the retention time of bucillamine is about 5 minutes.

Time span of measurement: About 7 times as long as the retention time of bucillamine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the stan-

dard solution add the mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of bucillamine obtained with 20 μ L of this solution is equivalent to 7 to 13% of that obtained with 20 μ L of the standard solution.

System performance: Dissolve 0.10 g of bucillamine and 10 mg of 4-fluorobenzoic acid in 100 mL of methanol. To 10 mL of this solution add water to make exactly 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, bucillamine and 4-fluorobenzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of bucillamine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

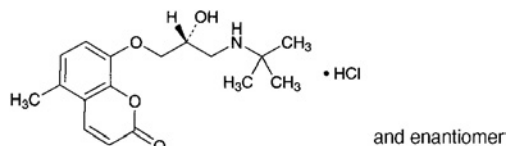
Assay Weigh accurately about 0.25 g of Bucillamine, dissolve in 35 mL of methanol, add 15 mL of water, and titrate <2.50> with 0.05 mol/L iodine VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 11.17 mg of C₇H₁₃NO₃S₂

Containers and storage Containers—Tight containers.

Bucumolol Hydrochloride

ブクモロール塩酸塩



C₁₇H₂₃NO₄.HCl: 341.83

8-[(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-5-methylchromen-2-one monohydrochloride [36556-75-9]

Bucumolol Hydrochloride, when dried, contains not less than 99.0% of C₁₇H₂₃NO₄.HCl.

Description Bucumolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in methanol and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in diethyl ether.

Melting point: about 228°C (with decomposition).

Identification (1) Dissolve 0.01 g of Bucumolol Hydrochloride in 10 mL of diluted ethanol (95) (1 in 2), and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Render this solution alkaline by adding sodium hydroxide TS: the fluorescence disappears. Acidify the solution by adding dilute hydrochloric acid: the fluorescence reappears.

(2) Dissolve 0.1 g of Bucumolol Hydrochloride in 5 mL

of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Bucumolol Hydrochloride (1 in 60,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Bucumolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Bucumolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (296 nm): 330 – 360 (after drying, 40 mg, water, 2500 mL).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Bucumolol Hydrochloride in 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Bucumolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bucumolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Bucumolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia-ammonium chloride buffer solution, pH 10.7, (30:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

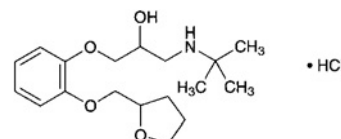
Assay Weigh accurately about 0.4 g of Bucumolol Hydrochloride, previously dried, add 45 mL of acetic acid (100), dissolve by warming at 60°C, and cool. Add 105 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 34.18 \text{ mg of } \text{C}_{17}\text{H}_{23}\text{NO}_4\cdot\text{HCl} \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Bufetolol Hydrochloride

ブフェトロール塩酸塩



$\text{C}_{18}\text{H}_{29}\text{NO}_4\cdot\text{HCl}$; 359.89

1-(1,1-Dimethylethyl)amino-3-[2-(tetrahydrofuran-2-ylmethoxy)phenoxy]propan-2-ol monohydrochloride
[35108-88-4]

Bufetolol Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_{18}\text{H}_{29}\text{NO}_4\cdot\text{HCl}$.

Description Bufetolol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Bufetolol Hydrochloride (1 in 10) is optically inactive.

Identification (1) To 5 mL of a solution of Bufetolol Hydrochloride (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Bufetolol Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bufetolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bufetolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 153 – 157°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Bufetolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Bufetolol Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bufetolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.20 g of Bufetolol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot

10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone, ethanol (95) and ammonia solution (28) (40:20:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

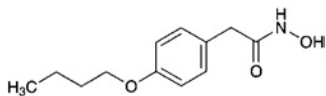
Assay Weigh accurately about 0.4 g of Bufetolol Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.99 mg of $C_{18}H_{29}NO_4 \cdot HCl$

Containers and storage Containers—Tight containers.

Bufexamac

ブフェキサマク



$C_{12}H_{17}NO_3$: 223.27

2-(4-Butyloxyphenyl)-*N*-hydroxy acetamide [2438-72-4]

Bufexamac, when dried, contains not less than 98.0% of $C_{12}H_{17}NO_3$.

Description Bufexamac occurs as white to pale yellowish white crystals or crystalline powder. It has a faint, characteristic odor, and is tasteless.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Melting point: about 162°C (with decomposition).

Identification (1) To 5 mL of a solution of Bufexamac in methanol (1 in 5000) add 1 drop of iron (III) chloride-methanol TS, and shake: a dark red color develops.

(2) Determine the absorption spectrum of a solution of Bufexamac in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bufexamac as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Bufexamac in 20 mL of ethanol (95): the solution is clear

and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Bufexamac according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bufexamac according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Bufexamac in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Use a plate of silica gel with fluorescent indicator for thin-layer chromatography, moisten the surface of the plate evenly by spraying with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and dry at 110°C for about 30 minutes. Spot 15 μ L each of the sample solution and standard solution on the plate. Develop the plate with a mixture of chloroform, cyclohexane, methanol and acetic acid (100) (6:4:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Bufexamac, previously dried, dissolve in 40 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide-methanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide-methanol VS
= 22.33 mg of $C_{12}H_{17}NO_3$

Containers and storage Containers—Tight containers.

Bufexamac Cream

ブフェキサマククリーム

Bufexamac Cream contains not less than 90% and not more than 110% of the labeled amount of bufexamac ($C_{12}H_{17}NO_3$: 223.27).

Method of preparation Prepared as directed under Ointments, with Bufexamac.

Description Bufexamac Cream is white.
pH: 4.0 – 6.0

Identification To a quantity of Bufexamac Cream, equivalent to 0.05 g of Bufexamac according to the labeled amount, add 10 mL of tetrahydrofuran, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.05 g of bufexamac for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Use a plate of silica gel for thin-layer

chromatography, moisten the surface of the plate evenly by spraying with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and dry the plate at 110°C for about 30 minutes. Spot 5 μ L each of the sample solution and standard solution on the plate. Develop the plate with a mixture of pentane, ethyl acetate and acetic acid (100) (7:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: the spot from the sample solution and that from the standard solution show a red-brown color and the same Rf value.

Assay Weigh accurately a quantity of Bufexamac Cream, equivalent to about 50 mg of bufexamac ($C_{12}H_{17}NO_3$), dissolve in 40 mL of methanol, and add methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and add the mobile phase to make 100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of bufexamac for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of bufexamac to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of bufexamac (C}_{12}\text{H}_{17}\text{NO}_3\text{)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of bufexamac for assay

Internal standard solution—A solution of diphenylimidazole in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of sodium 1-octane sulfonate and 0.6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 850 mL of water, and add 400 mL of methanol, 400 mL of acetonitrile and 8 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of bufexamac is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, bufexamac and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bufexamac to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bufexamac Ointment

ブフェキサマク軟膏

Bufexamac Ointment contains not less than 90% and not more than 110% of the labeled amount of bufexamac ($C_{12}H_{17}NO_3$; 223.27).

Method of preparation Prepare as directed under Ointments, with Bufexamac.

Identification To a quantity of Bufexamac Ointment, equivalent to 0.05 g of Bufexamac according to the labeled amount, add 5 mL of tetrahydrofuran, shake well, add 5 mL of ethanol (99.5), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.05 g of bufexamac for assay in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Use a plate of silica gel for thin-layer chromatography, moisten the surface of the plate evenly by spraying with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and dry the plate at 110°C for about 30 minutes. Spot 5 μ L each of the sample solution and standard solution on the plate. Develop the plate with a mixture of pentane, ethyl acetate and acetic acid (100) (7:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: the spot from the sample solution and that from the standard solution show a red-brown color and the same Rf value.

Assay Weigh accurately a quantity of Bufexamac Ointment, equivalent to about 50 mg of bufexamac ($C_{12}H_{17}NO_3$), add 40 mL of tetrahydrofuran, warm to 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and filter, if necessary, through a membrane filter of 0.45- μ m porosity. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of bufexamac for assay, previously dried at 105°C for 4 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of bufexamac to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of bufexamac (C}_{12}\text{H}_{17}\text{NO}_3\text{)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of bufexamac for assay

Internal standard solution—A solution of diphenylimidazole in methanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle di-

ameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of sodium 1-octane sulfonate and 0.6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 850 mL of water, and add 400 mL of methanol, 400 mL of acetonitrile and 8 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of bumetanide is about 6 minutes.

System suitability—

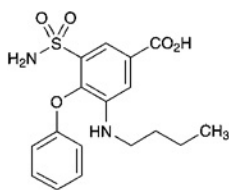
System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, bumetanide and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 µL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of bumetanide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Bumetanide

ブメタニド



$C_{17}H_{20}N_2O_5S$: 364.42

3-Butylamino-4-phenoxy-5-sulfamoylbenzoic acid
[28395-03-1]

Bumetanide, when dried, contains not less than 98.5% of $C_{17}H_{20}N_2O_5S$.

Description Bumetanide occurs as white crystals or crystalline powder.

It is freely soluble in pyridine, soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in potassium hydroxide TS.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Bumetanide in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, shake, add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light blue color develops in the chloroform layer.

(2) Dissolve 0.04 g of Bumetanide in 100 mL of phosphate buffer solution, pH 7.0, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bumetanide, previously dried, as directed in the potassium

bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 232 – 237°C

Purity (1) Clarity and color of solution—Dissolve 50 mg of Bumetanide in 2 mL of a solution of potassium hydroxide (1 in 30) and 8 mL of water: the solution is clear, and is not more colored than the following control solution.

Control solution: Pipet 0.5 mL each of Cobalt (II) Chloride Colorimetric Stock Solution, Iron (III) Chloride Colorimetric Stock Solution and Copper (II) Sulfate Colorimetric Stock Solution, mix them, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Chloride <1.03>—Mix well 0.5 g of Bumetanide with 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, transfer, in small portions, to a red-hot platinum crucible, and heat to red-hot until the reaction is complete. After cooling, to the residue add 14 mL of dilute sulfuric acid and 6 mL of water, boil for 5 minutes, filter, wash the residue with 10 mL of water, combine the filtrate and the washing, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Bumetanide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bumetanide according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Bumetanide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetic acid (100), cyclohexane and methanol (32:4:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Bumetanide, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

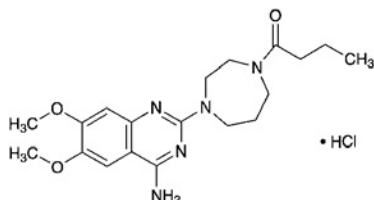
Each mL of 0.1 mol/L sodium hydroxide VS
= 36.44 mg of $C_{17}H_{20}N_2O_5S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Bunazosin Hydrochloride

ブナゾシン塩酸塩



$C_{19}H_{27}N_5O_3 \cdot HCl$: 409.91

4-Amino-2-(4-butanoyl-1,4-diazepan-1-yl)-6,7-dimethoxyquinazoline monohydrochloride [72712-76-2]

Bunazosin Hydrochloride, when dried, contains not less than 98.0% of $C_{19}H_{27}N_5O_3 \cdot HCl$.

Description Bunazosin Hydrochloride occurs as a white crystalline powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Melting point: about 273°C (with decomposition).

Identification (1) Dissolve 0.1 g of Bunazosin Hydrochloride in 10 mL of 0.2 mol/L hydrochloric acid TS, and boil for 3 minutes over a flame: butyric acid like odor is perceptible.

(2) Determine the infrared absorption spectrum of Bunazosin Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Bunazosin Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Bunazosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Bunazosin Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of bunazosin from the sample solution is not larger than the peak area of bunazosin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in parti-

cle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.44 g of sodium lauryl sulfate in a suitable amount of water, add 10 mL of acetic acid (100), 500 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of bunazosin is about 5 minutes.

Selection of column: Proceed with 20 μ L of a mixture of the standard solution and a solution of procaine hydrochloride in the mobile phase (1 in 20,000) (1:1) under the above operating conditions, and calculate the resolution. Use a column giving elution of procaine and bunazosin in this order with the resolution between these peaks being not less than 3.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of bunazosin obtained from 20 μ L of the standard solution is 20 to 60% of the full-scale.

Time span of measurement: About 6 times of the retention time of bunazosin.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Bunazosin Hydrochloride, previously dried, dissolve in 6 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid, and heat for 20 minutes on a water bath. After cooling, add 20 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

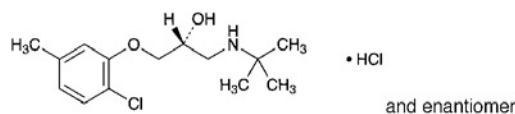
Each mL of 0.1 mol/L perchloric acid VS
= 40.99 mg of $C_{19}H_{27}N_5O_3 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Bupranolol Hydrochloride

ブプラノロール塩酸塩



$C_{14}H_{22}ClNO_2 \cdot HCl$: 308.24

(2*RS*)-3-(2-Chloro-5-methylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol monohydrochloride [15148-80-8]

Bupranolol Hydrochloride, when dried, contains not less than 98.0% of $C_{14}H_{22}ClNO_2 \cdot HCl$.

Description Bupranolol Hydrochloride occurs as a white, crystalline powder.

It is sparingly soluble in methanol, slightly soluble in water, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Bupranolol Hydrochloride (1 in

1000) is between 5.2 and 6.2.

Identification (1) Take 0.01 g of Bupranolol Hydrochloride in a test tube, mix with 25 mg of potassium iodide and 25 mg of oxalic acid dihydrate, cover the mouth of the test tube with filter paper moistened with a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 100), and heat gently for several minutes. Expose the filter paper to ammonia gas: the filter paper acquires a blue color.

(2) Determine the absorption spectrum of a solution of Bupranolol Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Bupranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Bupranolol Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (275 nm): 57 – 60 (after drying, 50 mg, 0.1 mol/L hydrochloric acid TS, 500 mL).

Melting point <2.60> 223 – 226°C

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Bupranolol Hydrochloride in 15 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.10 g of Bupranolol Hydrochloride in 15 mL of freshly boiled and cooled water, and add 1 drop of methyl red TS: a light red color develops. To this solution add 0.05 mL of 0.01 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Sulfate <1.14>—Perform the test with 0.10 g of Bupranolol Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.168%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Bupranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Bupranolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.30 g of Bupranolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of polyamide with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and water (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

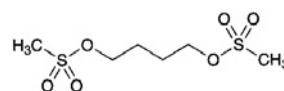
Assay Weigh accurately about 0.18 g of Bupranolol Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.82 mg of $\text{C}_{14}\text{H}_{22}\text{ClNO}_2 \cdot \text{HCl}$

Containers and storage Containers—Well-closed containers.

Busulfan

ブスルファン



$\text{C}_6\text{H}_{14}\text{O}_6\text{S}_2$: 246.30

Tetramethylenedimethanesulfonate [55-98-1]

Busulfan contains not less than 98.5% of $\text{C}_6\text{H}_{14}\text{O}_6\text{S}_2$, calculated on the dried basis.

Description Busulfan occurs as a white, crystalline powder.

It is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Identification (1) To 0.1 g of Busulfan add 10 mL of water and 5 mL of sodium hydroxide TS, dissolve by heating, and use this solution as the sample solution.

(i) To 7 mL of the sample solution add 1 drop of potassium permanganate TS: the red-purple color of potassium permanganate TS changes from blue-purple through blue to green.

(ii) Acidify 7 mL of the sample solution with dilute sulfuric acid, and add 1 drop of potassium permanganate TS: the color of potassium permanganate TS remains.

(2) Determine the infrared absorption spectrum of Busulfan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 115 – 118°C

Purity (1) Sulfate <1.14>—To 1.0 g of Busulfan add 40 mL of water, and dissolve by heating. Cool in ice for 15 minutes, and filter. Wash the residue with 5 mL of water, combine the washings with the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Busulfan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum,

phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Busulfan, add 40 mL of water, and boil gently under a reflux condenser for 30 minutes. Cool, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

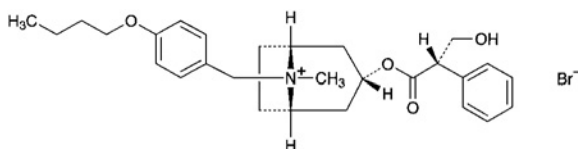
Each mL of 0.1 mol/L sodium hydroxide VS
= 12.32 mg of C₆H₁₄O₆S₂

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Butropium Bromide

ブトロピウム臭化物



C₂₈H₃₈BrNO₄: 532.51

(1*R*,3*r*,5*S*)-8-(4-Butoxybenzyl)-3-[(2*S*)-hydroxy-2-phenylpropanoyloxy]-8-methyl-8-azoniabicyclo[3.2.1]octane bromide [29025-14-7]

Butropium Bromide, when dried, contains not less than 98.0% of C₂₈H₃₈BrNO₄.

Description Butropium Bromide occurs as white crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in methanol, soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether and in acetic anhydride.

Identification (1) To 1 mg of Butropium Bromide add 3 drops of fuming nitric acid, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Butropium Bromide in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Butropium Bromide in methanol (1 in 20) responds to the Qualitative Tests <1.09> (1) for bromide.

Optical rotation <2.49> [α]_D²⁰: -14.0 - -17.0° (after drying, 0.5 g, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Butropium Bromide in 40 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test, using this solution as the test solution. Prepare the control solution

with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Butropium Bromide in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the peak area having a ratio of the retention time about 0.5 to butropium from the sample solution is not larger than 1/4 of the peak area from the standard solution, and the total area of all peaks other than the peak eluted first, the peak having a ratio of the retention time to butropium about 0.5 and butropium peak from the sample solution is not larger than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.15 g of sodium lauryl sulfate in 1000 mL of a mixture of acetonitrile and 0.005 mol/L sulfuric acid (3:2).

Flow rate: Adjust the flow rate so that the retention time of butropium is about 5 minutes.

Selection of column: Dissolve 0.50 g of Butropium Bromide in 9 mL of ethanol (99.5) and 1 mL of 0.1 mol/L potassium hydroxide-ethanol TS, and heat at 70°C for 15 minutes. After cooling, to 1 mL of this solution add the mobile phase to make 100 mL. Proceed with 5 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the peak of butropium and the peak having a ratio of the retention time about 0.7 to butropium with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the butropium obtained from 5 μ L of the standard solution is between 10 mm and 30 mm.

Time span of measurement: About twice as long as the retention time of butropium.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.8 g of Butropium Bromide, previously dried, dissolve in 5 mL of formic acid, add 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

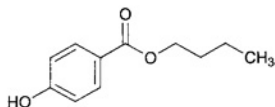
Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 53.25 mg of C₂₈H₃₈BrNO₄

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Butyl Parahydroxybenzoate

パラオキシ安息香酸ブチル



$C_{11}H_{14}O_3$: 194.23
Butyl 4-hydroxybenzoate [94-26-8]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Butyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of $C_{11}H_{14}O_3$.

♦**Description** Butyl Parahydroxybenzoate occurs as colorless crystals or white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and practically insoluble in water. ◆

Identification (1) The melting point <2.60> of Butyl Parahydroxybenzoate is between 68°C and 71°C.

♦(2) Determine the infrared absorption spectrum of Butyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. ◆

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 10 mL of ethanol (95); the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Butyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

♦(3) Heavy metals <1.07>—Dissolve 1.0 g of Butyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm). ◆

(4) Related substances—Dissolve 0.10 g of Butyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of

methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Butyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 194.2 mg of $C_{11}H_{14}O_3$

♦**Containers and storage** Containers—Well-closed containers. ◆

Cacao Butter

Oleum Cacao

カカオ脂

Cacao Butter is the fat obtained from the seed of *Theobroma cacao* Linné (*Sterculiaceae*).

Description Cacao Butter occurs as a yellowish white, hard, brittle mass. It has a slight, chocolate-like odor, and has no odor of rancidity.

It is freely soluble in diethyl ether and in petroleum ether, soluble in boiling ethanol (99.5), and very slightly soluble in ethanol (95).

Congeeing point of the fatty acids: 45 – 50°C

Melting point 31–35°C (Cram the sample into a capillary tube without melting the sample).

Specific gravity <1.13> d_{20}^{40} : 0.895 – 0.904

Acid value <1.13> Not more than 3.0.

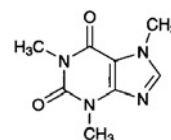
Saponification value <1.13> 188 – 195

Iodine value <1.13> 35 – 43

Containers and storage Containers—Well-closed containers.

Anhydrous Caffeine

無水カフェイン



$C_8H_{10}N_4O_2$: 194.19
1,3,7-Trimethyl-1*H*-purine-2,6-(3*H*,7*H*)-dione
[58-08-2]

Anhydrous Caffeine, when dried, contains not less

than 98.5% of $C_8H_{10}N_4O_2$.

Description Anhydrous Caffeine occurs as white crystals or powder. It is odorless, and has a bitter taste.

It is freely soluble in chloroform, sparingly soluble in water, in acetic anhydride and in acetic acid (100), and slightly soluble in ethanol (95) and in diethyl ether.

The pH of a solution of Anhydrous Caffeine (1 in 100) is between 5.5 and 6.5

Identification (1) To 2 mL of a solution of Anhydrous Caffeine (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Anhydrous Caffeine add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Anhydrous Caffeine in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting point <2.60> 235 – 238°C

Purity (1) Chloride <1.03>—Dissolve 2.0 g of Anhydrous Caffeine in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate <1.14>—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Anhydrous Caffeine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Anhydrous Caffeine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances <1.15>—Perform the test using 0.5 g of Anhydrous Caffeine: the solution is not

more colored than Matching Fluid D.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

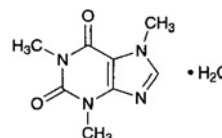
Assay Weigh accurately about 0.4 g of Anhydrous Caffeine, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.42 mg of $C_8H_{10}N_4O_2$

Containers and storage Containers—Tight containers.

Caffeine Hydrate

カフェイン水和物



$C_8H_{10}N_4O_2 \cdot H_2O$: 212.21
1,3,7-Trimethyl-1*H*-purine-2,6-(3*H*,7*H*)-dione
monohydrate [5743-12-4]

Caffeine Hydrate, when dried, contains not less than 98.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

Description Caffeine Hydrate occurs as white, soft crystals or powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, sparingly soluble in water, in acetic acid (100) and in acetic anhydride, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

The pH of a solution of Caffeine Hydrate (1 in 100) is between 5.5 and 6.5.

It effloresces in dry air.

Identification (1) To 2 mL of a solution of Caffeine Hydrate (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(2) To 0.01 g of Caffeine Hydrate add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(3) Dissolve 0.01 g of Caffeine Hydrate in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

Melting point <2.60> 235 – 238°C (after drying).

Purity (1) Chloride <1.03>—Dissolve 2.0 g of Caffeine Hydrate in 80 mL of hot water, cool rapidly to 20°C, add water to make 100 mL, and use this solution as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(2) Sulfate <1.14>—To 40 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Caffeine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Caffeine Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(5) Readily carbonizable substances <1.15>—Perform the test using 0.5 g of Caffeine Hydrate: the solution is not more colored than Matching Fluid D.

Loss on drying <2.41> 0.5 – 8.5% (1 g, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Caffeine Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the solution changes from purple through green to yellow (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.42 mg of C₈H₁₀N₄O₂

Containers and storage Containers—Tight containers.

Caffeine and Sodium Benzoate

安息香酸ナトリウムカフェイン

Caffeine and Sodium Benzoate, when dried, contains not less than 48.0% and not more than 50.0% of caffeine (C₈H₁₀N₄O₂: 194.19), and not less than 50.0% and not more than 52.0% of sodium benzoate (C₇H₅NaO₂: 144.10).

Description Caffeine and Sodium Benzoate occurs as a

white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, soluble in acetic acid (100) and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 1 g of Caffeine and Sodium Benzoate in 10 mL of water in a separator, add 1 drop of phenolphthalein TS, and add carefully 0.01 mol/L sodium hydroxide VS dropwise until a faint red color develops. Extract with three 20-mL portions of chloroform by thorough shaking, and separate the chloroform layer from the water layer. [Use the water layer for test (2).] Filter the combined chloroform extracts, evaporate the filtrate to dryness on a water bath, and proceed the following tests with the residue:

(i) To 2 mL of a solution of the residue (1 in 500) add tannic acid TS dropwise: a white precipitate, which dissolves upon the dropwise addition of tannic acid TS, is produced.

(ii) To 0.01 g of the residue add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, evaporate to dryness on a water bath: the residue acquires a yellow-red color. Invert the residue over a vessel containing 2 to 3 drops of ammonia TS: the color turns red-purple, and disappears upon the addition of 2 to 3 drops of sodium hydroxide TS.

(iii) Dissolve 0.01 g of the residue in water to make 50 mL. To 5 mL of this solution add 3 mL of diluted acetic acid (31) (3 in 100) and 5 mL of a solution of pyridine (1 in 10), mix, add 2 mL of diluted sodium hypochlorite TS (1 in 5), and allow to stand for 1 minute. Add 2 mL of sodium thiosulfate TS and 5 mL of sodium hydroxide TS to the solution: a yellow color develops.

(2) To 5 mL of the water layer obtained in (1) add 5 mL of water: the solution responds to the Qualitative Tests <1.09> (2) for benzoate.

(3) Heat Caffeine and Sodium Benzoate: white fumes are evolved. Ignite furthermore, and to the residue add hydrochloric acid: bubbles are produced, and the solution responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 20 mL of water, and add 1 or 2 drops of phenolphthalein TS: no red color develops.

(3) Chloride <1.03>—Dissolve 0.5 g of Caffeine and Sodium Benzoate in 10 mL of water, and add 30 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95) and water to make 50 mL (not more than 0.050%).

(4) Chlorinated compounds—Dissolve 1.0 g of Caffeine and Sodium Benzoate in 40 mL of water, add 10 mL of dilute sulfuric acid, and extract with two 20-mL portions of diethyl ether. Allow the combined diethyl ether extracts to evaporate at room temperature to dryness. Place this residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite at about 600°C, dissolve the residue in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, and add water to make 50 mL. To this solution add 0.5 mL of silver nitrate TS: the solution is not more turbid than the following control solution to which 0.5 mL of silver nitrate TS has been added.

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, and add 1.2 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL.

(5) Heavy metals <1.07>—Dissolve 2.0 g of Caffeine and Sodium Benzoate in 47 mL of water, add slowly, with vigorous stirring, 3 mL of dilute hydrochloric acid, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL of the filtrate with ammonia TS, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Caffeine and Sodium Benzoate according to Method 1, and perform the test (not more than 2 ppm).

(7) Phthalic acid—To 0.10 g of Caffeine and Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

(8) Readily carbonizable substances <1.15>—Proceed with 0.5 g of Caffeine and Sodium Benzoate, and perform the test: the solution is not more colored than Matching Fluid A.

Loss on drying <2.41> Not more than 3.0% (2 g, 80°C, 4 hours).

Assay (1) Sodium benzoate—Weigh accurately about 0.2 g of Caffeine and Sodium Benzoate, previously dried, dissolve by warming in 50 mL of a mixture of acetic anhydride and acetic acid for nonaqueous titration (6:1), cool, and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS to the first equivalence point (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 14.41 mg of C₇H₅NaO₂

(2) Caffeine—Continue the titration <2.50> in (1) with 0.1 mol/L perchloric acid-dioxane VS from the first equivalence point to the second equivalence point (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 19.42 mg of C₈H₁₀N₄O₂

Containers and storage Containers—Well-closed containers.

Precipitated Calcium Carbonate

沈降炭酸カルシウム

CaCO₃: 100.09

Precipitated Calcium Carbonate, when dried, contains not less than 98.5% of calcium carbonate (CaCO₃).

Description Precipitated Calcium Carbonate occurs as a white, fine crystalline powder. It is odorless and tasteless.

It is practically insoluble in water, but its solubility in water is increased in the presence of carbon dioxide.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves with effervescence in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid.

Identification (1) Dissolve 0.5 g of Precipitated Calcium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

(2) Precipitated Calcium Carbonate responds to the Qualitative Tests <1.09> (1) for carbonate.

Purity (1) Acid-insoluble substances—To 5.0 g of Precipitated calcium Carbonate add 50 mL of water, then add 20 mL of hydrochloric acid dropwise with stirring, boil for 5 minutes, cool, add water to make 200 mL, and filter through filter paper for quantitative analysis. Wash the residue until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 10.0 mg.

(2) Heavy metals <1.07>—Mix 2.0 g of Precipitated Calcium Carbonate with 5 mL of water, add slowly 6 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Barium—Mix 1.0 g of Precipitated Calcium Carbonate with 10 mL of water, add dropwise 4 mL of hydrochloric acid with stirring, boil for 5 minutes, cool, add water to make 40 mL, and filter. With the filtrate, perform the test as directed under Flame Coloration Test <1.04> (1): no green color appears.

(4) Magnesium and alkali metals—Dissolve 1.0 g of Precipitated Calcium Carbonate in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS, and add ammonium oxalate TS until precipitation of calcium oxalate is completed. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake well, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue is not more than 5.0 mg.

(5) Arsenic <1.11>—Moisten 0.40 g of Precipitated Calcium Carbonate with 1 mL of water, then dissolve in 4 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 180°C, 4 hours).

Assay Weigh accurately about 0.12 g of Precipitated Calcium Carbonate, previously dried, and dissolve in 20 mL of water and 3 mL of dilute hydrochloric acid. Add 80 mL of water, 15 mL of a solution of potassium hydroxide (1 in 10) and 0.05 g of NN indicator, and titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 5.004 mg of CaCO₃

Containers and storage Containers—Tight containers.

Calcium Chloride Hydrate

塩化カルシウム水和物

CaCl₂·2H₂O: 147.01

Calcium Chloride Hydrate contains not less than 96.7% and not more than 103.3% of CaCl₂·2H₂O.

Description Calcium Chloride Hydrate occurs as white granules or masses. It is odorless.

It is very soluble in water, and soluble in ethanol (95), and practically insoluble in diethyl ether.

It is deliquescent.

Identification A solution of Calcium Chloride Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for calcium salt and for chloride.

pH <2.54> The pH of a solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of freshly boiled and cooled water is between 4.5 and 9.2.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Calcium Chloride Hydrate in 20 mL of water is clear and colorless.

(2) Sulfate <1.14>—Take 1.0 g of Calcium Chloride Hydrate, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Hypochlorite—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 to 3 drops of dilute hydrochloric acid and 2 to 3 drops of zinc iodide-starch TS: no blue color develops immediately.

(4) Heavy metals <1.07>—Proceed with 2.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Iron, aluminum or phosphate—Dissolve, in a Nessler tube, 1.0 g of Calcium Chloride Hydrate in 20 mL of water and 1 drop of dilute hydrochloric acid, boil, then cool, add 3 drops of ammonia TS, and heat the solution to boil: no turbidity or precipitate is produced.

idity or precipitate is produced.

(6) Barium—Dissolve 0.5 g of Calcium Chloride Hydrate in 5 mL of water, add 2 drops of dilute hydrochloric acid and 2 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(7) Arsenic <1.11>—Prepare the test solution with 1.0 g of Calcium Chloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.4 g of Calcium Chloride Hydrate, and dissolve in water to make exactly 200 mL. Measure exactly 20 mL of this solution, add 40 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate <2.50> immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.940 mg of CaCl₂·2H₂O

Containers and storage Containers—Tight containers.

Calcium Chloride Injection

塩化カルシウム注射液

Calcium Chloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of calcium chloride (CaCl₂: 110.98).

The concentration of Calcium Chloride Injection is expressed as the quantity of calcium chloride (CaCl₂).

Method of preparation Prepare as directed under Injection, with Calcium Chloride Hydrate.

Description Calcium Chloride Injection is a clear, colorless liquid.

Identification Calcium Chloride Injection responds to the Qualitative Tests <1.09> for calcium salt and for chloride.

pH <2.54> 4.5 – 7.5

Bacterial endotoxins <4.01> Less than 0.30 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Calcium Chloride Injection, equivalent to about 0.4 g of calcium chloride (CaCl₂), and proceed as directed in the Assay under Calcium Chloride Hydrate.

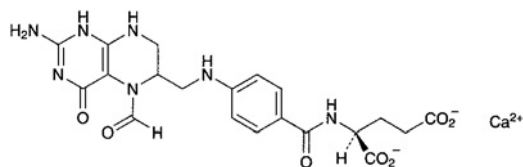
Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.220 mg of CaCl₂

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Calcium Folate

Calcium Leucovorin

ホリナートカルシウム



$C_{20}H_{21}CaN_7O_7$: 511.50

Monocalcium *N*-{4-[(2-amino-5-formyl-4-oxo-1,4,5,6,7,8-hexahydropteridin-6-yl)methylamino]benzoyl}-*L*-glutamate [1492-18-8]

Calcium Folate contains not less than 95.0% and not more than 102.0% of $C_{20}H_{21}CaN_7O_7$, calculated on the anhydrous basis.

Description Calcium Folate occurs as a light yellow to yellow powder. It is odorless and tasteless.

It is very soluble in water, freely soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Calcium Folate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Calcium Folate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Calcium Folate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Calcium Folate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Calcium Folate (1 in 100) responds to the Qualitative Tests <1.09> (2), (3) and (4) for calcium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Calcium Folate in 10 mL of water: the solution is clear and yellow.

(2) Heavy metals <1.07>—Proceed with 0.40 g of Calcium Folate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 50 ppm).

Water <2.48> Weigh accurately about 0.2 g of Calcium Folate in a dried titration flask, and dissolve in 25 mL of acetic acid (100). Add 10.0 mL of Standard Water-Methanol Solution, titrate with Karl Fischer TS to the end point and perform the test: it is not more than 17.0%. Perform a blank determination, and make any necessary correction.

Assay Weigh accurately about 20 mg of Calcium Folate, dissolve in the mobile phase to make exactly 100 mL, and use

this solution as the sample solution. Separately, weigh accurately about 17.5 mg of Calcium Folate Reference Standard, calculated on the anhydrous basis, dissolve in the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of folinate in each solution.

$$\begin{aligned} \text{Amount (mg) of } C_{20}H_{21}CaN_7O_7 \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Calcium Folate Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: To 860 mL of water add 100 mL of acetonitrile and 15 mL of tetrabutylammonium hydroxide-methanol TS, adjust the pH to 7.5 with 2 mol/L sodium dihydrogenphosphate TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of folinate is about 10 minutes.

Selection of column: Dissolve 17.5 mg of folic acid in 100 mL of the mobile phase, and to 5 mL of this solution add 20 mL of the standard solution. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of folinate and folic acid in this order with the resolution of these peaks being not less than 3.6.

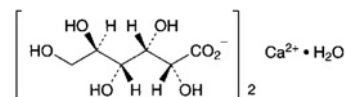
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of each peak area of folinate is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Calcium Gluconate Hydrate

グルコン酸カルシウム水和物



$C_{12}H_{22}CaO_{14} \cdot H_2O$: 448.39

Monocalcium di-D-gluconate monohydrate [299-28-5]

Calcium Gluconate Hydrate, when dried, contains not less than 99.0% and not more than 104.0% of $C_{12}H_{22}CaO_{14} \cdot H_2O$.

Description Calcium Gluconate Hydrate occurs as a white, crystalline powder or granules.

It is soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To separately 10 mg each of Calcium Gluconate Hydrate and calcium gluconate for thin-layer chromatography add 1 mL of water, dissolve by warming, and use these solutions as the sample solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), water, ammonia solution (28) and ethyl acetate (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and heat the plate at 110°C for 20 minutes. After cooling, spray evenly hexaammonium heptamolybdate-cerium (IV) sulfate TS on the plate, air-dry, and heat at 110°C for 10 minutes: the spots with the sample solution and the standard solution are the same in the R_f value and color tone.

(2) A solution of Calcium Gluconate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +6 – +11° (after drying, 0.5 g, water, warming, after cooling, 25 mL, 100 mL).

pH <2.54> Dissolve 1.0 g of Calcium Gluconate Hydrate in 20 mL of water by warming: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Calcium Gluconate Hydrate in 50 mL of water by warming: the solution is clear and colorless.

(2) Chloride <1.03>—Take 0.40 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Sulfate <1.14>—Take 1.0 g of Calcium Gluconate Hydrate, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048 %).

(4) Heavy metals <1.07>—Dissolve 1.0 g of Calcium Gluconate Hydrate in 30 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 0.6 g of Calcium Gluconate Hydrate in 5 mL of water by warming, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, and concentrate on a water bath to 5 mL. Perform the test with this solution as the test solution (not more than 3.3 ppm).

(6) Sucrose and reducing sugars—To 0.5 g of Calcium Gluconate Hydrate add 10 mL of water and 2 mL of dilute hydrochloric acid, and boil the solution for 2 minutes. After cooling, add 5 mL of sodium carbonate TS, allow to stand for 5 minutes, add water to make 20 mL, and filter. To 5 mL of the filtrate add 2 mL of Fehling's TS, and boil for 1 minute: no orange-yellow to red precipitate is formed immediately.

Loss on drying <2.41> Not more than 1.0% (1 g, 80°C, 2 hours).

Assay Weigh accurately about 0.4 g of Calcium Gluconate Hydrate, previously dried, dissolve in 100 mL of water, add 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN in-

dicator, and titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 22.42 mg of $\text{C}_{12}\text{H}_{22}\text{CaO}_{14}\cdot\text{H}_2\text{O}$

Containers and storage Containers—Well-closed containers.

Calcium Hydroxide

Slaked Lime

水酸化カルシウム

$\text{Ca}(\text{OH})_2$: 74.09

Calcium Hydroxide contains not less than 90.0% of $\text{Ca}(\text{OH})_2$.

Description Calcium Hydroxide occurs as a white powder. It has a slightly bitter taste.

It is slightly soluble in water, very slightly soluble in boiling water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid, in dilute hydrochloric acid and in dilute nitric acid.

It absorbs carbon dioxide from air.

Identification (1) Mix Calcium Hydroxide with 3 to 4 times its mass of water: the mixture is slushy and is alkaline.

(2) Dissolve 1 g of Calcium Hydroxide in 30 mL of dilute acetic acid, and boil. After cooling, neutralize with ammonia TS: the solution responds to the Qualitative tests <1.09> (2) and (3) for calcium salt.

Purity (1) Acid-insoluble substances—To 5 g of Calcium Hydroxide add 100 mL of water, add hydrochloric acid dropwise with string until the solution becomes acidic, and further add 1 mL of hydrochloric acid. Boil this solution for 5 minutes, cool, and filter through a tared glass filter (G4). Wash the residue with boiling water until the last washing exhibits no turbidity upon addition of silver nitrate TS, and dry at 105°C to constant mass: the mass is not more than 25 mg.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Calcium Hydroxide in 10 mL of dilute hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 40 mL of water, and filter. To 20 mL of the filtrate add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of dilute hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Hydroxide in a mixture of 20 mL of water and 10 mL of dilute hydrochloric acid, boil, neutralize with ammonia TS, and precipitate calcium oxalate completely by adding dropwise ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, shake, and filter. To 50 mL of the filtrate add 0.5 mL of sul-

furic acid, evaporate to dryness, and ignite at 600°C to constant mass: the mass of the residue does not exceed 24 mg.

(4) Arsenic <1.11>—Dissolve 0.5 g of Calcium Hydroxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 4 ppm).

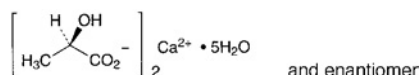
Assay Weigh accurately about 1 g of Calcium Hydroxide, dissolve by adding 10 mL of dilute hydrochloric acid, and add water to make 100 mL. Measure 10 mL of this solution, add 90 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS, shake, allow to stand for 3 to 5 minutes, and then add 0.1 g of NN indicator. Titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the red-purple color of the solution changes to blue.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 3.705 mg of Ca(OH)₂

Containers and storage Containers—Tight containers.

Calcium Lactate Hydrate

乳酸カルシウム水和物



C₆H₁₀CaO₆·5H₂O: 308.29

Monocalcium bis[(2*RS*)-2-hydroxypropanoate] pentahydrate [63690-56-2]

Calcium Lactate Hydrate, when dried, contains not less than 97.0% of calcium lactate (C₆H₁₀CaO₆: 218.22).

Description Calcium Lactate Hydrate occurs as white powder or granules. It is odorless, and has a slightly acid taste.

A 1 g portion of it dissolves gradually in 20 mL of water, and it is slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is partly efflorescent at ordinary temperature, and yields the anhydride at 120°C.

Identification A solution of Calcium Lactate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for calcium salt and for lactate.

Purity (1) Clarity of solution—Dissolve 1.0 g of Calcium Lactate Hydrate in 20 mL of water by warming: the solution is clear.

(2) Acidity or alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color is produced. Then add 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Calcium Lactate Hydrate in 30 mL of water and 5 mL of dilute acetic acid by warming, cool, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 20 ppm).

(4) Magnesium or alkali metals—Dissolve 1.0 g of Calci-

um Lactate Hydrate in 40 mL of water, add 0.5 g of ammonium chloride, boil, then add 20 mL of ammonium oxalate TS. Heat the mixture on a water bath for 1 hour, cool, dilute with water to 100 mL, and filter. To 50 mL of the filtrate add 0.5 mL of sulfuric acid, evaporate to dryness, and ignite between 450°C and 550°C to constant mass: the mass of the residue is not more than 5 mg.

(5) Arsenic <1.11>—Dissolve 0.5 g of Calcium Lactate Hydrate in 2 mL of water and 3 mL of hydrochloric acid, and perform the test with this solution as the test solution (not more than 4 ppm).

(6) Volatile fatty acid—Warm 1.0 g of Calcium Lactate Hydrate with 2 mL of sulfuric acid: an odor of acetic acid or butyric acid is not perceptible.

Loss on drying <2.41> 25.0 – 30.0% (1 g, 80°C, 1 hour at first, then 120°C, 4 hours).

Assay Weigh accurately about 0.5 g of Calcium Lactate, previously dried, add water, dissolve by heating on a water bath, cool, and add water to make exactly 100 mL. Pipet 20 mL of this solution, then 80 mL of water and 1.5 mL of 8 mol/L potassium hydroxide TS, and allow to stand for 3 to 5 minutes. Add 0.1 g of NN indicator, and titrate <2.50> immediately with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.364 mg of C₆H₁₀CaO₆

Containers and storage Containers—Tight containers.

Calcium Oxide

Quick Lime

酸化カルシウム

CaO: 56.08

Calcium Oxide, when incinerated, contains not less than 98.0% of CaO.

Description Calcium Oxide occurs as hard, white masses, containing a powder. It is odorless.

It is very slightly soluble in boiling water, and practically insoluble in ethanol (95).

One gram of Calcium Oxide dissolves almost completely in 2500 mL of water.

It slowly absorbs moisture and carbon dioxide from air.

Identification (1) Moisten Calcium Oxide with water: heat is generated and a white powder is obtained. Mix the powder with about 5 times its mass of water: the mixture is alkaline.

(2) Dissolve 1 g of Calcium Oxide in 20 mL of water by adding a few drops of acetic acid (31): the solution responds to the Qualitative Tests <1.09> for calcium salt.

Purity (1) Acid-insoluble substances—Disintegrate 5.0 g of Calcium Oxide with a small amount of water, add 100 mL of water, add dropwise hydrochloric acid with stirring until the solution becomes acidic, and further add 1 mL of

hydrochloric acid. Boil the solution for 5 minutes, cool, filter through a glass filter (G4), wash the residue with boiling water until no turbidity is produced when silver nitrate TS is added to the last washing, and dry at 105°C to constant mass: the mass of the residue is not more than 10.0 mg.

(2) Carbonate—Disintegrate 1.0 g of Calcium Oxide with a small amount of water, mix thoroughly with 50 mL of water, allow to stand for a while, remove most of the supernatant milky liquid by decantation, and add an excess of dilute hydrochloric acid to the residue: no vigorous effervescence is produced.

(3) Magnesium and alkali metals—Dissolve 1.0 g of Calcium Oxide in 75 mL of water by adding dropwise hydrochloric acid, and further add 1 mL of hydrochloric acid. Boil for 1 to 2 minutes, neutralize with ammonia TS, add dropwise an excess of hot ammonium oxalate TS, heat the mixture on a water bath for 2 hours, cool, add water to make 200 mL, mix thoroughly, and filter. Evaporate 50 mL of the filtrate with 0.5 mL of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass of the residue is not more than 15 mg.

Loss on ignition <2.43> Not more than 10.0% (1 g, 900°C, constant mass).

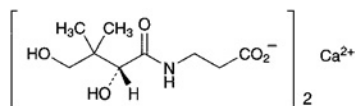
Assay Weigh accurately about 0.7 g of Calcium Oxide, previously incinerated at 900°C to constant mass and cooled in a desiccator (silica gel), and dissolve in 50 mL of water and 8 mL of diluted hydrochloric acid (1 in 3) by heating. Cool, and add water to make exactly 250 mL. Pipet 10 mL of the solution, add 50 mL of water, 2 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator, and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the red-purple color of the solution changes to blue.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.122 mg of CaO

Containers and storage Containers—Tight containers.

Calcium Pantothenate

パントテン酸カルシウム



$C_{18}H_{32}CaN_2O_{10}$: 476.53

Monocalcium bis{3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoate} [137-08-6]

Calcium Pantothenate, when dried, contains not less than 5.7% and not more than 6.0% of nitrogen (N: 14.01), and not less than 8.2% and not more than 8.6% of calcium (Ca: 40.08).

Description Calcium Pantothenate occurs as a white powder. It is odorless, and has a bitter taste.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Calcium Pantothenate (1 in 20) is

between 7.0 and 9.0.

It is hygroscopic.

Identification (1) Dissolve 0.05 g of Calcium Pantothenate in 5 mL of sodium hydroxide TS, and filter. To the filtrate add 1 drop of copper (II) sulfate TS: a deep blue color develops.

(2) To 0.05 g of Calcium Pantothenate add 5 mL of sodium hydroxide TS, and boil for 1 minute. After cooling, add diluted hydrochloric acid (1 in 10) to adjust the solution to a pH between 3 and 4, and add 2 drops of iron (III) chloride TS: a yellow color is produced.

(3) A solution of Calcium Pantothenate (1 in 10) responds to the Qualitative Tests <1.09> for calcium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +25.0 – +28.5° (after drying, 1 g, water, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Calcium Pantothenate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Calcium Pantothenate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Alkaloids—Dissolve 0.05 g of Calcium Pantothenate in 5 mL of water, add 0.5 mL of hexaammonium heptamolybdate TS and 0.5 mL of a solution of phosphoric acid (1 in 10): no white turbidity is produced.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 4 hours).

Assay (1) Nitrogen—Proceed with about 50 mg of Calcium Pantothenate, previously dried and accurately weighed, as directed under Nitrogen Determination <1.08>.

(2) Calcium—Weigh accurately about 0.4 g of Calcium Pantothenate, previously dried, and dissolve in 30 mL of water by warming. After cooling, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, then 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L magnesium chloride VS until the color of the solution changes from blue-purple to red-purple. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.004 mg of Ca

Containers and storage Containers—Tight containers.

Calcium Paraaminosalicylate Granules

Pas-calcium Granules

パラアミノサリチル酸カルシウム顆粒

Calcium Paraaminosalicylate Granules contain not less than 95.0% and not more than 105.0% of the labeled amount of calcium paraaminosalicylate hydrate

($C_7H_5CaNO_3 \cdot 3 \frac{1}{2}H_2O$: 254.25).

Method of preparation Prepare as directed under Granules, with Calcium Paraaminosalicylate Hydrate.

Identification Powder Calcium Paraaminosalicylate Granules, weigh a portion of the powder, equivalent to 50 mg of Calcium Paraaminosalicylate Hydrate according to the labeled amount, add 100 mL of water, shake, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

Particle size <6.03> It meets the requirement.

Assay Powder Calcium Paraaminosalicylate Granules, weigh accurately a portion of the powder, equivalent to about 0.2 g of calcium paraaminosalicylate hydrate ($C_7H_5CaNO_3 \cdot 3 \frac{1}{2}H_2O$), add 60 mL of water and 0.75 mL of dilute hydrochloric acid, and dissolve by heating on a water bath. After cooling, add water to make exactly 100 mL, and filter. Pipet 30 mL of the filtrate, transfer to an iodine flask, and proceed as directed in the Assay under Calcium Paraaminosalicylate Hydrate.

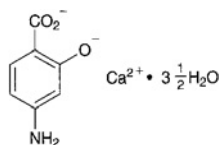
Each mL of 0.05 mol/L bromine VS
= 4.238 mg of $C_7H_5CaNO_3 \cdot 3 \frac{1}{2}H_2O$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Calcium Paraaminosalicylate Hydrate

Pas-calcium Hydrate

パラアミノサリチル酸カルシウム水和物



$C_7H_5CaNO_3 \cdot 3 \frac{1}{2} H_2O$: 254.25

Monocalcium 4-amino-2-oxidobenzoate hemiheptahydrate
[133-15-3, anhydride]

Calcium Paraaminosalicylate Hydrate contains not less than 97.0% and not more than 103.0% of calcium para-aminosalicylic acid ($C_7H_5CaNO_3$: 191.20), calculated on the anhydrous basis.

Description Calcium Paraaminosalicylate Hydrate occurs as a white to slightly colored powder. It has a slightly bitter taste.

It is very slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

It is gradually colored to brown by light.

Identification (1) To 50 mg of Calcium Paraaminosalicylate Hydrate add 100 mL of water, shake well, and filter. To 10 mL of the filtrate add 1 mL of 1 mol/L hydrochloric acid TS, shake, and add 1 drop of iron (III) chloride TS: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Calci-

um Paraaminosalicylate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 g of Calcium Paraaminosalicylate Hydrate add 15 mL of ammonium chloride TS and 15 mL of water, heat on a water bath until almost dissolved, and filter after cooling: the filtrate responds to the Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Calcium Paraaminosalicylate Hydrate in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.025%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Calcium Paraaminosalicylate Hydrate according to method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 0.40 g of Calcium Paraaminosalicylate Hydrate in 20 mL of 0.1 mol/L hydrochloric acid TS by warming on a water bath, use this solution as the test solution, and perform the test (not more than 5 ppm).

(4) 3-Aminophenol—To 0.10 g of Calcium Paraaminosalicylate Hydrate add 5 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, previously cooled in ice-water, and dissolve by shaking vigorously. Add immediately 3 mL of ammonia-ammonium chloride buffer solution, pH 11.0, previously cooled in ice water, and shake. Add 2 mL of 4-amino-*N,N*-diethylaniline sulfate TS, shake, add 10.0 mL of cyclohexane and 4 mL of diluted potassium hexacyanoferrate (III) TS (1 in 10), and shake immediately for 20 seconds. Centrifuge this solution, wash the separated cyclohexane layer with two 5-mL portions of diluted ammonia TS (1 in 14), add 1 g of anhydrous sodium sulfate, shake, and allow to stand for 5 minutes: the clear cyclohexane layer is not more colored than the following control solution.

Control solution: Dissolve 50 mg of 3-aminophenol in water, and dilute with water to exactly 500 mL. Measure exactly 20 mL of this solution, and add water to make exactly 100 mL. Take 5.0 mL of this solution, add 3 mL of ammonia-ammonium chloride buffer solution, pH 11.0, previously cooled in ice-water, and treat this solution in the same manner as the sample.

Water <2.48> 23.3 – 26.3% (0.1 g, volumetric titration, direct titration)

Assay Weigh accurately about 0.2 g of Calcium Paraaminosalicylate Hydrate, dissolve in 60 mL of water and 0.75 mL of dilute hydrochloric acid by warming on a water bath. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 30 mL of the sample solution, transfer to an iodine flask, and add exactly 25 mL of 0.05 mol/L bromine VS and 20 mL of a solution of potassium bromide (1 in 4). Add immediately 14 mL of a mixture of acetic acid (100) and hydrochloric acid (5:2), stopper the flask immediately, and allow to stand for 10 minutes with occasional shaking. Add cautiously 6 mL of potassium iodide TS, and shake gently. After 5 minutes, titrate <2.50> the produced iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a

blank determination.

Each mL of 0.05 mol/L bromine VS
= 3.187 mg of $C_7H_5CaNO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dibasic Calcium Phosphate Hydrate

リン酸水素カルシウム水和物

$CaHPO_4 \cdot 2H_2O$: 172.09

Dibasic Calcium Phosphate Hydrate, when dried, contains not less than 98.0% of dibasic calcium phosphate ($CaHPO_4$: 136.06).

Description Dibasic Calcium Phosphate Hydrate occurs as a white, crystalline powder. It is colorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification (1) Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 10 mL of diluted hydrochloric acid (1 in 6) by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming for 1 to 2 minutes at 70°C: a yellow precipitate is produced.

Purity (1) Acid-insoluble substance—Dissolve 5.0 g of Dibasic Calcium Phosphate Hydrate in 40 mL of water and 10 mL of hydrochloric acid, and boil for 5 minutes. After cooling, collect the insoluble substance using filter paper for assay. Wash with water until no more turbidity of the washing is produced when silver nitrate is added. Ignite to incinerate the residue and filter paper: the mass is not more than 2.5 mg (not more than 0.05%).

(2) Chloride <1.03>—Dissolve 0.20 g of Dibasic Calcium Phosphate Hydrate in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.248%).

(3) Sulfate <1.14>—Dissolve by warming 1.0 g of Dibasic Calcium Phosphate Hydrate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 30 mL of the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.160%).

(4) Carbonate—Mix 1.0 g of Dibasic Calcium Phosphate Hydrate with 5 mL of water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

(5) Heavy metals <1.07>—Dissolve 0.65 g of Dibasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add

ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Barium—Heat 0.5 g of Dibasic Calcium Phosphate Hydrate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter, if necessary. Add 2 mL of potassium sulfate TS to the filtrate, and allow to stand for 10 minutes: no turbidity forms.

(7) Arsenic <1.11>—Dissolve 1.0 g of Dibasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <2.41> 19.5 – 22.0% (1 g, 200°C, 3 hours).

Assay Weigh accurately about 0.4 g of Dibasic Calcium Phosphate Hydrate, previously dried, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc acetate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen
ethylenediamine tetraacetate VS
= 2.721 mg of $CaHPO_4$

Containers and storage Containers—Well-closed containers.

Anhydrous Dibasic Calcium Phosphate

無水リン酸水素カルシウム

$CaHPO_4$: 136.06

Anhydrous Dibasic Calcium Phosphate, when dried, contains not less than 98.0% of $CaHPO_4$.

Description Anhydrous Dibasic Calcium Phosphate occurs as white, crystalline powder or granules. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification (1) Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 10 mL of diluted hydrochloric acid (1 in 6) by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming for 1 to 2 minutes at 70°C: a yellow precipitate is produced.

Purity (1) Acid-insoluble substances—Dissolve 5.0 g of Anhydrous Dibasic Calcium Phosphate in 40 mL of water and 10 mL of hydrochloric acid, and boil for 5 minutes. After cooling, collect the insoluble substance using filter paper for assay. Wash with water until no more turbidity of the washing is produced when silver nitrate is added. Ignite to incinerate the residue with the filter paper: the mass is not more than 2.5 mg (not more than 0.05%).

(2) Chloride <1.03>—Dissolve 0.20 g of Anhydrous Dibasic Calcium Phosphate in 20 mL of water and 13 mL of dilute nitric acid, add water to make 100 mL, and filter, if necessary. Perform the test using a 50-mL portion of this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.248%).

(3) Sulfate <1.14>—Dissolve by warming 0.80 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of water and 5 mL of dilute hydrochloric acid, add water to make 100 mL, and filter, if necessary. Take 30 mL of the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.200%).

(4) Carbonate—Mix 1.0 g of Anhydrous Dibasic Calcium Phosphate with 5 mL of water, and add immediately 2 mL of hydrochloric acid: no effervescence occurs.

(5) Heavy metals <1.07>—Dissolve 0.65 g of Anhydrous Dibasic Calcium Phosphate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Barium—Heat 0.5 g of Anhydrous Dibasic Calcium Phosphate with 10 mL of water, add 1 mL of hydrochloric acid dropwise with stirring, and filter, if necessary. Add 2 mL of potassium sulfate TS to the filtrate, and allow to stand for 10 minutes: no turbidity forms.

(7) Arsenic <1.11>—Dissolve 0.5 g of Anhydrous Dibasic Calcium Phosphate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 200°C, 3 hours).

Assay Weigh accurately about 0.4 g of Anhydrous Dibasic Calcium Phosphate, previously dried, dissolve in 12 mL of dilute hydrochloric acid, and add water to make exactly 200 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02

mol/L zinc acetate VS (indicator: 0.025 g of eriochrome black T-sodium chloride indicator). Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.721 mg of CaHPO₄

Containers and storage Containers—Well-closed containers.

Monobasic Calcium Phosphate Hydrate

リン酸二水素カルシウム水和物

Ca(H₂PO₄)₂·H₂O: 252.07

Monobasic Calcium Phosphate Hydrate, when dried, contains not less than 90.0% of Ca(H₂PO₄)₂·H₂O.

Description Monobasic Calcium Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless and has an acid taste.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

It is slightly deliquescent.

Identification (1) Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 10 mL of diluted hydrochloric acid (1 in 6) by warming, add 2.5 mL of ammonia TS dropwise with shaking, and add 5 mL of ammonium oxalate TS: a white precipitate is produced.

(2) Dissolve 0.1 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute nitric acid, and add 2 mL of hexaammonium heptamolybdate TS after warming for 1 to 2 minutes at 70°C: a yellow precipitate is produced.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 19 mL of water and 2 mL of diluted hydrochloric acid (3 in 4), and heat on a water bath for 5 minutes with occasional shaking: the solution is clear and colorless.

(2) Dibasic phosphate and acid—Triturate 1.0 g of Monobasic Calcium Phosphate Hydrate with 3 mL of water, and add 100 mL of water and 1 drop of methyl orange TS: a red color develops. Then add 1.0 mL of 1 mol/L sodium hydroxide VS: the color changes to yellow.

(3) Chloride <1.03>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 12 mL of dilute nitric acid, add water to make exactly 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <1.14>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 20 mL of water and 1 mL of hydrochloric acid, add water to make 100 mL, and filter, if necessary. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—Dissolve 0.65 g of Monobasic Calcium Phosphate Hydrate in a mixture of 5 mL of water and 5 mL of dilute hydrochloric acid by warming, cool, and add ammonia TS until precipitates begin to form in the solution. Dissolve the precipitates by adding a small amount of dilute hydrochloric acid dropwise, add 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 10 mL of hydrochloric acid-ammonium acetate buffer solution, pH 3.5, add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 31 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of Monobasic Calcium Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 3.0% (1 g, silica gel, 24 hours).

Assay Weigh accurately about 0.4 g of Monobasic Calcium Phosphate Hydrate, previously dried, dissolve in 3 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.02 mol/L zinc acetate VS (indicator: 25 mg of eriochrome black T-sodium chloride indicator). Perform a blank determination.

Each mL of 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 5.041 mg of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$

Containers and storage Containers—Tight containers.

Calcium Polystyrene Sulfonate

ポリスチレンスルホン酸カルシウム

Calcium Polystyrene Sulfonate is a cation exchange resin prepared as the calcium form of the sulfonated styrene divinylbenzene copolymer.

When dried, it contains not less than 7.0% and not more than 9.0% of calcium (Ca: 40.08).

Each g of Calcium Polystyrene Sulfonate, when dried, exchanges with 0.053 to 0.071 g of potassium (K: 39.10).

Description Calcium Polystyrene Sulfonate occurs as a pale yellowish white to light yellow powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Calcium Polystyrene Sulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Mix 0.5 g of Calcium Polystyrene Sulfonate with 10

mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

Purity (1) Ammonium—Place 1.0 g of Calcium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside, and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue (not less than 5 ppm).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Calcium Polystyrene Sulfonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Calcium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 2 ppm).

(4) Styrene—To 10.0 g of Calcium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, dilute with acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak heights, H_T and H_S , of styrene in each solution: H_T is not larger than H_S .

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter and 2 m in length, having polyethylene glycol 20 M coated at the ratio of 15% on siliceous earth for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Carrier gas: Nitrogen

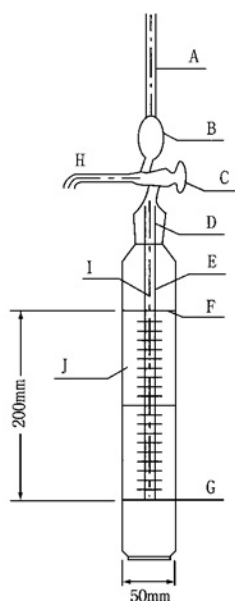
Flow rate: Adjust the flow rate so that the retention time of styrene is about 9 minutes.

System suitability—

System performance: Mix 10 mg of styrene with 1000 mL of acetone. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of styrene are not less than 800 and 0.8 to 1.2, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of styrene is not more than 5%.

(5) Sodium—Pipet 2 mL of the 50-mL solution obtained in the Assay (1), add 0.02 mol/L hydrochloric acid TS to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately 0.2542 g of sodium chloride, previously dried at 130°C for 2 hours, and dissolve in 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet a suitable volume of this solution, and dilute with 0.02 mol/L hydrochloric acid TS to make a solution containing 1 to 3 μg of sodium (Na: 22.99) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the amount of sodium in the sample solution using the calibration curve obtained from the stan-



Actual volume to the mark of 20 cm at which the sedimentation tube is inserted: 550 mL

Single suction volume: 10 mL

- A: Mark of pipet bulb
- B: Pipet bulb for suction
- C: Two-way stopcock
- D: Vent-hole
- E: Suction part of pipet
- F: Mark of 20 cm
- G: Base line of 0 cm
- H: Outlet of pipet
- I: Capillary tube of pipet
- J: Sedimentation tube

Fig. Andreasen pipet

dard solutions: the amount of sodium is not more than 1%.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: A sodium hollow-cathode lamp

Wavelength: 589.0 nm

Loss on drying <2.41> Not more than 10.0% (1 g, in vacuum, 80°C, 5 hours).

Microparticles (i) Apparatus: Use an apparatus as shown in the illustration.

(ii) Procedure: Weigh accurately about 5.5 g of Calcium Polystyrene Sulfonate, previously dried, add 300 mL of water of 25°C, and mix for 5 minutes. Transfer this turbid solution to the sedimentation tube J, keeping a temperature at 25°C, add water of 25°C to 2 mm below the mark F of 20 cm of the sedimentation tube J, and then insert the pipet. Open the two-way stopcock C, exhaust air, add exactly water from the vent-hole D to the mark F of 20 cm, and close the two-way stopcock C. Shake the apparatus well vertically and horizontally, disperse Calcium Polystyrene Sulfonate in water, and then open the two-way stopcock, and allow to stand at 25 ± 1°C for 5 hours and 15 minutes.

Then, draw exactly the meniscus of the turbid solution in sedimentation tube J up to the mark of pipet bulb A by suction, open the two-way stopcock C to the outlet of pipet H,

and transfer exactly measured 20 mL of the turbid solution to a weighing bottle. Repeat the procedure, and combine exactly measured 20 mL of the turbid solution. Evaporate 20 mL of this turbid solution on a water bath to dryness, dry to constant mass at 105°C, and weigh the residue as W_S (g). Pipet 20 mL of used water, and weigh the residue in the same manner as W_B (g). Calculate the difference mi (g) between W_S and W_B , and calculate the amount of microparticles (S) by the following equation: the amount of microparticles is not more than 0.1%.

$$S (\%) = \{(mi \times V)/(20 \times W_T)\} \times 100$$

W_T : Amount (g) of Calcium Polystyrene Sulfonate

V : Actual volume (mL) to the mark of 20 cm at which the suction part of pipet is inserted.

Assay (1) Calcium—Weigh accurately about 1 g of Calcium Polystyrene Sulfonate, previously dried, and disperse in 5 mL of 3 mol/L hydrochloric acid TS. Transfer this mixture, and wash out completely with the aid of a small quantity of 3 mol/L hydrochloric acid TS to a column 12 mm in inside diameter and 70 mm in length, packed with a pledget of fine glass wool in the bottom of it, placing a 50-mL volumetric flask as a receiver under the column. Then collect about 45 mL of eluate, adding 3 mol/L hydrochloric acid TS to the column, and add water to make exactly 50 mL. Pipet 20 mL of this solution, adjust with ammonia TS to a pH of exactly 10. Titrate <2.50> immediately with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution disappears, and a blue color develops (indicator: 0.04 g eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} &\text{Each mL of 0.05 mol/L disodium dihydrogen} \\ &\text{ethylenediamine tetraacetate VS} \\ &= 2.004 \text{ mg of Ca} \end{aligned}$$

(2) Potassium exchange capacity—Pipet 50 mL of Standard Potassium Stock Solution into a glass-stoppered flask containing about 1.0 g of dried Calcium Polystyrene Sulfonate, accurately weighed, stir for 120 minutes, filter, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, and add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 1000 mL, and use this solution as the sample solution. Separately, measure exactly a suitable volume of Standard Potassium Stock Solution, dilute with 0.02 mol/L hydrochloric acid TS to make solutions containing 0.5 to 2.5 μg of potassium (K: 39.10) per mL, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the amount, Y (mg), of potassium in 1000 mL of the sample solution, using the calibration curve obtained from the standard solutions. The exchange quantity for potassium per g of dried Calcium Polystyrene Sulfonate is 53 to 71 mg, calculating by the following equation.

$$\begin{aligned} &\text{Exchange quantity (mg) for potassium (K) per g of} \\ &\text{dried Calcium Polystyrene Sulfonate} \\ &= (X - 100 Y)/W \end{aligned}$$

X : The amount (mg) of potassium in 50 mL of Standard

Potassium Stock Solution before exchange.

W: The amount (g) of dried Calcium Polystyrene Sulfonate.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: A potassium hollow-cathode lamp

Wavelength: 766.5 nm

Containers and storage Containers—Tight containers.

Calcium Stearate

ステアリン酸カルシウム

Calcium Stearate mainly consists of calcium salts of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Calcium Stearate, when dried, contains not less than 6.4% and not more than 7.1% of calcium (Ca: 40.08).

Description Calcium Stearate occurs as a white, light, bulky powder. It feels smooth when touched, and is adhesive to the skin. It is odorless or has a faint, characteristic odor.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Shake vigorously 3 g of Calcium Stearate with 20 mL of diluted hydrochloric acid (1 in 2) and 30 mL of diethyl ether for 3 minutes, and allow to stand: the separated aqueous layer responds to the Qualitative Tests <1.09> (1), (2) and (4) for calcium salt.

(2) Wash the diethyl ether layer obtained in (1) with 20 mL and 10 mL of dilute hydrochloric acid and 20 mL of water successively, and evaporate the diethyl ether on a water bath: the residue melts <1.13> at a temperature not below 54°C.

Purity (1) Heavy metals <1.07>—Heat gently 1.0 g of Calcium Stearate with caution at the beginning, and heat further, gradually raising the temperature, to incineration. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, warm the residue with 20 mL of water and 2 mL of dilute acetic acid for 2 minutes, cool, filter, and wash the residue with 15 mL of water. Combine the filtrate and the washings, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution by evaporating 2 mL of hydrochloric acid on a water bath to dryness and by adding 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—To 1.0 g of Calcium Stearate add 5 mL of diluted hydrochloric acid (1 in 2) and 20 mL of chloroform, shake vigorously for 3 minutes, allow to stand, and separate the water layer. Perform the test with the water layer as the test solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 4.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.5 g of Calcium Stearate, previously dried, heat gently with caution at first, and then ignite gradually to ash. Cool, add 10 mL of dilute hydrochloric acid to the residue, warm for 10 minutes on a water bath, and transfer the contents to a flask with the aid of 10-mL, 10-mL,

and 5-mL portions of hot water. Add sodium hydroxide TS until the solution becomes slightly turbid, and then add 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, 4 drops of eriochrome black T TS and 5 drops of methyl yellow TS, and titrate <2.50> rapidly the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L magnesium chloride VS, until the green color of the solution disappears and a red color develops. Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.004 mg of Ca

Containers and storage Containers—Well-closed containers.

Camellia Oil

Oleum Camelliae

ツバキ油

Camellia Oil is the fixed oil obtained from the peeled seeds of *Camellia japonica* Linné (*Theaceae*).

Description Camellia Oil is a colorless or pale yellow, clear oil. It is nearly odorless and tasteless.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95).

It congeals partly at -10°C , and completely at -15°C .

Specific gravity d_{25}^{25} : 0.910 – 0.914

Identification To 2 mL of Camellia Oil add dropwise 10 mL of a mixture of fuming nitric acid, sulfuric acid, and water (1:1:1), previously cooled to room temperature: a bluish green color develops at the zone of contact.

Acid value <1.13> Not more than 2.8.

Saponification value <1.13> 188 – 194

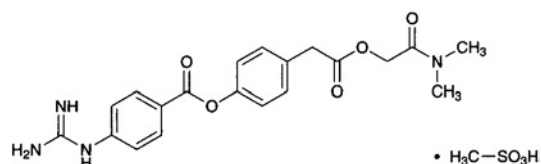
Unsaponifiable matters <1.13> Not more than 1.0%.

Iodine value <1.13> 78 – 83

Containers and storage Containers—Tight containers.

Camostat Mesilate

カモスタットメシル酸塩



$C_{20}H_{22}N_4O_5 \cdot \text{CH}_4\text{O}_3\text{S}$: 494.52

Dimethylcarbamoylmethyl

4-(4-guanidinobenzoyloxy)phenylacetate

monomethanesulfonate [59721-29-8]

Camostat Mesilate, when dried, contains not less

than 98.5% of $C_{20}H_{22}N_4O_5 \cdot CH_4O_3S$.

Description Camostat Mesilate occurs as white crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 4 mL of a solution of Camostat Mesilate (1 in 2000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.

(2) Determine the absorption spectrum of a solution of Camostat Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Camostat Mesilate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 0.1 g of Camostat Mesilate add 0.2 g of sodium hydroxide, fuse by gentle heating, and continue to heat for 20 to 30 seconds. After cooling, add 0.5 mL of water and 3 mL of dilute hydrochloric acid, and heat: the gas evolved changes moistened potassium iodate-starch paper to blue.

Melting point <2.60> 194 – 198°C

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Camostat Mesilate in 40 mL of water by warming, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid (not more than 20 ppm).

(2) Arsenic <1.11>—Dissolve 2.0 g of Camostat Mesilate in 20 mL of 2 mol/L hydrochloric acid TS by heating in a water bath, and continue to heat for 20 minutes. After cooling, centrifuge, take 10 mL of the supernatant liquid, and use this solution as the test solution. Perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 30 mg of Camostat Mesilate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand overnight in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, silica gel, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 50 mg each of Camostat Mesilate and Camostat Mesilate Reference Standard, previously dried, and dissolve each in water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 2 μ L each of the sample solution and standard

solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of camostat to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of } C_{20}H_{22}N_4O_5 \cdot CH_4O_3S \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Camostat Mesilate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in ethanol (95) (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, a solution of sodium 1-heptane sulfonate (1 in 500), a solution of sodium lauryl sulfate (1 in 1000) and acetic acid (100) (200:100:50:1).

Flow rate: Adjust the flow rate so that the retention time of camostat is about 10 minutes.

System suitability—

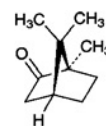
System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, camostat and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of camostat to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

d-Camphor

d-カンフル



$C_{10}H_{16}O$: 152.23
(1*R*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
[464-49-3]

d-Camphor contains not less than 96.0% of $C_{10}H_{16}O$.

Description *d*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and a slightly bitter taste, followed by a pleasant, cooling sensation.

It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water.

It slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of *d*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +41.0 – +43.0° (5 g, ethanol (95), 50 mL, 100 mm).

Melting point <2.60> 177 – 182°C

Purity (1) Water—Shake 1.0 g of *d*-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *d*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) Non-volatile residue—Heat 2.0 g of *d*-Camphor on a water bath until sublimation is complete, then dry the residue at 105°C for 3 hours: the mass of the residue does not exceed 1.0 mg.

Assay Weigh accurately about 0.1 g each of *d*-Camphor and *d*-Camphor Reference Standard, add exactly 5 mL each of the internal standard solution, dissolve in dehydrated methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 2 μ L each of these solutions as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of *d*-camphor to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{10}H_{16}O \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of *d*-Camphor Reference Standard

Internal standard solution—A solution of methyl salicylate in ethanol (99.5) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, which is packed with 10% of polyethylene glycol 20 M for gas chromatography supported on 180 to 250 μ m mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 160°C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of *d*-camphor is about 6 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, *d*-camphor and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating con-

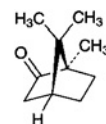
ditions, the relative standard deviation of the ratios of the peak area of *d*-camphor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

dl-Camphor

Synthetic Camphor

dl-カンフル



and enantiomer

$C_{10}H_{16}O$: 152.23
(1*RS*,4*RS*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
[76-22-2]

dl-Camphor contains not less than 96.0% of $C_{10}H_{16}O$.

Description *dl*-Camphor occurs as colorless or white, translucent crystals, crystalline powder or masses. It has a characteristic, agreeable odor, and has a slightly bitter taste followed by a pleasant, cooling sensation.

It is freely soluble in ethanol (95), in diethyl ether and in carbon disulfide, and slightly soluble in water.

It slowly volatilizes at room temperature.

Identification Dissolve 0.1 g of *dl*-Camphor in 2 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for 5 minutes on a water bath: an orange-red precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: –1.5 – +1.5° (5 g, ethanol (95), 50 mL, 100 mm).

Melting point <2.60> 175 – 180°C

Purity (1) Water—Shake 1.0 g of *dl*-Camphor with 10 mL of carbon disulfide: the solution is clear.

(2) Chlorinated compounds—Mix 0.20 g of finely powdered *dl*-Camphor with 0.4 g of sodium peroxide in a dried porcelain crucible. Heat the crucible gently by the open flame until the incineration is complete. Dissolve the residue in 20 mL of warm water, acidify with 12 mL of dilute nitric acid, and filter the solution into a Nessler tube. Wash the filter paper with three 5-mL portions of hot water, adding the washings to the filtrate. After cooling, add water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes: the turbidity of the solution does not exceed that of the following control solution.

Control solution: Prepare in the same manner as described above, using 0.20 mL of 0.01 mol/L hydrochloric acid VS.

(3) Non-volatile residue—Heat 2.0 g of *dl*-Camphor on a water bath until sublimation is complete, then dry the residue at 105°C for 3 hours: the mass of the residue does not exceed 1.0 mg.

Assay Weigh accurately about 0.1 g each of *dl*-Camphor and *dl*-Camphor Reference Standard, add exactly 5 mL each of the internal standard solution, dissolve in dehydrated methanol to make 100 mL, and use these solutions as the

sample solution and standard solution, respectively. Perform the test with 2 μL each of these solutions as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of *dl*-camphor to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{10}H_{16}O \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of *dl*-Camphor Reference Standard

Internal standard solution—A solution of methyl salicylate in ethanol (99.5) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, which is packed with 10% of polyethylene glycol 20 M for gas chromatography supported on 180 to 250 μm mesh silanized siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of *dl*-camphor is about 6 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, *dl*-camphor and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of *dl*-camphor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Capsules

カプセル

Capsules are made of gelatin or a suitable material, and their shape is a pair of cylinders with one end closed which can be overlapped on each other.

Method of preparation Dissolve Gelatin or the like in water by warming, add Glycerin or D-Sorbitol, emulsifier, preservatives, coloring substances and so forth, if necessary, to make a thick gluey solution, and form into capsules while warm.

Capsules may be coated with a lubricant, if necessary.

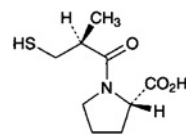
Description Capsules are odorless and elastic.

Purity Odor, solubility, and acidity or alkalinity—Place, without overlapping of the parts, 1 piece (1 pair) of Capsules in a 100-mL conical flask, add 50 mL of water, and shake often, keeping the temperature at $37 \pm 2^\circ\text{C}$. Perform this test 5 times: they all dissolve within 10 minutes. All these solutions are odorless, and neutral or slightly acidic.

Containers and storage Containers—Well-closed containers.

Captopril

カプトプリル



$C_9H_{15}NO_3S$: 217.29

(2*S*)-1-[(2*S*)-2-Methyl-3-sulfanylpropanoyl]pyrrolidine-2-carboxylic acid [62571-86-2]

Captopril contains not less than 98.0% of $C_9H_{15}NO_3S$, calculated on the dried basis.

Description Captopril occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), and soluble in water.

Identification Determine the infrared absorption spectrum of Captopril as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: $-125 - -134^\circ$ (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> $105 - 110^\circ\text{C}$

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Captopril according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Captopril according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Captopril in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 15 mg of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-dipropine in methanol to make exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of toluene and acetic acid (100) (13:7) to a distance of about 15 cm, and air-dry the plate. Place the plate in a chamber filled with iodine vapor, and allow to stand for 30 minutes: the number of the spots other than the spot corresponding to that from the standard solution and the principal spot from the sample solution is not more than two, and they are not more intense than the spot from the standard solution.

(4) 1,1'-[3,3'-Dithiobis(2-methyl-1-oxopropyl)]-L-dipropine—Dissolve 0.10 g of Captopril in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-dipropine in methanol to make exactly 250 mL, and use this solution as the standard solution. Perform the

test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak area, A_T and A_S , of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline of these solutions: A_T is not larger than A_S .

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol and phosphoric acid (1000:1000:1).

Flow rate: Adjust the flow rate so that the retention time of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is about 10 minutes.

System suitability—

System performance: Dissolve 25 mg each of Captopril and 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline in 200 mL of methanol. When the procedure is run with 20 μL of this solution under the above operating conditions, captopril and 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of 1,1'-[3,3'-dithiobis(2-methyl-1-oxopropyl)]-L-diproline is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

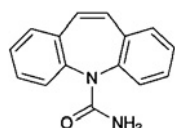
Assay Weigh accurately about 0.3 g of Captopril, dissolve in 100 mL of water, add 20 mL of dilute sulfuric acid and 1 g of potassium iodide, and shake. Titrate <2.50> with 1/60 mol/L potassium iodate VS (indicator: 2 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 1/60 mol/L potassium iodate VS
= 21.73 mg of $\text{C}_9\text{H}_{15}\text{NO}_3\text{S}$

Containers and storage Containers—Tight containers.

Carbamazepine

カルバマゼピン



$\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}$: 236.27

5*H*-Dibenz[*b,f*]azepine-5-carboxamide [298-46-4]

Carbamazepine, when dried, contains not less than 97.0% and not more than 103.0% of $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}$.

Description Carbamazepine occurs as a white to slightly yellowish white powder. It is odorless and tasteless at first, and leaves a slightly bitter aftertaste.

It is freely soluble in chloroform, sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water and in diethyl ether.

Identification (1) To 0.1 g of Carbamazepine add 2 mL of nitric acid, and heat on a water bath for 3 minutes: an orange-red color is produced.

(2) To 0.1 g of Carbamazepine add 2 mL of sulfuric acid, and heat on a water bath for 3 minutes: a yellow color is produced with a green fluorescence.

(3) Examine Carbamazepine under ultraviolet light: the solution shows an intense blue fluorescence.

(4) Determine the absorption spectrum of the solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 189 – 193°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Carbamazepine in 10 mL of chloroform: the solution is clear and colorless to pale yellow.

(2) Acidity—To 2.0 g of Carbamazepine add exactly 40 mL of water, stir well for 15 minutes, and filter through a glass filter (G3). To 10 mL of this filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(3) Alkalinity—To 10 mL of the filtrate obtained in (2) add 1 drop of methyl red TS and 0.50 mL of 0.01 mol/L hydrochloric acid VS: a red color is produced.

(4) Chloride <1.03>—Dissolve 0.25 g of Carbamazepine in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.20 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Carbamazepine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.25 g of Carbamazepine in 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 5.0 mg of iminodibenzyl in chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and methanol (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spots other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 50 mg of Carbamazepine, previously

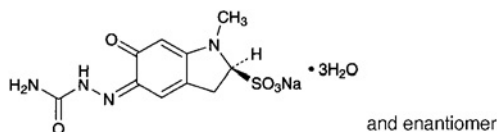
dried and accurately weighed, in ethanol (95) to make exactly 250 mL. Dilute 5 mL of this solution with ethanol (95) to exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 285 nm.

$$\text{Amount (mg) of } C_{15}H_{12}N_2O = (A/490) \times 50,000$$

Containers and storage Containers—Tight containers.

Carbazochrome Sodium Sulfonate Hydrate

カルバゾクロムスルホン酸ナトリウム水和物



$C_{10}H_{11}N_4NaO_5S \cdot 3H_2O$: 376.32

Monosodium (2*RS*)-1-methyl-6-oxo-5-semicarbazono-2,3,5,6-tetrahydroindole-2-sulfonate trihydrate [52422-26-5, anhydride]

Carbazochrome Sodium Sulfonate Hydrate contains not less than 98.0% and not more than 102.0% of carbazochrome sodium sulfonate ($C_{10}H_{11}N_4NaO_5S$: 322.27), calculated on the anhydrous basis.

Description Carbazochrome Sodium Sulfonate Hydrate occurs as orange-yellow, crystals or crystalline powder.

It is sparingly soluble in water, very slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Carbazochrome Sodium Sulfonate (1 in 100) shows no optical rotation.

Melting point: about 210°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbazochrome Sodium Sulfonate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Carbazochrome Sodium Sulfonate Hydrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 0.8 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and cool: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity of solution—Dissolve 1.0 g of Carbazochrome Sodium Sulfonate Hydrate in 50 mL of water by warming, and allow to cool: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible

Spectrophotometry <2.24>: the absorbance at 590 nm is not more than 0.070.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Carbazochrome Sodium Sulfonate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Carbazochrome Sodium Sulfonate Hydrate in 100 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of carbazochrome sulfonate from the sample solution is not larger than the peak area of carbazochrome sulfonate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.2 g of ammonium dihydrogen phosphate in 1000 mL of water, and filter through a membrane filter (0.4 μ m in pore size) if necessary. To 925 mL of this solution add 75 mL of ethanol (95), shake, and adjust the pH to 3 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbazochrome sulfonate is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of carbazochrome sulfonate beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbazochrome sulfonate obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of carbazochrome sulfonate obtained from 10 μ L of the standard solution.

System performance: Dissolve 10 mg each of Carbazochrome Sodium Sulfonate and carbazochrome in 100 mL of water by warming. When the procedure is run with 10 μ L of this solution under the above operating conditions, carbazochrome sulfonate and carbazochrome are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbazochrome sulfonate is not more than 2.0%.

Water <2.48> 13.0 – 16.0% (0.3 g, direct titration).

Assay Weigh accurately about 0.25 g of Carbazochrome Sodium Sulfonate Hydrate, dissolve in 50 mL of water, apply to a chromatographic column, 10 mm in diameter, previously prepared with 20 mL of strongly acidic ion exchange resin for column chromatography (type H), and allow to flow at a rate

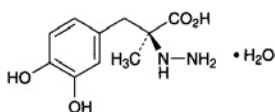
of 4 mL per minute. Wash the column with 150 mL of water, combine the washing and the former effluent solution, and titrate <2.50> with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 16.11 mg of C₁₀H₁₁N₄NaO₅S

Containers and storage Containers—Well-closed containers.

Carbidopa Hydrate

カルビドパ水和物



C₁₀H₁₄N₂O₄·H₂O: 244.24
(2*S*)-2-(3,4-Dihydroxybenzyl)-2-hydrazinopropanoic acid monohydrate [38821-49-7]

Carbidopa Hydrate contains not less than 98.0% of C₁₀H₁₄N₂O₄·H₂O.

Description Carbido-pa Hydrate occurs as a white to yellowish white powder.

It is sparingly soluble in methanol, slightly soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 197°C (with decomposition).

Identification (1) Dissolve 0.01 g of Carbido-pa Hydrate in 250 mL of a solution of hydrochloric acid in methanol (9 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24> at the wavelengths between 240 nm and 300 nm, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carbido-pa Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carbido-pa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Carbido-pa Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -21.0 - -23.5° (1 g, aluminum (III) chloride TS, 100 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Carbido-pa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Carbido-pa Hydrate in 70 mL of the mobile phase, by warming and using ultrasonication, if necessary. After cooling, add the mobile phase to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each

of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the total area of all peaks other than the peak of carbido-pa from the sample solution is not larger than the peak area of carbido-pa from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carbido-pa.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of carbido-pa obtained from 20 μL of this solution is equivalent to 7 to 13% of that of carbido-pa obtained from 20 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Loss on drying <2.41> 6.9 - 7.9% (1 g, in vacuum not exceeding 0.67 kPa, 100°C, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Carbido-pa Hydrate and Carbido-pa Reference Standard (determine separately the loss on drying <2.41> in the same manner as Carbido-pa Hydrate), and dissolve each in 70 mL of the mobile phase, by warming and using ultrasonication if necessary. After cooling, add the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A*_T and *A*_S, of carbido-pa in each solution.

$$\begin{aligned} \text{Amount (mg) of C}_{10}\text{H}_{14}\text{N}_2\text{O}_4\cdot\text{H}_2\text{O} \\ = W_S \times (A_T/A_S) \times 1.0796 \end{aligned}$$

*W*_S: Amount (mg) of Carbido-pa Reference Standard, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 950 mL of 0.05 mol/L sodium dihydrogen phosphate TS add 50 mL of ethanol (95), and adjust the pH to 2.7 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of carbido-pa is about 6 minutes.

System suitability—

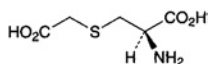
System performance: Dissolve 50 mg each of Carbido-pa and methyl-dopa in 100 mL of the mobile phase. When the procedure is run with 20 μL of this solution under the above operating conditions, methyl-dopa and carbido-pa are eluted in this order with the resolution between these peaks being not less than 0.9.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carbidopa is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

L-Carbocisteine

L-カルボシステイン



$\text{C}_5\text{H}_9\text{NO}_4\text{S}$: 179.19
(2*R*)-2-Amino-3-carboxymethylsulfanylpropanoic acid
[638-23-3]

L-Carbocisteine, when dried, contains not less than 98.5% of $\text{C}_5\text{H}_9\text{NO}_4\text{S}$.

Description L-Carbocisteine occurs as a white crystalline powder. It is odorless, and has a slightly acid taste.

It is very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid or in sodium hydroxide TS.

Melting point: about 186°C (with decomposition).

Identification (1) To 0.2 g of L-Carbocisteine add 1 mL of lead acetate TS and 3 mL of water, shake, add 0.2 g of sodium hydroxide, and heat over a flame for 1 minute: a dark brown to black precipitate is formed.

(2) Determine the infrared absorption spectrum of L-Carbocisteine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: $-33.5 - -36.5^\circ$ Weigh accurately about 5 g of L-Carbocisteine, previously dried, dissolve in 20 mL of water and a suitable amount of a solution of sodium hydroxide (13 in 100), and adjust the pH with 1 mol/L hydrochloric acid TS or 0.1 mol/L hydrochloric acid TS to 6.0, and add water to make exactly 50 mL. Determine the optical rotation of this solution in a 100-mm cell.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Carbocisteine in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.20 g of L-Carbocisteine in 10 mL of water and 20 mL of nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(3) Ammonium <1.02>—Perform the test with 0.25 g of L-Carbocisteine using the distillation under reduced pressure. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of L-Carbocisteine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solu-

tion (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Carbocisteine according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.30 g of L-Carbocisteine in 10 mL of 0.2 mol/L sodium hydroxide TS, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 100 mL. Pipet 1 mL of this solution, and add 0.2 mol/L sodium hydroxide TS to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution, in 15 mm length along the starting line on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of L-Carbocisteine, previously dried, dissolve in exactly 20 mL of 0.1 mol/L perchloric acid VS and 50 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 17.92 mg of $\text{C}_5\text{H}_9\text{NO}_4\text{S}$

Containers and storage Containers—Tight containers.

Carbon Dioxide

二酸化炭素

CO_2 : 44.01

Carbon Dioxide contains not less than 99.5 vol% of CO_2 .

Description Carbon Dioxide is a colorless gas at room temperature and under atmospheric pressure. It is odorless.

A 1 mL volume of Carbon Dioxide dissolves in 1 mL of water, and the solution is slightly acid.

1000 mL of Carbon Dioxide at 0°C and under a pressure of 101.3 kPa weighs about 1.978 g.

Identification (1) Put a flaming wood splinter into Carbon Dioxide: the flame is extinguished immediately.

(2) Pass Carbon Dioxide into calcium hydroxide TS: a white precipitate is produced. Collect the precipitate, and add acetic acid (31): it dissolves with effervescence.

Purity Maintain containers of Carbon Dioxide between 18°C and 22°C for not less than 6 hours prior to the test, and correct the volume of Carbon Dioxide to 20°C and under a

pressure of 101.3 kPa.

(1) Acidity—Place 50 mL of freshly boiled and cooled water in a Nessler tube, and pass 1000 mL of Carbon Dioxide into it for 15 minutes through an introducing tube about 1 mm in diameter extending to 2 mm from the bottom of the Nessler tube, then add 0.10 mL of methyl orange TS: the solution is not more colored than the following control solution.

Control solution: To 50 mL of freshly boiled and cooled water in a Nessler tube add 0.10 mL of methyl orange TS and 1.0 mL of 0.01 mol/L hydrochloric acid VS.

(2) Hydrogen phosphide, hydrogen sulfide or reducing organic substances—Place 25 mL of silver nitrate-ammonia TS and 3 mL of ammonia TS in each of two Nessler tubes A and B, and designate the solution in each tube as solution A and solution B, respectively. Pass 1000 mL of Carbon Dioxide into solution A in the same manner as directed in (1): the turbidity and color of this solution are the same as that of solution B.

(3) Carbon monoxide—Introduce 5.0 mL of Carbon Dioxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this according to the Gas Chromatography <2.02> under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in porous size).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

Selection of column: To 0.1 mL each of carbon monoxide and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving elution of oxygen, nitrogen and carbon monoxide in this order with a well-resolving of their peaks.

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

(4) Oxygen and nitrogen—Introduce 1.0 mL of Carbon Dioxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure with a pressure-reducing valve through a directly connected polyvinyl chloride tube. Perform the test as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of air. Separately, introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, allow to mix thoroughly, and use this mixture as the standard gas mixture. Perform the test with 1.0 mL of this mixture in the same manner as directed in the case of Carbon Dioxide, and determine the peak area A_S of nitrogen: A_T is smaller than A_S , and no other peak appears.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and

about 3 m in length, packed with silica gel for gas chromatography (300 to 500 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

Selection of column: Collect 0.5 mL of nitrogen in a gas mixer, add Carbon Dioxide to make 100 mL, mix well, and proceed with 1.0 mL of the mixture under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and Carbon Dioxide in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of nitrogen obtained from 1.0 mL of the standard gas mixture composes about 50% of the full scale.

Assay For the withdrawing of Carbon Dioxide, proceed as directed in the Purity. Place 125 mL of a solution of potassium hydroxide (1 in 2) in a gas pipet of suitable capacity. Measure exactly about 100 mL of Carbon Dioxide in a 100-mL gas buret filled with water. Force the entire volume of gas into the gas pipet, and shake for 5 minutes. Draw some of the unabsorbed gas into the gas buret, measure the volume, force the residual back upon the surface of the liquid in the gas pipet, and repeat this procedure until a constant volume of the residual reading is obtained. Determine the volume V (mL) of the residual gas, and correct its volume V to 20°C and under a pressure of 101.3 kPa.

Volume (mL) of CO_2

= calculated volume (mL) of the sample

– calculated volume V (mL)

Containers and storage Containers—Pressure resistant metal cylinders.

Storage—Not exceeding 40°C.

Carmellose

Carboxymethylcellulose

CMC

カルメロース

Carmellose is a polycarboxymethylether of cellulose.

Description Carmellose occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It swells with water to form suspension.

It becomes viscid in sodium hydroxide TS.

The pH of a suspension, obtained by shaking 1.0 g of Carmellose with 100 mL of water, is between 3.5 and 5.0.

It is hygroscopic.

Identification (1) Shake well 0.1 g of Carmellose with 10 mL of water, add 2 mL of sodium hydroxide TS, shake, and allow to stand for 10 minutes. Use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of concentrated disodium chlorotropeate TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) Shake 5 mL of the sample solution obtained in (1)

with 10 mL of acetone: a white, flocculent precipitate is produced.

(3) Shake 5 mL of the sample solution obtained in (1) with 1 mL of iron (III) chloride TS: a brown, flocculent precipitate is produced.

Purity (1) Chloride <1.03>—Shake well 0.8 g of Carmellose with 50 mL of water, dissolve in 10 mL of sodium hydroxide TS, and add water to make 100 mL. Heat 20 mL of this solution with 10 mL of dilute nitric acid on a water bath until a flocculent precipitate is produced, cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Take 25 mL of this solution, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.360%).

(2) Sulfate <1.14>—Shake well 0.40 g of Carmellose with 25 mL of water, dissolve in 5 mL of sodium hydroxide TS, and add 20 mL of water. Heat this solution with 2.5 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, and add water to make 100 mL. Filter this solution, discard 5 mL of the first filtrate, take 25 mL of the subsequent filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.5 mL of 0.005 mol/L sulfuric acid VS (not more than 0.720%).

(3) Silicate—Weigh accurately about 1 g of Carmellose, ignite in a platinum dish, add 20 mL of dilute hydrochloric acid, cover with a watch glass, and boil gently for 30 minutes. Remove the watch glass, and evaporate on a water bath to dryness with the aid of a current of air. Continue heating further for 1 hour, add 10 mL of hot water, stir well, and filter through a filter paper for quantitative analysis. Wash the residue with hot water, dry the residue together with the filter paper when no turbidity is produced on the addition of silver nitrate TS to the last washing, and ignite to constant mass: the amount of residue is not more than 0.5%.

(4) Heavy metals <1.07>—Proceed with 1.0 g of Carmellose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Take 1.0 g of Carmellose, prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 8.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 1.5% (after drying, 1 g).

Containers and storage Containers—Tight containers.

Carmellose Calcium

Carboxymethylcellulose Calcium CMC Calcium

カルメロースカルシウム

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Carmellose Calcium is the calcium salt of a polycarboxymethylether of cellulose.

♦**Description** Carmellose Calcium occurs as a white to yellowish white powder.

It is practically insoluble in ethanol (95) and in diethyl ether.

It swells with water to form a suspension.

The pH of a suspension, obtained by shaking 1.0 g of Carmellose Calcium with 100 mL of water, is between 4.5 and 6.0.

It is hygroscopic. ◆

Identification (1) Shake thoroughly 0.1 g of Carmellose Calcium with 10 mL of water, followed by 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of chromotropic acid TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) Shake 5 mL of the sample solution obtained in (1) with 10 mL of acetone: a white, flocculent precipitate is produced.

(3) Shake 5 mL of the sample solution obtained in (1) with 1 mL of iron (III) chloride TS: a brown, flocculent precipitate is produced.

(4) Ignite 1 g of Carmellose Calcium to ash, dissolve the residue in 10 mL of water and 6 mL of acetic acid (31), and filter, if necessary. Boil the filtrate, cool, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> (1) and (3) for calcium salt.

Purity (1) Alkalinity—Shake thoroughly 1.0 g of Carmellose Calcium with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops.

(2) Chloride <1.03>—Shake thoroughly 0.80 g of Carmellose Calcium with 50 mL of water, add 10 mL of sodium hydroxide TS to dissolved, add water to make 100 mL, and use this solution as the sample solution. Heat 20 mL of the sample solution with 10 mL of 2 mol/L nitric acid TS on a water bath until a flocculent precipitate is produced. After cooling, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Take 25 mL of this solution, and add 1 mL of nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.36%).

(3) Sulfate <1.14>—Heat 10 mL of the sample solution

obtained in (2) with 1 mL of hydrochloric acid in a water bath until a flocculent precipitate is produced. Cool, centrifuge, and take out the supernatant liquid. Wash the precipitate with three 10-mL portions of water by centrifuging each time, combine the supernatant and the washings, and add water to make 100 mL. Perform the test with 25 mL this solution as the test solution. Prepare the control solution with 0.42 mL of 0.005 mol/L sulfuric acid VS. To the test solution and the control solution add 1 mL of 3 mol/L hydrochloric acid and 3 mL of barium chloride TS, then add water to make 50 mL, and mix. Allow to stand for 10 minutes, and compare the turbidity of these solutions: the turbidity obtained with the test solution is not more than that obtained with the control solution (not more than 1.0%).

♦(4) Heavy metals <1.07>—Proceed with 1.0 g of Carmellose Calcium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).♦

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> 10 – 20% (after drying 1 g).

♦**Containers and storage** Containers—Tight containers.♦

Carmellose Sodium

Carboxymethylcellulose Sodium CMC Sodium

カルメロースナトリウム

Carmellose Sodium is the sodium salt of a polycarboxymethylether of cellulose.

It, when dried, contains not less than 6.5% and not more than 8.5% of sodium (Na: 22.99).

Description Carmellose Sodium occurs as a white to yellowish white powder or granules. It has no taste.

It is practically insoluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether.

It forms a viscid solution in water and in warm water.

It is hygroscopic.

Identification (1) Dissolve 0.2 g of Carmellose Sodium in 20 mL of warm water with stirring, cool, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 5 mL. To 1 drop of this solution add 0.5 mL of concentrated disodium chlomotropate TS, and heat in a water bath for 10 minutes: a red-purple color develops.

(2) To 10 mL of the sample solution obtained in test (1) add 1 mL of copper (II) sulfate TS: a blue flocculent precipitate is produced.

(3) To 3 g of Carmellose Sodium add 20 mL of methanol and 2 mL of dilute hydrochloric acid, boil gently on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, and add 20 mL of water to the residue: the solution responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54> Add 1.0 g of Carmellose Sodium in small portions to 100 mL of warm water with stirring, dissolve, and cool: the pH of this solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Firmly attach a

glass plate of good quality 2 mm in thickness, to the bottom of a glass column 250 mm in height, 25 mm in inner diameter and 2 mm in thickness. This is used as an outer tube. Similarly prepare an inner tube by attaching a glass plate of good quality 2 mm in thickness to the bottom of a glass column 300 mm in height, 15 mm in inner diameter and 2 mm in thickness. Dissolve 1.0 g of Carmellose Sodium in 100 mL of water, pour this solution into the outer tube, and place on a piece of white paper on which 15 parallel black lines 1 mm in width and 1 mm in interval are drawn. Moving the inner tube up and down and observing from the upper part, determine the height of the solution up to the lower edge of the inner tube when the distinction of the lines becomes impossible. Repeat the operation 3 times, and calculate the mean value: it is larger than that calculated from the similar operation, using the following control solution.

Control solution: To 5.50 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 5 mL of ethanol (95) and water to make 50 mL. Add 2 mL of barium chloride TS, mix well, and allow to stand for 10 minutes. Shake well this solution before use.

(2) Chloride <1.03>—Dissolve 0.5 g of Carmellose Sodium in 50 mL of water, and use this solution as the sample solution. Shake 10 mL of the sample solution with 10 mL of dilute nitric acid, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the supernatant liquid with the washings, and dilute with water to 200 mL. Perform the test using 50 mL of this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.640%).

(3) Sulfate <1.14>—Add 1 mL of hydrochloric acid to 10 mL of the sample solution obtained in (2), shake well, heat to produce a flocculent precipitate in a water bath, cool, and centrifuge. Separate the supernatant liquid, wash the precipitate with three 10-mL portions of water, centrifuging each time, combine the washings with the supernatant liquid mentioned above, and dilute to 50 mL with water. Take 10 mL of this solution, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.960%).

(4) Silicate—Weigh accurately about 1 g of Carmellose Sodium, ignite in a platinum dish, add 20 mL of dilute hydrochloric acid, cover with a watch glass, and boil gently for 30 minutes. Remove the watch glass, and evaporate on a water bath to dryness with the aid of a current of air. Continue heating for further 1 hour, add 10 mL of hot water, stir well, and filter through a filter paper for quantitative analysis. Wash the residue with hot water, dry together with the filter paper after no turbidity is produced on the addition of silver nitrate TS to the last washing, and then ignite to constant mass: the mass of the residue is not more than 0.5%.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Carmellose Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—To 1.0 g of Carmellose Sodium add 20 mL of nitric acid, heat gently until it becomes fluid, cool, add 5 mL of sulfuric acid, and heat until white fumes are evolved. Add, if necessary, 5 mL of nitric acid after cooling, and heat again. Repeat this operation until the solution

becomes colorless or slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again, cool, and dilute with water to 25 mL. Take 5 mL of this solution as the test solution, and perform the test. The solution has no more color than the following standard stain.

Standard stain: Without using Carmellose Sodium, proceed in the same manner, then transfer 5 mL of this solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed as directed for the test with the test solution (not more than 10 ppm).

(7) Starch—Add 2 drops of iodine TS to 10 mL of the sample solution obtained in (2): no blue color develops.

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 4 hours).

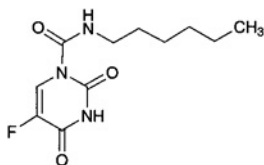
Assay Weigh accurately about 0.5 g of Carmellose Sodium, previously dried, add 80 mL of acetic acid (100), connect with a reflux condenser, and heat in an oil bath maintained at 130°C for 2 hours. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 2.299 mg of Na

Containers and storage Containers—Tight containers.

Carmofur

カルモフル



$C_{11}H_{16}FN_3O_3$; 257.26

5-Fluoro-1-(hexylaminocarbonyl)uracil [61422-45-5]

Carmofur, when dried, contains not less than 98.0% of $C_{11}H_{16}FN_3O_3$.

Description Carmofur occurs as a white crystalline powder.

It is very soluble in *N,N*-dimethylformamide, freely soluble in acetic acid (100), soluble in diethyl ether, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Melting point: about 111°C (with decomposition).

Identification (1) Proceed with 5 mg of Carmofur as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Carmofur in a mixture of methanol and phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0, (9:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorp-

tion at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carmofur, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Carmofur according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.20 g of Carmofur in 10 mL of a mixture of methanol and acetic acid (100) (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetic acid (100) (99:1) to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 15 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene and acetone (5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. After exposure of the plate to bromine vapor for 30 second, spray evenly a solution of fluorescein in ethanol (95) (1 in 2500): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 50°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Carmofur, previously dried, dissolve in 20 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide-methanol VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS).

Each mL of 0.1 mol/L tetramethylammonium hydroxide-methanol VS
= 25.73 mg of $C_{11}H_{16}FN_3O_3$

Containers and storage Containers—Tight containers.

Carnauba Wax

Cera Carnauba

カルナウバロウ

Carnauba Wax is the wax obtained from the leaves of *Copernicia cerifera* Mart (*Palmae*).

Description Carnauba Wax occurs as light yellow to light brown, hard and brittle masses or white to light yellow powder. It has a slight, characteristic odor. It is tasteless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in xylene.

Specific gravity d_{20}^{20} : 0.990 – 1.002

Melting point: 80 – 86°C

Acid value <1.13> Not more than 10.0. Use a mixture of xylene and ethanol (95) (2:1) as solvent.

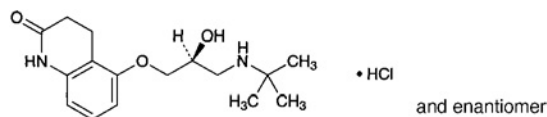
Saponification value <1.13> 78 – 95 Weigh accurately about 3 g of Carnauba Wax in a 300-mL flask, add 25 mL of xylene, and dissolve by warming. To this solution add 50 mL of ethanol (95) and exactly 25 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and proceed as directed in the Saponification value. The time of heating should be 2 hours and the titration should be done by warming.

Iodine value <1.13> 5 – 14 (Dissolve the sample by shaking a glass-stoppered flask in warm water.)

Containers and storage Containers—Well-closed containers.

Carteolol Hydrochloride

カルテオロール塩酸塩



$C_{16}H_{24}N_2O_3 \cdot HCl$: 328.83

5-[(2*RS*)-3-(1,1-Dimethylethyl)amino-2-hydroxypropyloxy]-3,4-dihydroquinolin-2(1*H*)-one monohydrochloride [51781-21-6]

Carteolol Hydrochloride, when dried, contains not less than 99.0% of $C_{16}H_{24}N_2O_3 \cdot HCl$.

Description Carteolol Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Carteolol Hydrochloride (1 in 100) is between 5.0 and 6.0.

The solution of Carteolol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 277°C (with decomposition).

Identification (1) Dissolve 0.1 g of Carteolol Hydrochloride in 5 mL of water, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Carteolol Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Carteolol Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Carteolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Carteolol Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Carteolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Carteolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Carteolol Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (50:20:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

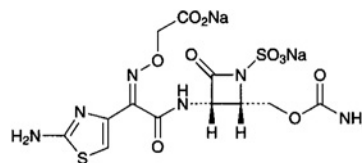
Assay Weigh accurately about 0.5 g of Carteolol Hydrochloride, previously dried, add 30 mL of acetic acid (100), dissolve by heating on a water bath, and cool. After adding 70 mL of acetic anhydride, titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $C_{16}H_{24}N_2O_3 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Carumonam Sodium

カルモナムナトリウム



$C_{12}H_{12}N_6Na_2O_{10}S_2$: 510.37

Disodium (*Z*)-{(2-aminothiazol-4-yl)[(2*S*,3*S*)-2-carbamoyloxymethyl-4-oxo-1-sulfonatoazetidin-3-ylcarbamoyl]methyleneaminoxy}acetate [86832-68-0]

Carumonam Sodium contains not less than 850 μ g (potency) and not more than 920 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Carumonam Sodium is expressed as mass (potency) of carumonam ($C_{12}H_{14}N_6O_{10}S_2$: 466.40).

Description Carumonam Sodium occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in formamide, very slightly soluble in methanol, and practically insoluble in acetic acid (100) and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Carumonam Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Carumonam Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Carumonam Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Carumonam Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Carumonam Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ5.5 ppm, and a single signal B at around δ7.0 ppm. The ratio of the integrated intensity of these signals, A:B, is about 1:1.

(4) Carumonam Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +18.5 – +21.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Carumonam Sodium in 10 mL of water is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Carumonam Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Carumonam Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Carumonam Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substance 1—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration

method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 4.0%, and each amount of the related substances other than the related substance having the relative retention time of 0.7 to the peak of carumonam is not more than 1.0%.

$$\begin{aligned} \text{Amount (\% of related substance)} \\ = (W_S/W_T) \times (A_T/A_S) \end{aligned}$$

W_S : Amount (g) of Carumonam Sodium Reference Standard

W_T : Amount (g) of the sample

A_S : Peak area of carumonam from the standard solution

A_T : Each peak area other than carumonam from the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of carumonam.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 3 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

(5) Related substance 2—Weigh accurately about 0.1 g of Carumonam Sodium, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Carumonam Sodium Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, and add the mobile phase to make exactly 25 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of each related substance is not more than 1.0%.

$$\begin{aligned} \text{Amount (\% of related substance)} \\ = (W_S/W_T) \times (A_T/A_S) \end{aligned}$$

W_S : Amount (g) of Carumonam Sodium Reference Standard

W_T : Amount (g) of the sample

A_S : Peak area of carumonam from the standard solution

A_T : Each area of the peaks appeared after the peak of carumonam from the sample solution

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (74:25:1).

Flow rate: Dissolve 0.01 g of phthalic acid in the mobile phase to make 100 mL. Adjust the flow rate so that the retention time of phthalic acid is about 6.5 minutes when the procedure is run with 10 μ L of this solution.

Time span of measurement: About 10 times as long as the retention time of carumonam.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of carumonam obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the standard solution.

System performance: Dissolve 40 mg of Carumonam Sodium in 20 mL of the mobile phase. To 5 mL of this solution add 5 mL of a solution of resorcinol in the mobile phase (9 in 1000) and the mobile phase to make 25 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, resorcinol and carumonam are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of carumonam is not more than 2.0%.

(6) Total amount of related substances—The total of the amounts of the related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.0%.

Water <2.48> Not more than 2.0% (0.2 g, volumetric titration, direct titration; Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Carumonam Sodium and Carumonam Sodium Reference Standard, equivalent to about 40 mg (potency), and dissolve each in the mobile phase to make exactly 20 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of carumonam to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of carumonam (C}_{12}\text{H}_{14}\text{N}_6\text{O}_{10}\text{S}_2) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Carumonam Sodium Reference Standard

Internal standard solution—A solution of resorcinol in the mobile phase (9 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 10,000), methanol and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of carumonam is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and carumonam are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of carumonam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Castor Oil

Oleum Ricini

ヒマシ油

Castor Oil is the fixed oil obtained by compression from the seeds of *Ricinus communis* Linné (*Euphorbiaceae*).

Description Castor Oil is a colorless or pale yellow, clear, viscous oil. It has a slight, characteristic odor, and has a bland at first, and afterwards slightly acrid taste.

It is miscible with ethanol (99.5) and with diethyl ether.

It is freely soluble in ethanol (95), and practically insoluble in water.

When cooled to 0°C, it becomes more viscous, and turbidity is gradually formed.

Identification To 3 g of Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a characteristic odor is perceptible. Dissolve the fused matter in 30 mL of water, add an excess of magnesium oxide, and filter. Acidify the filtrate with hydrochloric acid: white crystals is produced.

Specific gravity <1.13> d_{25}^{25} : 0.953 – 0.965

Acid value <1.13> Not more than 1.5.

Saponification value <1.13> 176 – 187

Hydroxyl value <1.13> 155 – 177

Iodine value <1.13> 80 – 90

Purity Adulteration—Shake to mix 1.0 g of Castor Oil with 4.0 mL of ethanol (95): it dissolves clearly. Add 15 mL of ethanol (95): no turbidity is produced.

Containers and storage Containers—Tight containers.

Aromatic Castor Oil

加香ヒマシ油

Method of preparation

Castor Oil	990 mL
Orange Oil	5 mL
Mentha Oil	5 mL
To make 1000 mL	

Mix the above ingredients.

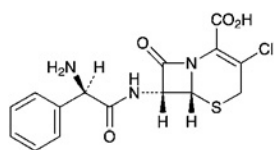
Description Aromatic Castor Oil is a colorless or yellowish, clear, viscous liquid. It has an aromatic odor.

Identification To 3 g of Aromatic Castor Oil add 1 g of potassium hydroxide, and heat the mixture carefully to fuse: a characteristic odor is perceptible. Dissolve the fused matter in 30 mL of water, add an excess of magnesium oxide, and filter. Acidify the filtrate with hydrochloric acid: white crystals are produced.

Containers and storage Containers—Tight containers.

Cefaclor

セファクロル



$C_{15}H_{14}ClN_3O_4S$: 367.81

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetamino]-3-chloro-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [53994-73-3]

Cefaclor contains not less than 950 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefaclor is expressed as mass (potency) of cefaclor ($C_{15}H_{14}ClN_3O_4S$).

Description Cefaclor occurs as a white to yellowish white crystalline powder.

It is slightly soluble in water and in methanol, and practically insoluble in *N,N*-dimethylformamide and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefaclor (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefaclor as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 40 mg of Cefaclor in 0.5 mL of heavy water

for nuclear magnetic resonance spectroscopy and 1 drop of deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits an AB type quartet signal A at around δ 3.7 ppm, and a single signal or a sharp multiple signal B at around δ 7.6 ppm. The ratio of the integrated intensity of each signal, A:B, is about 2:5.

(4) Perform the test with Cefaclor as directed under Flame Coloration Test <1.04> (2): a green color appears.

Optical rotation <2.49> $[\alpha]_D^{20}$: +105 – +120° (0.1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefaclor according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution by suspending 1.0 g of Cefaclor in 10 mL of *N,N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Cefaclor in 10 mL of sodium dihydrogen phosphate TS, pH 2.5, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas other than cefaclor from the sample solution are not more than 1/2 of the peak area of cefaclor from the standard solution, and the total of the peak areas other than cefaclor from the sample solution is not more than 2 times of the peak area of cefaclor from the standard solution. If necessary, proceed with 20 μ L of sodium dihydrogen phosphate TS, pH 2.5 in the same manner as above to compensate the base line.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.8 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water, and adjust the pH to 4.0 with phosphoric acid.

Mobile phase B: To 550 mL of the mobile phase A add 450 mL of acetonitrile for liquid chromatography.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	95 → 75	5 → 25
30 – 45	75 → 0	25 → 100
45 – 55	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefaclor beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add sodium dihydrogen phosphate TS, pH 2.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained from 20 μ L of this solution is equivalent to 4 to 6% of that from 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 steps and 0.8 to 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviations of the peak areas and the retention times of cefaclor are not more than 2.0%, respectively.

Water <2.48> Not more than 6.5% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefaclor and Cefaclor Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefaclor to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S} \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.4 with diluted phosphoric acid (3 in 500). To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefaclor is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cefaclor and the internal standard are eluted in

this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefaclor to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefaclor Capsules

セファクロルカプセル

Cefaclor Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefaclor ($\text{C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S}$; 367.81).

Method of preparation Prepare as directed under Capsules, with Cefaclor.

Identification Shake vigorously a quantity of the contents of one capsule of Cefaclor Capsules, equivalent to 20 mg (potency) of Cefaclor according to the labeled amount, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of Cefaclor Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same R_f value.

Purity Related substances—Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.25 g (potency) of Cefaclor, shake with 40 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45- μ m pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor Reference Standard, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Pipet 2.5 mL of this solution, add the same buffer solution to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related sub-

stance is not more than 0.5%, and the total amount of the related substances is not more than 2.5%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 20 μ L of 0.1 mol/L phosphate buffer solution, pH 4.5.

Amount (%) of each related substance

$$= (W_S/W_T) \times (A_{Ti}/A_S) \times (W_M/C) \times (25/2)$$

Total amount (%) of the related substances

$$= (W_S/W_T) \times (\Sigma A_{Tn}/A_S) \times (W_M/C) \times (25/2)$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

W_T : Amount (mg) of the contents of Cefaclor Capsules

W_M : Average mass (mg) of the contents in 1 capsule

A_{Ti} : Area of each peak other than cefaclor and solvent from the sample solution

A_S : Peak area of cefaclor from the standard solution

C : Labeled potency [mg (potency)] of Cefaclor in 1 capsule

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 20 μ L of this solution is equivalent to 3.5 to 6.5% of that of cefaclor obtained with 20 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Purity (3) under Cefaclor.

Water <2.48> Not more than 8.0% (0.2 g, volumetric titration, back titration).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with one Cefaclor Capsules at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 15 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.5 μ m. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μ g (potency) of Cefaclor according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor Reference Standard, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S : Amount [mg (potency)] of Cefaclor Reference Stan-

ard

C : Labeled amount [mg (potency)] of Cefaclor in 1 capsule

Assay Weigh accurately not less than 5 Cefaclor Capsules, open the capsules and carefully take out the contents, mix well, and powder, if necessary. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow the capsules to stand at room temperature to vaporize adhering diethyl ether, and weigh accurately the capsules to calculate the mass of the contents. Weigh accurately a quantity of the contents, equivalent to about 0.1 g (potency) of Cefaclor according to the labeled amount, shake vigorously with 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefaclor Reference Standard, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefaclor.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) \\ &= W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefaclor Fine Granules

セファクロル細粒

Cefaclor Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$; 367.81).

Method of preparation Prepare fine granules as directed under Powders, with Cefaclor.

Identification Shake vigorously a quantity of Cefaclor Fine Granules, equivalent to 20 mg (potency) of Cefaclor according to the labeled amount, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg (potency) of Cefaclor Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same R_f value.

Purity Related substances—Weigh accurately a quantity of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g (potency) of Cefaclor according to the labeled amount, shake with 40 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 50 mL, and filter through a 0.45- μ m pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor Reference Standard, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Pipet 2 mL of this solution, add the same buffer solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.5%, and the total amount of the related substances is not more than 3.0%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μ L of 0.1 mol/L phosphate buffer solution, pH 4.5.

Amount (%) of each related substance

$$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 5$$

Total amount (%) of the related substances

$$= (W_S/W_T) \times (\Sigma A_T/A_S) \times (1/C) \times 5$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

W_T : Amount (g) of sample

A_T : Area of the peak other than cefaclor and the solvent from the sample solution

A_S : Peak area of cefaclor from the standard solution

C : Labeled potency [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$) per g of the sample

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 50 μ L of this solution is equivalent to 3.5 to 6.5% of that of cefaclor obtained with 50 μ L of the standard solution.

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water <2.48> Not more than 1.5% (1 g, volumetric titration, back titration).

Uniformity of dosage units <6.02> The granules in single-unit container meet the requirement of the Mass variation

test.

Dissolution <6.10> Perform the test according to the following method: It meets requirement.

Perform the test with an accurately weighed quantity of Cefaclor Fine Granules, equivalent to about 0.25 g (potency) of Cefaclor according to the labeled amount, at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 15 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μ m. Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 20 μ g (potency) of Cefaclor according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor Reference Standard, and dissolve in water to make exactly 20 mL. Pipet 1 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 15 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of cefaclor ($C_{15}H_{14}ClN_3O_4S$)

$$= (W_S/W_T) \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

W_T : Amount [mg (potency)] of sample

C : Labeled amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$) per g of Cefaclor Fine Granules

Particle size <6.03> It meets the requirement of fine granules of the Powders.

Assay Weigh accurately a quantity of Cefaclor Fine Granules after powdered if necessary, equivalent to about 0.1 g (potency) of Cefaclor according to the labeled amount, shake vigorously with 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 10 minutes, add the same buffer solution to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefaclor Reference Standard, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as standard solution. Proceed as directed in the Assay under Cefaclor.

$$\text{Amount [mg (potency)] of cefaclor } (C_{15}H_{14}ClN_3O_4S) \\ = W_S \times (Q_T/Q_S) \times 2$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cefaclor Compound Granules

セファクロル複合顆粒

Cefaclor Compound Granules contain gastric granules and enteric granules in one pack.

It contains cefaclor ($C_{15}H_{14}ClN_3O_4S$; 367.81) equivalent to not less than 90.0% and not more than 110.0% of the labeled total potency and the labeled potency of gastric granule, respectively.

Method of preparation Prepare as directed under Granules, with Cefaclor, and divide into packs.

Identification Shake vigorously a quantity of Cefaclor Compound Granules, equivalent to 20 mg (potency) of Cefaclor according to the labeled total potency, with 10 mL of water, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of Cefaclor Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetonitrile, water, ethyl acetate and formic acid (30:10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the principal spot from the sample solution and the spot from the standard solution show the same *R_f* value.

Purity Related substances—Weigh accurately not less than 5 Cefaclor Compound Granules, transfer their total contents to a mortar, add a little amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind well, add the same buffer solution to make exactly *V* mL so that each mL contains about 2 mg (potency) of Cefaclor according to the labeled total potency, and filter through a 0.45- μ m pore-size membrane filter. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefaclor Reference Standard, and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Pipet 2 mL of this solution, add the same buffer solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of each related substance by the following equation: the amount of each related substance is not more than 0.6%, and the total amount of the related substances is not more than 2.8%. If necessary, correct the fluctuation of the base line by performing the test in the same way with 50 μ L of 0.1 mol/L phosphate buffer solution, pH 4.5.

Amount (%) of each related substance

$$= W_s \times (A_{Ti}/A_s) \times \{V/(C \times T)\} \times 1/10$$

Total amount (%) of the related substances

$$= W_s \times (\sum A_{Ti}/A_s) \times \{V/(C \times T)\} \times 1/10$$

W_s: Amount [mg (potency)] of Cefaclor Reference Stan-

dard

A_{Ti}: Area of each peak other than cefaclor, solvent and excipient from the sample solution

A_s: Peak area of cefaclor from the standard solution

C: Labeled total potency [mg (potency)] of cefaclor in 1 pack

T: Number (pack) of sample

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefaclor.

System suitability—

Test for required detectability: Pipet 1 mL of standard solution, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 20 mL. Confirm that the peak area of cefaclor obtained with 50 μ L of this solution is equivalent to 3.5 to 6.5% of that of cefaclor obtained with 50 μ L of the standard solution.

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefaclor are not less than 40,000 and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefaclor is not more than 2.0%.

Water <2.48> Not more than 5.5% (0.3 g, volumetric titration, back titration).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

(1) Total potency—Take out the total contents of 1 Cefaclor Compound Granules, add a little amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind well, add the same buffer solutions to make exactly *V* mL so that each mL contains about 3.8 mg (potency) of Cefaclor according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 3 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

Amount [mg (potency)] of cefaclor ($C_{15}H_{14}ClN_3O_4S$)

$$= W_s \times (Q_T/Q_S) \times (V/15)$$

W_s: Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

(2) Potency of gastric granule—Stir gently the total contents of 1 Cefaclor Compound Granules with 60 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 5 minutes, add the same buffer solution to make exactly *V* mL so that each mL contains about 1.5 mg (potency) of Cefaclor according to

the labeled potency of gastric granule, and centrifuge. Pipet 7 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefaclor (C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S)} \\ &= W_S \times (Q_T/Q_S) \times (V/35) \end{aligned}$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 Cefaclor Compound Granules at 50 revolutions per minute according to the Paddle method using 900 mL of the 1st fluid for dissolution test as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 60 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add the 1st fluid for dissolution test to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled potency of gastric granule, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 20 mg (potency), and dissolve in the 1st fluid for dissolution test to make exactly 20 mL. Pipet 2 mL of this solution, add the 1st fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 60 minutes is between 35% and 45%.

Dissolution rate (%) of cefaclor (C₁₅H₁₄ClN₃O₄S) with respect to the labeled potency

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

Separately, perform the test with 1 Cefaclor Compound Granules at 50 revolutions per minute according to the Paddle method using 900 mL of 2nd fluid for dissolution method as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 60 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.5 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 0.01 mol/L hydrochloric acid TS to make exactly V' mL so that each mL contains about 20 μg (potency) of Cefaclor according to the labeled total potency, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Refer-

ence Standard, equivalent to about 20 mg (potency), dissolve in 2nd fluid for dissolution test to make exactly 100 mL, and warm at 37°C for 60 minutes. Pipet 2 mL of this solution, add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 265 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> using 0.01 mol/L hydrochloric acid TS as the blank. The dissolution rate in 60 minutes is not less than 70%.

Dissolution rate (%) of cefaclor (C₁₅H₁₄ClN₃O₄S) with respect to the labeled potency

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

C: Labeled total potency [mg (potency)] of Cefaclor in 1 pack

Particle size <6.03> It meets the requirement.

Assay (1) Total potency—Take out the total contents of not less than 5 Cefaclor Compound Granules, add a small amount of 0.1 mol/L phosphate buffer solution, pH 4.5, grind well, add the same buffer solution to make a solution containing about 5 mg (potency) of Cefaclor per mL according to the labeled total potency after shaking vigorously for 10 minutes, and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefaclor (C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S)} \\ &= W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

(2) Potency of gastric granule—Stir gently the total contents of not less than 5 Cefaclor Compound Granules with about 100 mL of 0.1 mol/L phosphate buffer solution, pH 4.5 for 5 minutes, the same buffer solution so that each mL containing about 2 mg (potency) of Cefaclor according to the labeled potency of gastric granule, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefaclor Reference Standard, equivalent to about 50 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the same buffer solution to make 50 mL, and use this solution as the standard solution. Then, proceed as directed in the Assay under Cefaclor.

$$\text{Amount [mg (potency)] of cefaclor (C}_{15}\text{H}_{14}\text{ClN}_3\text{O}_4\text{S)}$$

$$= W_S \times (Q_T/Q_S) \times 2$$

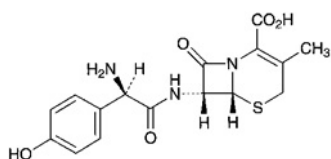
W_S : Amount [mg (potency)] of Cefaclor Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 700).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefadroxil

セファドロキシル



$C_{16}H_{17}N_3O_5S$: 363.39
(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl-amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[50370-12-2]

Cefadroxil contains not less than 950 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefadroxil is expressed as mass (potency) of cefadroxil ($C_{16}H_{17}N_3O_5S$).

Description Cefadroxil occurs as a white to light yellow-white powder.

It is sparingly soluble in water, slightly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefadroxil (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefadroxil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefadroxil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefadroxil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefadroxil in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits a single signal A at around δ 2.1 ppm, a double signal B at around δ 7.0 ppm, and a double signal C at around δ 7.5 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:2:2.

Optical rotation <2.49> $[\alpha]_D^{25}$: +164 – +182° (0.6 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefadroxil in 200 mL of water: pH of the solution is between 4.0 and 6.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefadroxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.1 g of Cefadroxil in 4 mL of a mixture of ethanol (99.5), water and diluted hydrochloric acid (1 in 5) (75:22:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (99.5), water and diluted hydrochloric acid (1 in 5) (75:22:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ethyl acetate, water, ethanol (99.5) and formic acid (14:5:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not less than 4.2% and not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefadroxil and Cefadroxil Reference Standard equivalent to about 50 mg (potency), dissolve each in water to make exactly 500 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefadroxil of the solutions.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of } C_{16}H_{17}N_3O_5S \\ = W_S \times (A_T/A_S) \times 1000$$

W_S : amount [mg (potency)] of Cefadroxil Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (17 in 12,500) and methanol (17:3).

Flow rate: Adjust the flow rate so that the retention time of cefadroxil is about 5 minutes.

System suitability—

System performance: Dissolve about 5 mg (potency) of cefadroxil and about 10 mg (potency) of propylene glycol cefatrizine in 50 mL of water. When the procedure is run with 10 μ L of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

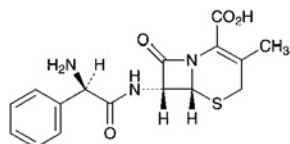
System repeatability: When the test is repeated 6 times with

10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefadroxil is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefalexin

セファレキシシ



$\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$: 347.39

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-phenylacetyl-amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [15686-71-2]

Cefalexin contains not less than 950 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalexin is expressed as mass (potency) of cefalexin ($\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$).

Description Cefalexin occurs as a white to light yellowish white, crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in ethanol (95) and in *N,N*-dimethylformamide.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefalexin (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalexin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefalexin in heavy water for nuclear magnetic resonance spectroscopy (1 in 200) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 1.8 ppm, and a single or a sharp multiple signal B at around δ 7.5 ppm. The ratio of integrated intensity of these signals, A:B, is about 3:5.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +144 – +158° (0.125 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefalexin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefalexin by suspending in 10 mL of *N,N*-dimethylformamide, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve about 25 mg of Cefalexin in a solution of potassium dihydrogenphosphate (9 in 500) to make 5 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method. If necessary, correct the change of the base-line due to the potassium dihydrogenphosphate solution by proceeding in the same manner with 20 μL of a solution of potassium dihydrogenphosphate (9 in 500): each peak area other than cefalexin from the sample solution is not more than the peak area of cefalexin from the standard solution, and the total of the peak areas which are bigger than 1/50 of the peak area of cefalexin from the standard solution and those other than cefalexin from the sample solution is not more than 5 times of the peak area of cefalexin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 1.0 g of sodium 1-pentanesulfonate in 1000 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid.

Mobile phase B: Dissolve 1.0 g of sodium 1-pentanesulfonate in 300 mL of water, add 15 mL of triethylamine, and adjust to pH 2.5 with phosphoric acid. To this solution add 350 mL of acetonitrile and 350 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 1	100	0
1 – 34.5	100→0	0→100
34.5 – 35.5	0	100

Flow rate: 1.0 mL per minute

Time span of measurement: About 2 times as long as the retention time of cefalexin beginning after the solvent peak.
System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add a solution of potassium dihydrogenphosphate (9 in 500) to make exactly 100 mL. Confirm that the peak area of cefalexin obtained from 20 μL of this solution is equivalent to 1.8 to 2.2% of that of cefalexin obtained from 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefalexin are not less than 150,000 steps and between 0.8 and 1.3, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the retention time and the peak areas of cefalexin are not more than 2.0%, respectively.

Water <2.48> Not more than 8.0% (0.2 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefalexin and Cefalexin Reference Standard, equivalent to about 0.1 g (potency), dissolve each in 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Pipet 10 mL of these solutions, add exactly 5 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution, pH 4.5 to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefalexin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : amount [mg (potency)] of Cefalexin Reference Standard

Internal standard solution—A solution of *m*-hydroxyacetophenone in 0.1 mol/L phosphate buffer solution, pH 4.5 (1 in 1500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 1000 mL of water, adjust to pH 3.0 with diluted phosphoric acid (3 in 500). To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefalexin is about 7 minutes.

System suitability—

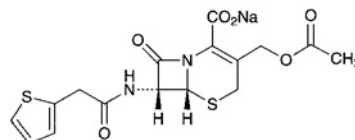
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefalexin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefalexin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefalotin Sodium

セファロチンナトリウム



$\text{C}_{16}\text{H}_{15}\text{N}_2\text{NaO}_6\text{S}_2$: 418.42

Monosodium (6*R*,7*R*)-3-acetoxymethyl-8-oxo-7-[2-(thiophen-2-yl)acetyl-amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [58-71-9]

Cefalotin Sodium contains not less than 910 μg (potency) and not more than 980 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefalotin Sodium is expressed as mass (potency) of cefalotin ($\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_6\text{S}_2$: 396.44).

Description Cefalotin Sodium occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Cefalotin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefalotin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefalotin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefalotin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefalotin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around $\delta 2.1$ ppm, a single or sharp multiple signal B at around $\delta 3.9$ ppm, and a multiple signal C at around $\delta 7.0$ ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:2.

(4) Cefalotin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{25}$: +124 – +134° (5 g, water, 100 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Cefalotin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Cefalotin Sodium in 5 mL of water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefalotin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefalotin Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Pipet 1 mL of the standard solution obtained in the Assay, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution obtained in the Assay and the standard solution prepared here as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefalotin from the sample solution is not more than the peak area of cefalotin from the standard solution, and the total area of the peaks other than cefalotin from the sample solution is not more than 3 times the peak area of cefalotin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefalotin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefalotin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the standard solution.

System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin, is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 3 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 2.0%.

Water <2.48> Not more than 1.0% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefalotin Sodium and Cefalotin Sodium Reference Standard, equivalent to about 25 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of cefalotin of the solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefalotin } (\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_6\text{S}_2) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefalotin Sodium Refer-

ence Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 17 g of sodium acetate trihydrate in 790 mL of water, and add 0.6 mL of acetic acid (100). If necessary adjust the pH to 5.9 ± 0.1 with 0.1 mol/L sodium hydrochloride TS or acetic acid (100). To this solution add 150 mL of acetonitrile and 70 mL of ethanol (95).

Flow rate: Adjust the flow rate so that the retention time of cefalotin is about 12 minutes.

System suitability—

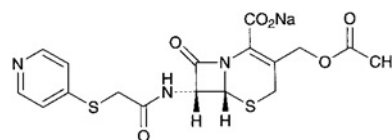
System performance: Heat the standard solution in a water bath of 90°C for 10 minutes, and cool. Measure exactly 2.5 mL of this solution, and add the mobile phase to make exactly 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, the resolution between the peak of cefalotin and the peak, having the relative retention time of about 0.5 with respect to cefalotin is not less than 9, and the symmetry factor of the peak of cefalotin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefalotin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefapirin Sodium

セファピリンナトリウム



$\text{C}_{17}\text{H}_{16}\text{N}_3\text{NaO}_6\text{S}_2$: 445.45
 Monosodium (6*R*,7*R*)-3-acetoxymethyl-8-oxo-7-[2-(pyridin-4-ylsulfanyl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
 [24356-60-3]

Cefapirin Sodium contains not less than 865 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefapirin Sodium is expressed as mass (potency) of cefapirin ($\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}_6\text{S}_2$: 423.46).

Description Cefapirin Sodium occurs as a white to yellowish white powder.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in acetone.

Identification (1) Determine the absorption spectrum of a solution of Cefapirin Sodium (3 in 200,000) as directed under

Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefapirin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefapirin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefapirin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefapirin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-(trimethylsilyl)propionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H): it exhibits a single signal A at around δ 2.2 ppm, and multiple signals, B and C, at around δ 7.3 ppm and at around δ 8.3 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C, is about 3:2:2.

(4) Cefapirin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{25}$: +157 – +175° (2 g calculated as the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefapirin Sodium in 10 mL of water: pH of the solution is between 6.5 and 8.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefapirin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefapirin Sodium according to Method 3, and perform the test (not more than 2 ppm). Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 25).

(3) Related substances—Dissolve 0.1 g of Cefapirin Sodium in 5 mL of a mixture of acetone and water (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetone and water (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, acetone, water and acetic acid (100) (5:2:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot at the original point from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 2.0% (0.7 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefapirin Sodium and Cefapirin Sodium Reference Standard equivalent to about 0.1 g (potency), dissolve each in phosphate buffer solution, pH 6.0 to make exactly 100 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution

and phosphate buffer solution, pH 6.0 to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefapirin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefapirin (C}_{17}\text{H}_{17}\text{N}_3\text{O}_6\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Cefapirin Sodium Reference Standard

Internal standard solution—A solution of vanillin (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 2.6 and acetonitrile (93:7).

Flow rate: Adjust the flow rate so that the retention time of cefapirin is about 7 minutes.

System suitability—

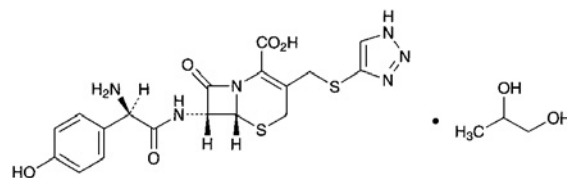
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, cefapirin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefapirin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefatrizine Propylene Glycolate

セファトリジンプロピレングリコール



$\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_2 \cdot \text{C}_3\text{H}_8\text{O}_2$: 538.60
(6*R*, 7*R*)-7-[(2*R*)-2-Amino-2-(4-hydroxyphenyl)acetyl-amino]-8-oxo-3-[2-(1*H*-1,2,3-triazol-4-yl)sulfanylmethyl]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monopropylene-1,2-diolate (1/1)
[51627-14-6, Cefatrizine]

Cefatrizine Propylene Glycolate contains not less than 785 μg (potency) and not more than 876 μg

(potency) per mg, calculated on the anhydrous basis. The potency of Cefatrizine Propylene Glycolate is expressed as mass (potency) of cefatrizine ($C_{18}H_{18}N_6O_5S_2$; 462.50).

Description Cefatrizine Propylene Glycolate occurs as a white to yellowish white powder.

It is sparingly soluble in water, and practically insoluble in methanol and in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefatrizine Propylene Glycolate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefatrizine Propylene Glycolate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefatrizine Propylene Glycolate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefatrizine Propylene Glycolate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefatrizine Propylene Glycolate in a mixture of heavy water for nuclear magnetic resonance spectroscopy and deuterated hydrochloric acid for nuclear magnetic resonance spectroscopy (3:1) (1 in 10), using sodium 3-(trimethylsilyl)propionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits a double signal A at around δ 1.2 ppm, a double signal B at around δ 7.0 ppm, a double signal C at around δ 7.5 ppm and a single signal D at around δ 8.3 ppm. The ratio of integrated intensity of these signals, A:B:C:D, is about 3:2:2:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +52 – +58° (2.5 g calculated on the anhydrous bases, 1 mol/L hydrochloric acid TS, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefatrizine Propylene Glycolate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefatrizine Propylene Glycolate according to Method 3, and perform the test (not more than 2 ppm). Use a solution of magnesium nitrate hexahydrate in ethanol (1 in 25).

(3) Related substances—Dissolve 25 mg of Cefatrizine Propylene Glycolate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly ninhydrin-citric acid-acetic acid TS on the plate, and heat at 100°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titra-

tion, direct titration).

Assay Weigh accurately an amount of Cefatrizine Propylene Glycolate and Cefatrizine Propylene Glycolate Reference Standard equivalent to about 0.1 g (potency), dissolve each in water to make exactly 500 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefatrizine of these solutions

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefatrizine } (C_{18}H_{18}N_6O_5S_2) \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefatrizine Propylene Glycolate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (17 in 12,500) and methanol (17:3).

Flow rate: Adjust the flow rate so that the retention time of cefatrizine is about 11 minutes.

System suitability—

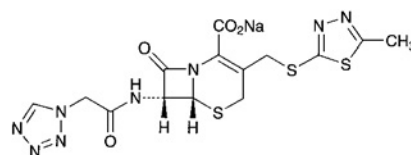
System performance: Dissolve about 5 mg (potency) of Cefadroxil and about 10 mg (potency) of Cefatrizine Propylene Glycolate in 50 mL of water. When the procedure is run with 10 μ L of this solution under the above operating conditions, cefadroxil and cefatrizine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefatrizine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefazolin Sodium

セファゾリンナトリウム



$C_{14}H_{13}N_8NaO_4S_3$; 476.49

Monosodium (6*R*,7*R*)-3-(5-methyl-1,3,4-thiadiazol-2-ylsulfanylmethyl)-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetylamino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [27164-46-1]

Cefazolin Sodium contains not less than 900 μ g (potency) and not more than 975 μ g (potency) per mg,

calculated on the anhydrous basis. The potency of Cefazolin Sodium is expressed as mass (potency) of cefazolin ($C_{14}H_{14}N_8O_4S_3$; 454.51).

Description Cefazolin Sodium occurs as a white to light yellow-white, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefazolin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethylsilylpropionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits single signals, A and B, at around δ 2.7 ppm and at around δ 9.3 ppm, respectively. The ratio of integrated intensity of these signals, A:B, is about 3:1.

(4) Cefazolin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-19 - -23^\circ$ (2.5 g calculated as the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefazolin Sodium in 10 mL of water: pH of the solution is between 4.8 and 6.3.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefazolin Sodium in 10 mL of water: the solution is clear and colorless to pale yellow, and its absorbance at 400 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.35. The test should be performed within 10 minutes after preparing of the solution.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefazolin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Cefazolin Sodium according to Method 3, and perform the test. When prepare the test solution, add 1.5 mL of hydrogen peroxide (30) after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), and then ignite (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Cefazolin Sodium in 20 mL of 0.1 mol/L phosphate buffer solution, pH 7.0 and use this solution as the sample solution. Prepare the sample solution before use. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the areas of a peak appeared at the relative retention time of about 0.2 to the retention time of cefazolin and peaks other than cefazolin by the automatic integration method, and calculate the amounts of these peak areas by the area percent-

tage method: the amount of each peak area is not more than 1.5%, and the total area of the peaks other than cefazolin is not more than 2.5%. The area of the peak appeared at the relative retention time of about 0.2 to the retention time of cefazolin obtained here is used after multiplying by its sensitivity coefficient, 1.43.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefazolin beginning after the solvent peak.

System suitability—

Test for required detection: Dissolve about 80 mg of Cefazolin Reference Standard in 0.1 mol/L phosphate buffer solution, pH 7.0 to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL. Confirm that the peak area of cefazolin obtained from 5 μ L of this solution is equivalent to 3 to 7% of that of cefazolin obtained from 5 μ L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefazolin is not more than 1.0%.

Water <2.48> Not more than 2.5% (1.0 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefazolin Sodium and Cefazolin Reference Standard, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefazolin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefazolin } (C_{14}H_{14}N_8O_4S_3) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Cefazolin Reference Standard

Internal standard solution—A solution of *p*-acetoanisidide in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL, and add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability—

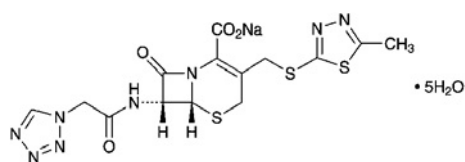
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, cefazolin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefazolin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefazolin Sodium Hydrate

セファゾリンナトリウム水和物



$C_{14}H_{13}N_8NaO_4S_3 \cdot 5H_2O$: 566.57

Monosodium (6*R*,7*R*)-3-(5-methyl-1,3,4-thiadiazol-2-ylsulfanylmethyl)-8-oxo-7-[2-(1*H*-tetrazol-1-yl)acetyl]amino-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate [115850-11-8]

Cefazolin Sodium Hydrate contains not less than 920 μ g (potency) and not more than 975 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefazolin Sodium Hydrate is expressed as mass (potency) of cefazolin ($C_{14}H_{14}N_8O_4S_3$: 454.51).

Description Cefazolin Sodium Hydrate occurs as white to pale yellowish white crystals.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Cefazolin Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 270 nm and 274 nm.

(2) Determine the infrared absorption spectrum of Cefazolin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1761 cm^{-1} , 1667 cm^{-1} , 1599 cm^{-1} , 1540 cm^{-1} and 1389 cm^{-1} .

(3) Determine the spectrum of a solution of Cefazolin Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10), using sodium 3-trimethylsilylpropionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits single signals, A and B, at around δ 2.7 ppm and at around δ 9.3 ppm. The ratio of integrated intensity of each signal, A:B, is about 3:1.

(4) Cefazolin Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Absorbance <2.24> $E_{1cm}^{1\%}$ (272 nm): 272 – 292 (80 mg calculated on the anhydrous basis, water, 5000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: –20 – –25° (2.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefazolin Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.8 and 6.3.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefazolin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Being specified separately.

(4) Related substances—Being specified separately.

(5) Residual solvents—Being specified separately.

Water <2.48> Not less than 13.7% and not more than 16.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Bacterial endotoxins <4.01> Less than 0.10 EU/mg (potency).

Assay Weigh accurately an amount of Cefazolin Sodium Hydrate and Cefazolin Reference Standard, equivalent to about 0.1 g (potency), dissolve in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefazolin to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefazolin } (C_{14}H_{14}N_8O_4S_3) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Cefazolin Reference Standard

Internal standard solution—A solution of *p*-acetoanisidine in 0.1 mol/L phosphate buffer solution, pH 7.0 (11 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.27 g of disodium hydrogen phosphate dodecahydrate and 0.47 g of citric acid monohydrate in water to make 935 mL. To this solution, add 65 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefazolin is about 8 minutes.

System suitability—

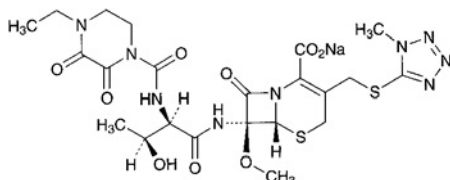
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, cefazolin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefazolin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Cefbuperazone Sodium

セフブペラゾンナトリウム



$\text{C}_{22}\text{H}_{28}\text{N}_9\text{NaO}_9\text{S}_2$: 649.63

Monosodium (6*R*,7*S*)-7-[(2*R*,3*S*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-3-hydroxybutanoylamino]-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[76648-01-6]

Cefbuperazone Sodium contains not less than 870 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefbuperazone Sodium is expressed as mass (potency) of cefbuperazone ($\text{C}_{22}\text{H}_{29}\text{N}_9\text{O}_9\text{S}_2$: 627.65).

Description Cefbuperazone Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol and in pyridine, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Cefbuperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.1 g of Cefbuperazone Sodium in 0.5 mL of deuterated pyridine for nuclear magnetic resonance spectroscopy and 1 drop of heavy water for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.1 ppm, and two doublet signals, B and C, at around δ 1.6 ppm and at around δ 5.1 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefbuperazone Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +48 – +56° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefbuperazone Sodium in 4 mL of water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefbuperazone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefbuperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefbuperazone Sodium in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the percentages of each peak area of related substances from the sample solution against 50 times of the peak area of cefbuperazone from the standard solution; the amount of related substance I having the relative retention time of about 0.2 to cefbuperazone is not more than 2.0%, the amount of related substance II having the relative retention time of about 0.6 to cefbuperazone is not more than 4.5% and the amount of related substance III having the relative retention time of about 1.6 to cefbuperazone is not more than 1.0%, and the total amount of these related substances is not more than 6.0%. For these calculations, use the values of the peak areas of the related substances I and III obtained by the automatic integration method after multiplying by each sensitivity coefficient, 0.72 and 0.69, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefbuperazone.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefbuperazone obtained from 25 μL of this solution is equivalent to 7 to 13% of that from 25 μL of the standard solution.

System performance: When the procedure is run with 25 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefbuperazone are not less than 5000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefbuperazone is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefbuperazone Sodium and Cefbuperazone Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL. Measure exactly 10 mL each of these solutions, add exactly 10 mL of the internal standard

solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefbuperazone to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefbuperazone (C}_{22}\text{H}_{29}\text{N}_9\text{O}_9\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Cefbuperazone Reference Standard

Internal standard solution—A solution of acetonitrile in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.0 g of tetra-*n*-propylammonium bromide in 1000 mL of a mixture of water, acetonitrile and acetic acid-sodium acetate buffer solution, pH 5.0 (83:13:4).

Flow rate: Adjust the flow rate so that the retention time of cefbuperazone is about 16 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and cefbuperazone are eluted in this order with the resolution between these peaks being not less than 3.

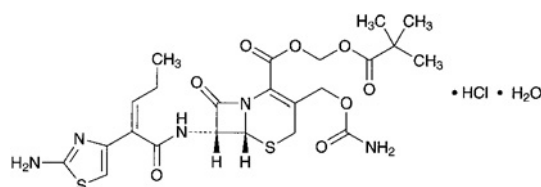
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefbuperazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Cefcapene Pivoxil Hydrochloride Hydrate

セフカペン ピボキシル塩酸塩水和物



$\text{C}_{23}\text{H}_{29}\text{N}_5\text{O}_8\text{S}_2 \cdot \text{HCl} \cdot \text{H}_2\text{O}$: 622.11

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(2*Z*)-2-(2-aminothiazol-4-yl)pent-2-enoylamino]-3-carbamoyloxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride monohydrate [147816-24-8]

Cefcapene Pivoxil Hydrochloride Hydrate contains not less than 722 μ g (potency) and not more than 764 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefcapene Pivoxil Hydrochloride Hydrate is expressed as mass (potency) of cefcapene ($\text{C}_{17}\text{H}_{19}\text{N}_5\text{O}_6\text{S}_2$; 453.49).

Description Cefcapene Pivoxil Hydrochloride Hydrate occurs as a white to pale yellowish white, crystalline powder or mass. It has slightly a characteristic odor.

It is freely soluble in *N,N*-dimethylformamide and in methanol, soluble in ethanol (99.5), slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Determine the infrared absorption spectra of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride Reference Standard as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the spectrum of a solution of Cefcapene Pivoxil Hydrochloride Hydrate in deuterated methanol for nuclear magnetic resonance spectroscopy (1 in 50) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 6.3 ppm, and a single signal B at around δ 6.7 ppm, and the ratio of integrated intensity of each signal, A:B, is about 1:1.

(3) Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate in 2 mL of a mixture of water and methanol (1:1), and add 1 drop of silver nitrate TS: a white precipitate is formed.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (265 nm): 255 – 285 (30 mg calculated on the anhydrous basis, a mixture of acetate buffer solution, pH 5.5 and methanol (1:1), 2000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: +51 – +54° (0.1 g calculated on the anhydrous basis, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefcapene Pivoxil Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substance I—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 10 mg (potency), in 2 mL of methanol, add a mixture of water and methanol (1:1) to make 50 mL, and use this solution as the sample solution. Perform the test with 30 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. If necessary, compensate the base-line by performing in the same manner as the test with 30 μ L of a mixture of water and methanol (1:1). Measure the amount of the peak other than cefcapene pivoxil by the area percentage method: the amounts of the peaks, having the relative retention times of about 1.5 and about 1.7 with respect to cefcapene pivoxil, are not more than 0.2%, respectively. The amount of the peak other than the peaks mentioned above is not more than 0.1%, and the total of them is not more than 1.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase A: Dissolve 5.99 g of potassium dihydrogen phosphate in water to make 1100 mL. To this solution add a solution prepared by dissolving 1.89 g of tetra-*n*-pentylammonium bromide in methanol to make 1000 mL.

Mobile phase B: A mixture of methanol and water (22:3).

Flowing of the mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 20	98	2
20 - 40	98 → 50	2 → 50
40 - 50	50	50

Flow rate: 0.8 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of cefcapene pivoxil.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add a mixture of water and methanol (1:1) to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mixture of water and methanol (1:1) to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 30 μ L of this solution is equivalent to 7 to 13% of that of cefcapene pivoxil obtained from 30 μ L of the solution for system suitability test.

System performance: Dissolve 10 mg of Cefcapene Pivoxil Hydrochloride Hydrate and 10 mg of propyl parahydroxybenzoate in 25 mL of methanol, and add water to make 50 mL. To 5 mL of this solution add the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 30 μ L of this solution under the above operating conditions, cefcapene pivoxil and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 3 times with 30 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of cefcapene pivoxil is not more than 4.0%.

(3) Related substance II—Dissolve an amount of Cefcapene Pivoxil Hydrochloride Hydrate, equivalent to about 2 mg (potency), in *N,N*-dimethylformamide for liquid chromatography to make 20 mL, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks which appear earlier than cefcapene pivoxil is not more than 1.7% of the total area of the peaks other than the solvent.

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 280 nm).

Column: A stainless steel column 7.8 mm in inside diameter and 30 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A solution of lithium bromide in *N,N*-dimethylformamide for liquid chromatography (13 in 5000).

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 22 minutes.

Time span of measurement: About 1.8 times as long as the retention time of cefcapene pivoxil.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add *N,N*-dimethylformamide for liquid chromatography to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 3 mL of the solution for system suitability test, and add *N,N*-dimethylformamide for liquid chromatography to make exactly 10 mL. Confirm that the peak area of cefcapene pivoxil obtained from 20 μ L of this solution is equivalent to 20 to 40% of that of cefcapene pivoxil obtained from 20 μ L of the solution for system suitability test.

System performance: When the procedure is run with 20 μ L of the sample solution under the above operating conditions, the number of theoretical plates of the peak of cefcapene pivoxil is not less than 12,000 steps.

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefcapene pivoxil is not more than 4.0%.

Water <2.48> Not less than 2.8% and not more than 3.7% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Hydrate and Cefcapene Pivoxil Hydrochloride Reference Standard, equivalent to about 20 mg (potency), and dissolve each in a mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to them to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefcapene pivoxil to that of the internal standard of these solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefcapene (C}_{17}\text{H}_{19}\text{N}_5\text{O}_6\text{S}_2) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride Reference Standard

Internal standard solution—A solution of *p*-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 3.0 mm in inside diameter and 7.5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.56 g of sodium dihydrogenphosphate dihydrate and 1.22 g of sodium 1-decanesulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile and 100 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefcapene pivoxil is about 5 minutes.

System suitability—

System performance: Dissolve 0.2 g of Cefcapene Pivoxil Hydrochloride Hydrate in 10 mL of methanol, and warm in a water bath at 60°C for 20 minutes. After cooling, pipet 1 mL of this solution, and add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, cefcapene pivoxil, *trans*-cefcapene pivoxil and the internal standard are eluted in this order, the ratios of the retention time of *trans*-cefcapene pivoxil and the internal standard to that of cefcapene pivoxil are about 1.7 and 2.0, respectively, and the resolution between the peaks of *trans*-cefcapene pivoxil and the internal standard is not less than 1.5.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefcapene pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 5°C.

Cefcapene Pivoxil Hydrochloride Fine Granules

セフカペン ピボキシル塩酸塩細粒

Cefcapene Pivoxil Hydrochloride Fine Granules contains not less than 90.0% and not more than 110.0% of cefcapene (C₁₇H₁₉N₅O₆S₂: 453.49).

Method of preparation Prepare to finely granulated form as directed under Powders, with Cefcapene Pivoxil Hydrochloride Hydrate.

Identification Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, and filter through a membrane filter with a pore size of 0.45 μ m. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

Purity (1) Related substances I—Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 1 mL of methanol, and shake. Add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 5 minutes, and filter through a membrane filter with a pore size of 0.45 μ m. Discard the first 3 mL of the

filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method. If necessary, compensate the base-line by performing in the same manner as the test with 30 μ L of a mixture of water and methanol (1:1). Calculate the amount of the peaks other than the peak of cefcapene pivoxil by the area percentage method: the amount of the substance, having the relative retention time of about 1.3 with respect to cefcapene pivoxil, is not more than 0.4%, the amount of the trans-isomer of cefcapene pivoxil, having the relative retention time of about 1.5, is not more than 1.1%, the amount of the substance other than that mentioned above is not more than 0.3%, and the total of these substances is not more than 2.8 %.

Operating conditions—

Proceed as directed in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

(2) Related substances II—Powder Cefcapene Pivoxil Hydrochloride Fine Granules. To a portion of the powder, equivalent to 2 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate, add 20 mL of *N,N*-dimethylformamide for liquid chromatography, shake vigorously for 10 minutes, and filter through a membrane filter with a pore size of 0.45 μ m. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks eluted before that of cefcapene pivoxil is not more than 4.0% of the total area of all peaks other than the solvent peak.

Operating conditions—

Proceed as directed in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

Water <2.48> Not more than 1.4% (0.5 g, volumetric titration, back titration). Perform the test without pulverizing the sample, and handling the sample under a relative humidity of less than 30%.

Uniformity of dosage units <6.02> The granules in single-unit container meet the requirement of the Mass variation test.

Dissolution Being specified separately.

Particle size <6.03> It meets the requirement of the fine granules of the Powders.

Assay Weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Fine Granules, equivalent to about 0.2 g (potency) of and Cefcapene Pivoxil Hydrochloride Hydrate, add 100 mL of the mixture of water and methanol (1:1), shake vigorously for 10 minutes, add the mixture of water and methanol (1:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with a pore size of 0.45 μ m, discard the first 1 mL of the filtrate, pipet the subsequent 2 mL of the filtrate,

add exactly 5 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefcapene Pivoxil Hydrochloride Reference Standard, and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil Hydrochloride Hydrate.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_5\text{O}_6\text{S}_2) \\ &= W_S \times (Q_T/Q_S) \times 10 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride Reference Standard

Internal standard solution—A solution of *p*-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefcapene Pivoxil Hydrochloride Tablets

セフカペン ピボキシル塩酸塩錠

Cefcapene Pivoxil Hydrochloride Tablets contain not less than 90.0% and not more than 105.0% of the labeled amount of cefcapene (C₁₇H₁₉N₅O₆S₂; 453.49).

Method of preparation Prepare as directed under Tablets, with Cefcapene Pivoxil Hydrochloride Hydrate.

Identification To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 10 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 40 mL of methanol, shake vigorously, and add methanol to make 50 mL. To 4 mL of this solution add methanol to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, and use the filtrate as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm.

Purity (1) Related substances I—To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 5 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 1 mL of methanol, and shake. Add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 5 minutes, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 30 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. If necessary, proceed with 30 μL of the mixture of water and methanol (1:1) in the same manner as the sample solution to compensate the base line. Calculate the amounts of the peaks other than cefcapene pivoxil by the area percentage method: the

peak, having the relative retention time of about 1.3 with respect to cefcapene pivoxil, is not more than 0.4%, the peak of cefcapene pivoxil trans-isomer, having the relative retention time of about 1.5, is not more than 0.5%, any other peaks are not more than 0.3%, respectively, and the total of these peaks is not more than 2.0%.

Operating conditions—

Proceed as directed in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the Purity (2) under Cefcapene Pivoxil Hydrochloride Hydrate.

(2) Related substances II—To an amount of powdered Cefcapene Pivoxil Hydrochloride Tablets, equivalent to 2 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 20 mL of *N,N*-dimethylformamide for liquid chromatography, shake vigorously for 10 minutes, and filter through a membrane filter with pore size of 0.45 μm. Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks which are eluted before cefcapene pivoxil is not more than 3.3% of the total area of the peaks other than the solvent peak.

Operating conditions—

Proceed as directed in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

System suitability—

Proceed as directed in the Purity (3) under Cefcapene Pivoxil Hydrochloride Hydrate.

Water <2.48> Not more than 3.9% (0.5 g, volumetric titration, back titration). Powdering of the sample tablets and handling of the powder are performed under the relative humidity of not exceeding 30%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cefcapene Pivoxil Hydrochloride Tablets add 5 mL of water, and shake vigorously for 5 minutes to disintegrate. Add 20 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 50 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm, and discard the first 1 mL of the filtrate. Pipet *V* mL of the subsequent filtrate, equivalent to about 6 mg (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add exactly 15 mL of the internal standard solution, add a mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Hereinafter, proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_5\text{O}_6\text{S}_2) \\ &= W_S \times (Q_T/Q_S) \times (15/V) \end{aligned}$$

W_S : Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride Reference Standard

Internal standard solution—A solution of *p*-benzylphenol in a mixture of water and methanol (1:1) (7 in 4000).

Dissolution Being specified separately.

Assay To an amount of Cefcapene Pivoxil Hydrochloride Tablets, equivalent to about 0.6 g (potency) of Cefcapene Pivoxil Hydrochloride Hydrate according to the labeled amount, add 20 mL of water, and shake for 5 minutes to disintegrate. Add 80 mL of methanol, shake vigorously for 5 minutes, add a mixture of methanol and water (4:1) to make exactly 200 mL, and centrifuge at 3000 rpm for 5 minutes. Filter the supernatant liquid through a membrane filter with pore size of 0.45 μm , and discard the first 1 mL of the filtrate. Pipet 2 mL of the subsequent filtrate, add exactly 15 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 75 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefcapene Pivoxil Hydrochloride Reference Standard, equivalent to about 20 mg (potency), and dissolve in the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add the mixture of water and methanol (1:1) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefcapene Pivoxil Hydrochloride Hydrate.

$$\begin{aligned} & \text{Amount [mg (potency)] of cefcapene (C}_{17}\text{H}_{19}\text{N}_5\text{O}_6\text{S}_2) \\ & = W_S \times (Q_T/Q_S) \times 30 \end{aligned}$$

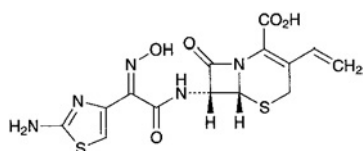
W_S : Amount [mg (potency)] of Cefcapene Pivoxil Hydrochloride Reference Standard

Internal standard solution—A solution of *p*-benzylphenol in the mixture of water and methanol (1:1) (7 in 4000).

Containers and storage Containers—Tight containers.

Cefdinir

セフジニル



$\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$: 395.41
(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(hydroxyimino)acetyl]amino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[91832-40-5]

Cefdinir contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg. The potency of Cefdinir is expressed as mass (potency) of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$).

Description Cefdinir occurs as a white to light yellow crystalline powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in 0.1 mol/L phosphate buffer solution, pH 7.0.

Identification (1) Determine the absorption spectra of solutions of Cefdinir and Cefdinir Reference Standard in 0.1 mol/L phosphate buffer solution, pH 7.0 (1 in 100,000) as

directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Cefdinir and Cefdinir Reference Standard as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefdinir in a mixture of deuterated dimethyl sulfoxide and heavy water for nuclear magnetic resonance spectroscopy (4:1) (1 in 10), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H): it exhibits multiple signals, A at around δ 5.0 – 6.1 ppm and B at around δ 6.4 – 7.5 ppm. The ratio of integrated intensity of each signal, A:B is about 2:1.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (287 nm): 570 – 610 (50 mg, 0.1 mol/L phosphate buffer solution, pH 7.0, 5000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: -58 – -66° (0.25 g, 0.1 mol/L phosphate buffer solution, pH 7.0, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefdinir according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve about 0.1 g of Cefdinir in 10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0. Pipet 3 mL of this solution, add tetramethylammonium hydroxide TS, pH 5.5 to make exactly 20 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the areas of each peak by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of E-isomer having the relative retention time 1.5 to cefdinir is not more than 0.8%, and the amount of total peak areas other than cefdinir is not more than 3.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: To 1000 mL of tetramethylammonium hydroxide TS, pH 5.5, add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Mobile phase B: To 500 mL of tetramethylammonium hydroxide TS, pH 5.5 add 300 mL of acetonitrile for liquid chromatography and 200 mL of methanol, and add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS.

Flowing of the mobile phase: Control the gradient by mixing the mobile A and B as directed in the following table.

Time after injection of the sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 2	95	5
2 – 22	95 → 75	5 → 25
22 – 32	75 → 50	25 → 50
32 – 37	50	50
37 – 38	50 → 95	50 → 5
38 – 58	95	5

Flow rate: 1.0 mL per minute. The retention time of cefdinir is about 22 minutes under this condition.

Time span of measurement: About 40 minutes after injection of the sample solution.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add tetramethylammonium hydroxide TS, pH 5.5 to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add tetramethylammonium hydroxide TS, pH 5.5 to make exactly 10 mL. Confirm that the peak area of cefdinir obtained from 10 μ L of this solution is equivalent to 7 to 13% of that obtained from 10 μ L of the test solution for system suitability.

System performance: Dissolve 0.03 g of Cefdinir Reference Standard and 2 mg of cefdinir lactam ring-cleavage lactones in 3 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, add tetramethylammonium hydroxide TS, pH 5.5, to make 20 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. Relative retention time of peak 3 of cefdinir lactam ring-cleavage lactone to the retention time of cefdinir is not less than 1.09. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 7000 steps and not more than 3.0, respectively.

System repeatability: When the test is repeated 3 times with 10 μ L of the test solution for system suitability under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 2.0%.

Water <2.48> Not more than 2.0% (1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefdinir and Cefdinir Reference Standard equivalent to about 20 mg (potency), dissolve each in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 5 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefdinir of the solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2 \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefdinir Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 1000 mL of tetramethylammonium hydroxide TS, pH 5.5, add 0.4 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS. To 900 mL of this solution add 60 mL of acetonitrile for liquid chromatography and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefdinir is about 8 minutes.

System suitability—

System performance: Dissolve 2 mg of Cefdinir Reference Standard and 5 mg of cefdinir lactam ring-cleavage lactones in 10 mL of 0.1 mol/L phosphate buffer solution, pH 7.0. When the procedure is run with 5 μ L of this solution under the above operating conditions, peak 1 and peak 2 of cefdinir lactam ring-cleavage lactones separated into 4 peaks, cefdinir, peak 3 and peak 4 of remaining cefdinir lactam ring-cleavage lactones are eluted in this order. The resolution between the peak 2 of cefdinir lactam ring-cleavage lactone and that of cefdinir is not less than 1.2. The number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefdinir is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefdinir Capsules

セフジニルカプセル

Cefdinir Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$: 395.41).

Method of preparation Prepare as directed under Capsules, with Cefdinir.

Identification To an amount of the contents of Cefdinir Capsules, equivalent to 10 mg (potency) of Cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage unit <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the follow-

ing method: it meets the requirement.

Perform the test with 1 capsule of Cefdinir Capsules at 50 revolutions per minute according to the Paddle method using a sinker, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test for a 50-mg capsule, or 45 minutes after for a 100-mg capsule, and filter through a membrane filter with pore size of not more than 0.5 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 56 μg (potency) of Cefdinir according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir Reference Standard, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 4 mL of this solution, add 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S , of cefdinir. The dissolution rate of a 50-mg capsule in 30 minutes is not less than 80% and that of a 100-mg capsule in 45 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$$

W_S : Amount [mg (potency)] of Cefdinir Reference Standard

C : Labeled amount [mg (potency)] of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$) in 1 capsule

Operating conditions—

Proceed as directed in the Assay under Cefdinir.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Assay Weigh accurately not less than 5 Cefdinir Capsules, take out the contents, and powder. Wash the empty capsules with a little amount of diethyl ether, if necessary, allow to stand at a room temperature to vaporize the adhering diethyl ether, and weigh accurately the mass of the capsules to calculate the mass of the contents. Weigh accurately an amount of the contents, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Centrifuge this solution at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefdinir Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

$$\text{Amount [mg (potency)] of cefdinir } (\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2) \\ = W_S \times (A_T/A_S) \times 5$$

W_S : Amount [mg (potency)] of Cefdinir Reference Standard

Containers and storage Containers—Tight containers.

Cefdinir Fine Granules

セフジニル細粒

Cefdinir Fine Granules contain not less than 93.0% and not more than 107.0% of the labeled amount of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$; 395.41).

Method of preparation Prepare to finely granulated form as directed under Powders, with Cefdinir.

Identification To an amount of Cefdinir Fine Granules, equivalent to 10 mg (potency) of Cefdinir according to the labeled amount, add 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, exposure to ultrasonic waves for 1 minute, and filter. To 2 mL of the filtrate add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 221 nm and 225 nm and between 285 nm and 289 nm.

Uniformity of dosage units <6.02> The granules in single-unit container meet the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with an accurate amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, at 50 revolutions per minute according to the Paddle method using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm . Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 28 mg (potency) of Cefdinir Reference Standard, and 2nd fluid for dissolution test to make exactly 50 mL. Pipet 4 mL of this solution, add 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S , of cefdinir. The dissolution rate in 30 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$)

$$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 360$$

W_S : Amount [mg (potency)] of Cefdinir Reference Standard

W_T : Amount (g) of sample

C : Labeled amount [mg (potency)] of cefdinir ($\text{C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2$) in 1 g

Operating conditions—

Proceed as directed in the Assay under Cefdinir.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefdinir are not less than 2000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefdinir is not more than 1.0%.

Particle size <6.03> It meets the requirement of fine granules of the Powders.

Assay Powder, if necessary, and weigh accurately an amount of Cefdinir Fine Granules, equivalent to about 0.1 g (potency) of Cefdinir according to the labeled amount, add 70 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, shake for 30 minutes, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Centrifuge at 3000 revolutions per minute for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefdinir Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefdinir.

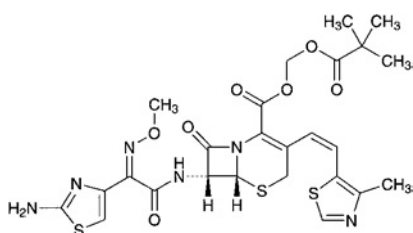
$$\begin{aligned} &\text{Amount [mg (potency)] of cefdinir (C}_{14}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2) \\ &= W_S \times (A_T/A_S) \times 5 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefdinir Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cefditoren Pivoxil

セフジトレン ピボキシル



$\text{C}_{25}\text{H}_{28}\text{N}_6\text{O}_7\text{S}_3$: 620.72

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl-amino]-3-[(1*Z*)-2-(4-methylthiazol-5-yl)ethenyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [I17467-28-4]

Cefditoren Pivoxil contains not less than 770 μ g (potency) and not more than 820 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefditoren Pivoxil is expressed as mass (potency) of cefditoren ($\text{C}_{19}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_3$: 506.58).

Description Cefditoren Pivoxil occurs as a light yellowish white to light yellow crystalline powder.

It is sparingly soluble in methanol, slightly soluble in acetonitrile and in ethanol (95), very slightly soluble in diethylether and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 5 mg of Cefditoren Pivoxil in 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefditoren Pivoxil in 1 mL of dilute hydrochloric acid and 4 mL of water, add 3 drops of sodium nitrite TS under ice-cooling, shake, and allow to stand for 2 minutes. Then add 1 mL of ammonium amidosulfate TS, shake well, and allow to stand for 1 minute, and add 1 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS: a purple color develops.

(3) Determine the absorption spectrum of a solution of Cefditoren Pivoxil in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefditoren Pivoxil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the spectrum of a solution of Cefditoren Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 50), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H): it exhibits single signals A, B and C, at around δ 1.1 ppm, at around δ 2.4 ppm and at around δ 4.0 ppm, double signals D and E, at around δ 6.4 ppm and at around δ 6.7 ppm, and a single signal F at around δ 8.6 ppm. The ratio of integrated intensity of each signal A:B:C:D:E:F, is about 9:3:3:1:1:1.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (231 nm): 340 – 360 (50 mg, methanol, 2500 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: – 45 – – 52° (50 mg, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefditoren Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately.

(3) Residual solvents—Being specified separately.

Water <2.48> Not more than 1.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition Being specified separately.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately an amount of Cefditoren Pivoxil and Cefditoren Pivoxil Reference Standard, equivalent to about 40 mg (potency), dissolve in 40 mL of acetonitrile, add exactly 10 mL each of the internal standard solution, and add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions,

and calculate the ratios, Q_T and Q_S , of the peak area of cefditoren pivoxil to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefditoren (C}_{19}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_3) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefditoren Pivoxil Reference Standard

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in acetonitrile (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.58 g of ammonium formate in 900 mL of water, adjust to pH 6.0 with diluted formic acid (1 in 250), and add water to make 1000 mL. To 450 mL of this solution add 275 mL of acetonitrile and 275 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefditoren pivoxil is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefditoren pivoxil are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefditoren pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefditoren Pivoxil Fine Granules

セフジトレン ピボキシル細粒

Cefditoren Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefditoren (C₁₉H₁₈N₆O₅S₃; 506.58).

Method of preparation Prepare in the form of very fine granules as directed under Powders, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to 0.1 g (potency) of Cefditoren Pivoxil according to the labeled amount, add 10 mL of acetonitrile, shake vigorously, and filter. To 1 mL of the filtrate add acetonitrile to make 50 mL. To 1 mL of this solution add acetonitrile to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 230 nm and 234 nm.

Purity Related substances—Being specified separately.

Loss on drying <2.41> Not more than 4.5% (0.5 g, reduced

pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <6.02> The granules in single-unit container meet the requirement of the Mass variation test.

Dissolution Being specified separately.

Particle size <6.03> It meets the requirement of fine granules of the Powders.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately an amount of powdered Cefditoren Pivoxil Fine Granules, equivalent to about 40 mg (potency) of Cefditoren Pivoxil according to the labeled amount, add 70 mL of diluted acetonitrile (3 in 4), and shake vigorously. To this solution add exactly 10 mL of the internal standard solution, then add acetonitrile to make 100 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil Reference Standard, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefditoren (C}_{19}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_3) \\ &= W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefditoren Pivoxil Reference Standard

Internal standard solution—A solution of propyl *p*-hydroxybenzoate in acetonitrile (1 in 200)

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefditoren Pivoxil Tablets

セフジトレン ピボキシル錠

Cefditoren Pivoxil Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of cefditoren (C₁₉H₁₈N₆O₅S₃; 506.58),

Method of preparation Prepare as directed under Tablets, with Cefditoren Pivoxil.

Identification To an amount of powdered Cefditoren Pivoxil Tablets, equivalent to 35 mg (potency) of Cefditoren Pivoxil according to the labeled amount, add 100 mL of methanol, shake, and filter. To 5 mL of the filtrate add methanol to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 229 nm and 233 nm.

Purity Related substances—Being specified separately.

Loss on drying <2.41> Not more than 4.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cefditoren Pivoxil Tablets add 12.5 mL of the 1st fluid for disintegration test, shake vigorously, add

about 25 mL of acetonitrile, shake again, and add acetonitrile to make exactly 50 mL. Pipet V mL of this solution, equivalent to about 20 mg (potency) of Cefditoren Pivoxil according to the labeled amount, add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil Reference Standard, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefditoren (C}_{19}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_3) \\ &= W_S \times (Q_T/Q_S) \times (50/V) \end{aligned}$$

W_S : Amount [mg (potency)] of Cefditoren Pivoxil Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200)

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Cefditoren Pivoxil Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of the 1st fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 20 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 11 μg (potency) of Cefditoren Pivoxil according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg (potency) of Cefditoren Pivoxil Reference Standard, dissolve in 20 mL of diluted acetonitrile (3 in 4), then add the 1st fluid for dissolution test to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 272 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control. The dissolution rate in 20 minutes is not less than 85%.

$$\begin{aligned} &\text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ &= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefditoren Pivoxil Reference Standard

C : Labeled amount [mg (potency)] of cefditoren pivoxil (C₂₅H₂₈N₆O₇S₃) in 1 tablet

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an amount of Cefditoren Pivoxil Tablets, equivalent to 0.5 g (potency) of Cefditoren Pivoxil according to the labeled amount, add 63 mL of the 1st fluid for disintegration test, shake vigorously, add about 125 mL of acetonitrile, shake again, and add acetonitrile to make exactly 250 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (3 in 4) to make 50 mL, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg (potency) of Cefditoren Pivoxil Reference Standard, dissolve in 20 mL of acetonitrile, add exactly 5 mL of the internal standard solution, then add acetonitrile to make

50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefditoren Pivoxil.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefditoren (C}_{19}\text{H}_{18}\text{N}_6\text{O}_5\text{S}_3) \\ &= W_S \times (Q_T/Q_S) \times 25 \end{aligned}$$

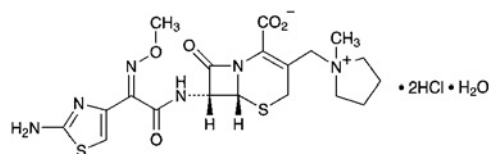
W_S : Amount [mg (potency)] of Cefditoren Pivoxil Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in acetonitrile (1 in 200)

Containers and storage Containers—Tight containers.

Cefepime Dihydrochloride Hydrate

セフェピム塩酸塩水和物



C₁₉H₂₄N₆O₅S₂·2HCl·H₂O: 571.50

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(1-methylpyrrolidinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride monohydrate
[123171-59-5]

Cefepime Dihydrochloride Hydrate contains not less than 835 μg (potency) and not more than 886 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefepime Dihydrochloride Hydrate is expressed as mass (potency) of cefepime (C₁₉H₂₄N₆O₅S₂: 480.56).

Description Cefepime Dihydrochloride Hydrate occurs as a white to yellowish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (95), and practically insoluble in diethylether.

Identification (1) Dissolve 0.02 g of Cefepime Dihydrochloride Hydrate in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectra of solutions (1 in 20,000) of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride Reference Standard as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the spectrum of a solution of Cefepime Dihydrochloride Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear

Magnetic Resonance Spectroscopy <2.21> (¹H), using sodium 3-trimethylsilylpropionate-d₄ for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.1 ppm and at around δ 7.2 ppm, respectively, and the ratio of integrated intensity of each signal, A:B, is about 3:1.

(5) Dissolve 15 mg of Cefepime Dihydrochloride Hydrate in 5 mL of water, and add 2 drops of silver nitrate TS: a white turbidity is produced.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (259 nm): 310 – 340 (50 mg calculated on the anhydrous basis, water, 1000 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +39 – +47° (60 mg calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 0.1 g of Cefepime Dihydrochloride Hydrate in 10 mL of water: the pH of this solution is between 1.6 and 2.1.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefepime Dihydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) *N*-Methylpyrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride Hydrate equivalent to about 80 mg (potency), dissolve in diluted nitric acid (2 in 3125) to make exactly 10 mL, and use this solution as the sample solution. Separately, put 30 mL of water in a 100-mL volumetric flask, weigh accurately the mass of flask, then add about 0.125 g of *N*-methylpyrrolidine, weigh accurately the mass of the flask again, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of *N*-methylpyrrolidine by the automatic integration method. Calculate the amount of *N*-methylpyrrolidine per 1 mg (potency) of Cefepime Dihydrochloride Hydrate by the following equation: not more than 0.5%. The sample solution must be tested within 20 minutes after preparation.

$$\begin{aligned} &\text{Amount (\%)} \text{ of } N\text{-methylpyrrolidine} \\ &= \{(W_S \times f) / W_T\} \times (A_T / A_S) \times (1/250) \end{aligned}$$

W_S : Amount (mg) of *N*-methylpyrrolidine

W_T : Amount [mg (potency)] of sample

f : Purity (%) of *N*-methylpyrrolidine

Operating conditions—

Detector: An electric conductivity detector

Column: A plastic tube 4.6 mm in inside diameter and 5 cm in length, packed with hydrophilic silica gel for liquid chromatography carrying sulfonic acid groups having the exchange capacity of about 0.3 meq per g (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 990 mL of diluted nitric acid (2 in 3125) add 10 mL of acetonitrile.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: To 20 mL of a solution of sodium chloride (3 in 1000) add 0.125 g of *N*-methylpyrrolidine, and add water to make 100 mL. To 4 mL of this solution add diluted nitric acid (2 in 3125) to make 100 mL. When the procedure is run with 100 μL of this solution under the above operating conditions, sodium and *N*-methylpyrrolidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N*-methylpyrrolidine is not more than 4.0%.

(4) Related substances—Dissolve about 0.1 g of Cefepime Dihydrochloride Hydrate in the mobile phase A to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak by the automatic integration method. Calculate the total of the peak areas other than cefepime: not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 0.57 g of ammonium dihydrogenphosphate in 1000 mL of water.

Mobile phase B: Acetonitrile

Flowing of the mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Time after injection of the sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0–25	100→75	0→25

Flow rate: Adjust the flow rate so that the retention time of cefepime is about 9.5 minutes.

Time span of measurement: About 2.5 times as long as the retention time of cefepime.

System suitability—

Test for required detection: To 1 mL of the sample solution add the mobile phase A to make 10 mL, and use this solution as the solution for system suitability test. To 1 mL of the solution for system suitability test add the mobile phase A to make 10 mL, and use this solution as the solution for test for required detection. Pipet 1 mL of the solution for test for required detection, add the mobile phase A to make exactly 10 mL. Conform that the peak area of cefepime obtained from 5 μL of this solution is equivalent to 7 to 13% of that of cefepime obtained from 5 μL of the solution for test for required detection.

System performance: When the procedure is run with 5 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 6000 steps.

System repeatability: When the test is repeated 3 times with

5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0%.

Water <2.48> Not less than 3.0% and not more than 4.5% (Weigh accurately about 50 mg, and add exactly 2 mL of methanol for water determination to dissolve. Use exactly 0.5 mL of this solution as the test solution; coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Bacterial endotoxins <4.01> Less than 0.04 EU/mg (potency).

Assay Weigh accurately an amount of Cefepime Dihydrochloride Hydrate and Cefepime Dihydrochloride Reference Standard, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of cefepime of each solution.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefepime (C}_{19}\text{H}_{24}\text{N}_6\text{O}_5\text{S}_2) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefepime Dihydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust a solution of sodium 1-pentanesulfonate (261 in 100,000) to pH 3.4 with acetic acid (100), then adjust this solution to pH 4.0 with a solution of potassium hydroxide (13 in 20). To 950 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefepime is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefepime is not less than 1500 steps.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefepime is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefepime Dihydrochloride for Injection

注射用セフェピム塩酸塩

Cefepime Dihydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 95.0% and not more than 110.0% of the labeled amount of cefepime (C₁₉H₂₄N₆O₅S₂: 480.56).

Method of preparation Prepare as directed under Injections, with Cefepime Dihydrochloride Hydrate.

Description Cefepime Dihydrochloride for Injection occurs as a white to pale yellow powder.

Identification (1) Dissolve 40 mg of Cefepime Dihydrochloride in 2 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Cefepime Dihydrochloride for Injection (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 233 nm and 237 nm and between 255 nm and 259 nm.

pH <2.54> The pH of a solution obtained by dissolving an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, in 5 mL of water is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve an amount of Cefepime Dihydrochloride for Injection, equivalent to 0.5 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, in 5 mL of water: the solution is clear and colorless or light yellow. The color is not darker than Matching Fluid I.

(2) *N*-Methylpyrrolidine—Weigh accurately an amount of Cefepime Dihydrochloride for Injection, equivalent to about 0.2 g (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, dissolve in diluted nitric acid (2 in 625) to make exactly 20 mL, and use this solution as the sample solution. Separately, transfer 30 mL of water into a 100-mL volumetric flask, weigh accurately the mass of the flask, add about 0.125 g of *N*-methylpyrrolidine, then weigh accurately the mass, and add water to make exactly 100 mL. Pipet 4 mL of this solution, add diluted nitric acid (2 in 3125) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of *N*-methylpyrrolidine, A_T and A_S , by the automatic integration method within 20 minutes after the sample solution is prepared. Calculate the amount of *N*-methylpyrrolidine per mg (potency) of Cefepime Dihydrochloride for Injection by the following formula: not more than 1.0%.

Amount (%) of *N*-methylpyrrolidine

$$= \{(W_S \times f) / W_T\} \times (A_T / A_S) \times (1/125)$$

W_S : Amount (mg) of *N*-methylpyrrolidine

W_T : Amount [mg (potency)] of cefepime in the sample

f : Purity (%) of *N*-methylpyrrolidine

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3) under Cefepime Dihydrochloride Hydrate.

System suitability—

Proceed as directed in the system suitability in the Purity (3) under Cefepime Dihydrochloride Hydrate.

Water <2.48> Not more than 4.0% (Weigh accurately about 50 mg of Cefepime Dihydrochloride for Injection, dissolve in exactly 2 mL of methanol for Karl Fischer method, and perform the test with exactly 0.5 mL of this solution. Coulometric titration).

Bacterial endotoxins <4.01> Less than 0.06 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Cefepime Dihydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 60 mg (potency) of Cefepime Dihydrochloride Hydrate according to the labeled amount, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefepime Dihydrochloride Reference Standard, equivalent to about 60 mg (potency), dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefepime Dihydrochloride Hydrate.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefepime (C}_{19}\text{H}_{24}\text{N}_6\text{O}_5\text{S}_2) \\ = W_S \times (A_T / A_S) \times 1000$$

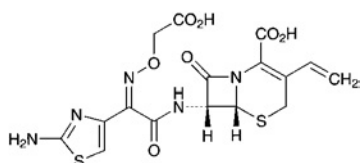
W_S : Amount [mg (potency)] of Cefepime Dihydrochloride Reference Standard

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Cefixime

セフィキシム



C₁₆H₁₅N₅O₇S₂: 453.45

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(carboxymethoxyimino)acetyl-amino]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
[79350-37-1]

Cefixime contains not less than 930 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefixime is expressed as mass (potency) of cefixime (C₁₆H₁₅N₅O₇S₂).

Description Cefixime occurs as a white to light yellow crystalline powder.

It is freely soluble in methanol and in dimethylsulfoxide, sparingly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefixime in 0.1 mol/L phosphate buffer solution, pH 7.0 (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefixime Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefixime as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefixime Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Cefixime in 0.5 mL of a mixture of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy and heavy water for nuclear magnetic resonance spectroscopy (4:1). Determine the spectrum of this solution, using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits a single signal A at around δ 4.7 ppm, and a multiple signal B between δ 6.5 ppm and δ 7.4 ppm. The ratio of integrated intensity of these signals, A:B, is about 1:1.

Optical rotation <2.49> [α]_D²⁰: −75 – −88° (0.45 g calculated on the anhydrous bases, a solution of sodium hydrogen carbonate (1 in 50), 50 mL, 100 mm).

Purity Dissolve 0.1 g of Cefixime in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, measure the areas of the peaks by the automatic integration method, and calculate the amounts of these peak areas by the area percentage method: the amount of each peak area other than cefixime is not more than 1.0%, and the total area of the peaks other than cefixime is not more than 2.5%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefixime beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, and add 0.1 mol/L phosphate buffer solution, pH 7.0 to

make exactly 100 mL. Confirm that the peak height of cefixime obtained from 10 μ L of this solution is equivalent to 20 to 60 mm.

System performance: Dissolve about 2 mg of Cefixime Reference Standard in 200 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the solution for system suitability test. When the procedure is run with 10 μ L of the solution according to the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefixime is not more than 2.0%.

Water <2.48> Not less than 9.0 and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Cefixime and Cefixime Reference Standard, equivalent to about 0.1 g (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL each. Pipet 10 mL each of these solutions, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL each, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefixime of these solutions.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } C_{16}H_{15}N_5O_7S_2 \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefixime Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 25 mL of a solution of tetrabutylammonium hydroxide TS (10 in 13) add water to make 1000 mL, adjust to pH 6.5 with diluted phosphoric acid (1 in 10). To 300 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefixime is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefixime are not less than 4000 and not more than 2.0, respectively.

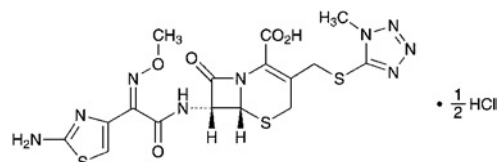
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of peak areas of cefixime is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefmenoxime Hydrochloride

セフメノキシム塩酸塩



$C_{16}H_{17}N_9O_5S_3 \cdot \frac{1}{2}HCl$: 529.79

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetamino]-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid hemihydrochloride [75738-58-8]

Cefmenoxime Hydrochloride contains not less than 890 μ g (potency) and not more than 975 μ g (potency) per mg, calculated on the dehydrated basis. The potency of Cefmenoxime Hydrochloride is expressed as mass (potency) of cefmenoxime ($C_{16}H_{17}N_9O_5S_3$: 511.56).

Description Cefmenoxime Hydrochloride occurs as white to light orange-yellow crystals or crystalline powder.

It is freely soluble in formamide and in dimethylsulfoxide, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefmenoxime Hydrochloride in 0.1 mol/L phosphate buffer solution, pH 6.8 (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefmenoxime Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmenoxime Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefmenoxime Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefmenoxime Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 3.9 ppm, and a single signal C at around δ 6.8 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Dissolve 10 mg of Cefmenoxime Hydrochloride in 1 mL of diluted sodium carbonate TS (1 in 20), add 5 mL of acetic acid (100) and 2 drops of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: -27 – -35° (1 g, 0.1 mol/L phosphate buffer solution, pH 6.8, 100 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Cefmenoxime Hydrochloride in 150 mL of water is between 2.8 and 3.3.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefmenoxime Hydrochloride in 10 mL of diluted sodium carbonate TS (1 in 4) is clear and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefmenoxime Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefmenoxime Hydrochloride according to Method 4 and adding 10 mL of dilute hydrochloric acid to the residue after cooling, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of 1-methyl-1*H*-tetrazol-5-thiol, and dissolve in the mobile phase to make exactly 100 mL. Pipet 4 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (1). Weigh accurately about 0.1 g of Cefmenoxime Hydrochloride Reference Standard, dissolve in 20 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution (2). Perform the test immediately after preparation of these solutions with exactly 10 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method, and calculate the amounts of 1-methyl-1*H*-tetrazol-5-thiol and the total related substance by the following formula: the amount of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%, and the total related substance is not more than 3.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of 1-methyl-1}H\text{-tetrazol-5-thiol} \\ = (W_{\text{Sa}}/W_{\text{T}}) \times (A_{\text{Ta}}/A_{\text{Sa}}) \times 20 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} \text{ of total related substances} \\ = \{(W_{\text{Sa}}/W_{\text{T}}) \times (A_{\text{Ta}}/A_{\text{Sa}}) \times 20\} \\ + \{(W_{\text{Sb}}/W_{\text{T}}) + (S_{\text{T}}/A_{\text{Sb}}) \times 5\} \end{aligned}$$

W_{Sa} : Amount (g) of 1-methyl-1*H*-tetrazol-5-thiol

W_{Sb} : Amount (g) of Cefmenoxime Hydrochloride Reference Standard

W_{T} : Amount (g) of the sample

A_{Sa} : Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the standard solution (1)

A_{Sb} : Peak area of cefmenoxime from the standard solution (2)

A_{Ta} : Peak area of 1-methyl-1*H*-tetrazol-5-thiol from the sample solution

S_{T} : Total area of the peaks other than 1-methyl-1*H*-tetrazol-5-thiol and other than cefmenoxime from the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of cefmenoxime.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution (1), add the mobile phase to make exactly 100 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazol-5-thiol obtained from 10 μ L of this solution is equivalent to 4.5 to 5.5% of that from the standard solution (1). Then, measure exactly 2 mL of the standard solution (2), add the mobile phase to make exactly 100 mL. Confirm that the peak area of cefmenoxime obtained from 10 μ L of this solution is equivalent to 1.5 to 2.5% of that from the standard solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazol-5-thiol is not more than 1.0%.

Water <2.48> Not more than 1.5% (1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (2:1)).

Assay Weigh accurately an amount of Cefmenoxime Hydrochloride and Cefmenoxime Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in 10 mL of 0.1 mol/L phosphate buffer solution, pH 6.8, and add the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, add exactly 20 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of the peak area of cefmenoxime to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefmenoxime (C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_3) \\ = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Cefmenoxime Hydrochloride Reference Standard

Internal standard solution—A solution of phthalimide in methanol (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:10:1).

Flow rate: Adjust the flow rate so that the retention time of cefmenoxime is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cefmenoxime and the internal standard are eluted

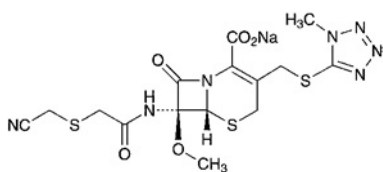
in this order with the resolution between these peaks being not less than 2.3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefmenoxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefmetazole Sodium

セフメタゾールナトリウム



$\text{C}_{15}\text{H}_{16}\text{N}_7\text{NaO}_5\text{S}_3$: 493.52

Monosodium (6*R*,7*R*)-7-

{[(cyanomethylsulfanyl)acetyl]amino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[56796-20-4]

Cefmetazole Sodium contains not less than 860 μg (potency) and not more than 965 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefmetazole Sodium is expressed as mass (potency) of cefmetazole ($\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3$: 471.53).

Description Cefmetazole Sodium occurs as a white to light yellowish white, powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in tetrahydrofuran.

Identification (1) Determine the absorption spectrum of a solution of Cefmetazole Sodium (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefmetazole Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefmetazole Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 3.6 ppm, at around δ 4.1 ppm and at around δ 5.2 ppm, respectively. The ratio of integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefmetazole Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +73 – +85° (0.25 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the pH of the solution is between 4.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefmetazole Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefmetazole Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefmetazole Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Cefmetazole Sodium in 2 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution (1). Separately, dissolve 0.10 g of 1-methyl-1*H*-tetrazole-5-thiol in water to make exactly 100 mL, and use this solution as the standard solution (2). Immediately perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of 1-butanol, water and acetic acid (100) (4:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spot obtained from the sample solution corresponding to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than this spot and other than the principal spot are not more intense than the spot from the standard solution (1).

Water <2.48> Not more than 1.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefmetazole Sodium and Cefmetazole Reference Standard, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 25 mL. Pipet 1 mL each of these solutions, add exactly 10 mL of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{T} and Q_{S} , of the peak area of cefmetazole to that of the internal standard of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefmetazole } (\text{C}_{15}\text{H}_{17}\text{N}_7\text{O}_5\text{S}_3) \\ = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Cefmetazole Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.75 g of ammonium dihydrogenphosphate in 700 mL of water, add 280 mL of methanol, 20 mL of tetrahydrofuran and 3.2 mL of 40% tetrabutylammonium hydroxide TS, and adjust to pH 4.5 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of cefmetazole is about 8 minutes.

System suitability—

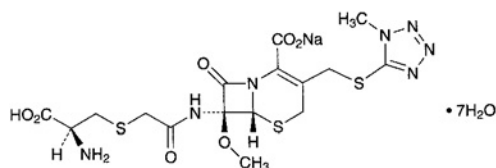
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cefmetazole and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefmetazole to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Cefminox Sodium Hydrate

セフミノクスナトリウム水和物



$C_{16}H_{20}N_7NaO_7S_3 \cdot 7H_2O$: 667.66

Monosodium (6R,7S)-7-[2-[(2S)-2-amino-2-carboxyethylsulfanyl]acetylamino]-7-methoxy-3-(1-methyl-1H-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate heptahydrate [75498-96-3]

Cefminox Sodium Hydrate contains not less than 900 μ g (potency) and not more than 970 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefminox Sodium Hydrate is expressed as mass (potency) of cefminox ($C_{16}H_{21}N_7O_7S_3$; 519.58).

Description Cefminox Sodium Hydrate occurs as a white to light yellow crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefminox Sodium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefminox Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefminox Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the

spectrum of Cefminox Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefminox Sodium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 30) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal, A, at around δ 3.2 ppm, a single signal, B, at around δ 3.5 ppm, a single signal, C, at around δ 4.0 ppm, and a single signal, D, at around δ 5.1 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 2:3:3:1.

(4) Cefminox Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +62 – +72° (50 mg, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.70 g of Cefminox Sodium Hydrate in 10 mL of water: the pH of the solution is between 4.5 and 6.0.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefminox Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Cefminox Sodium Hydrate according to Method 3, and perform the test (not more than 1 ppm).

Water <2.48> Not less than 18.0% and not more than 20.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—Use the medium iii in 3) under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solution—Weigh accurately an amount of Cefminox Sodium Reference Standard, equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 40 μ g (potency) and 20 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

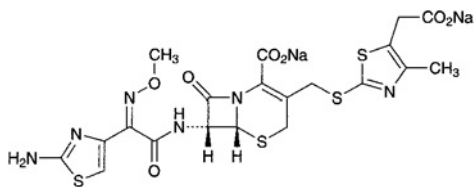
(iv) Sample solution—Weigh accurately an amount of Cefminox Sodium Hydrate equivalent to about 40 mg (potency), dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 40 μ g (potency) and 20 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

(v) Procedure—Incubate between 32°C and 35°C.

Containers and storage Containers—Hermetic containers.

Cefodizime Sodium

セフォジジムナトリウム



$C_{20}H_{18}N_6Na_2O_7S_4$: 628.63

Disodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl-amino]-3-[(5-carboxylatomethyl-4-methylthiazol-2-yl)sulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [86329-79-5]

Cefodizime Sodium contains not less than 890 μg (potency) per mg, calculated on the anhydrous basis and corrected by the ethanol amount. The potency of Cefodizime Sodium is expressed as mass (potency) of cefodizime ($C_{20}H_{20}N_6O_7S_4$: 584.67).

Description Cefodizime Sodium occurs as a white to light yellowish white crystalline powder.

It is very soluble in water, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefodizime Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefodizime Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefodizime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefodizime Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefodizime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ 2.3 ppm, at around δ 4.0 ppm, and at around δ 7.0 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:3:1.

(4) Cefodizime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-56 - -62^\circ$ (0.2 g calculated on the anhydrous basis and corrected by the ethanol amount, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefodizime Sodium in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Cefodizime Sodium in 10 mL of water: the solution is clear and pale yellow to light yellow.

(2) Heavy metals <1.07>—Weigh 1.0 g of Cefodizime Sodium in a crucible, cover loosely, and carbonize by gentle heating. After cooling, add 2 mL of sulfuric acid, heat gradually until the white fumes are no longer evolved, and ignite between 500°C and 600°C. Proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefodizime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 30 mg of Cefodizime Sodium in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cefodizime from the sample solution is not more than the peak area of cefodizime from the standard solution, and the total area of the peaks other than cefodizime from the sample solution is not more than 3 times the peak area of cefodizime from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefodizime beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefodizime obtained from 5 μL of this solution is equivalent to 7 to 13% of that from 5 μL of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(5) Ethanol—Weigh accurately about 1 g of Cefodizime Sodium, and dissolve in water to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 2 g of ethanol for gas chromatography, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and the standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ethanol to that of the internal standard: the amount of ethanol is not more than 2.0%.

$$\text{Amount (\%)} \text{ of ethanol} = (W_S/W_T) \times (Q_T/Q_S)$$

W_S : Amount (g) of ethanol for gas chromatography

W_T : Amount (g) of the sample

Internal standard solution—A solution of 1-propanol (1 in 400).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 3 m in length, packed with tetrafluoroethylene polymer for gas chromatography (180 – 250 μm in particle diameter) coated in 15% with polyethylene glycol 20 M.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water <2.48> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefodizime Sodium and Cefodizime Sodium Reference Standard, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefodizime to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefodizime (C}_{20}\text{H}_{20}\text{N}_6\text{O}_7\text{S}_4) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefodizime Sodium Reference Standard

Internal standard solution—A solution of anhydrous caffeine (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.80 g of potassium dihydrogen phosphate and 0.20 g of anhydrous disodium hydrogen phosphate in a suitable amount of water, and add 80 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefodizime is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cefodizime and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

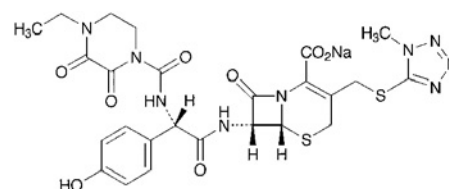
System repeatability: When the test is repeated 6 times with

10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefodizime to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefoperazone Sodium

セフォペラゾンナトリウム



$\text{C}_{25}\text{H}_{26}\text{N}_9\text{NaO}_8\text{S}_2$: 667.65

Monosodium (6*R*,7*R*)-7-[(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonylamino]-2-(4-hydroxyphenyl)acetyl-amino]-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [62893-20-3]

Cefoperazone Sodium contains not less than 871 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefoperazone Sodium is expressed as mass (potency) of cefoperazone ($\text{C}_{25}\text{H}_{27}\text{N}_9\text{O}_8\text{S}_2$: 645.67).

Description Cefoperazone Sodium occurs as a white to yellowish white crystalline powder.

It is very soluble in water, soluble in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefoperazone Sodium (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefoperazone Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a triplet signal A at around δ 1.2 ppm, and double signals, B and C, at around δ 6.8 ppm and at around δ 7.3 ppm. The ratio of integrated intensity of these signals, A:B:C, is about 3:2:2.

(3) Cefoperazone Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-15 - -25^\circ$ (1 g, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefoperazone Sodium in 4 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefoperazone Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefoper-

azone Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefoperazone Sodium according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Cefoperazone Sodium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method. Calculate the percentages of each peak area from the sample solution to 50 times of the peak area of cefoperazone from the standard solution: the related substance I with the retention time of about 8 minutes is not more than 5.0%, the related substance II with that of about 17 minutes is not more than 1.5%, and the total of all related substances is not more than 7.0%. Use the peak areas of the related substances I and II after multiplying by their relative response factor, 0.90 and 0.75, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of cefoperazone beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefoperazone obtained from 25 μ L of this solution is equivalent to 3.5 to 6.5% of that of cefoperazone obtained from 25 μ L of the standard solution.

System performance: When the procedure is run with 25 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefoperazone are not less than 5000 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefoperazone is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefoperazone Sodium equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefoperazone Reference Standard equivalent to about 0.1 g (potency), dissolve in 5 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefoperazone to that of the internal standard.

Amount [μ g (potency)] of cefoperazone ($C_{25}H_{27}N_9O_8S_2$)
 $= W_S \times (Q_T/Q_S) \times 1000$

W_S : Amount [mg (potency)] of Cefoperazone Reference Standard

Internal standard solution—A solution of acetanilide in a mixture of water and acetonitrile (43:7) (3 in 8000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 57 mL of acetic acid (100) add 139 mL of triethylamine and water to make 1000 mL. To 20 mL of this solution add 835 mL of water, 140 mL of acetonitrile and 5 mL of dilute acetic acid.

Flow rate: Adjust the flow rate so that the retention time of cefoperazone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and cefoperazone are eluted in this order with the resolution between these peaks being not less than 5.

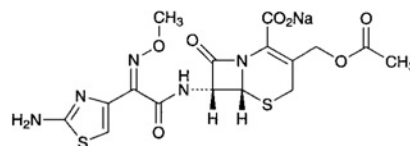
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefoperazone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place.

Cefotaxime Sodium

セフトキシムナトリウム



$C_{16}H_{16}N_5NaO_7S_2$: 477.45

Monosodium (6*R*,7*R*)-3-acetoxymethyl-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamin]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
 [64485-93-4]

Cefotaxime Sodium contains not less than 916 μ g (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Sodium is expressed as mass (potency) of cefotaxime ($C_{16}H_{17}N_5O_7S_2$: 455.47).

Description Cefotaxime Sodium occurs as white to light yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Dissolve 2 mg of Cefotaxime Sodium in 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotaxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefotaxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three single signals, A, B and C, at around δ 2.1 ppm, at around δ 4.0 ppm and at around δ 7.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(4) Cefotaxime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +58 – +64° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cefotaxime Sodium in 10 mL of water is clear and light yellow.

(2) Sulfate <1.14>—Dissolve 2.0 g of Cefotaxime Sodium in 40 mL of water, add 2 mL of dilute hydrochloric acid and water to make 50 mL, shake well, and filter. Discard first 10 mL of the filtrate, and to the subsequent 25 mL of the filtrate add water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Cefotaxime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefotaxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Perform the test with 10 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area obtained from the chromatogram by the automatic integration method: the each peak area other than cefotaxime is not more than 1.0% and the total of these peak areas is not more than 3.0%.

Operating conditions—

Detector, column, column temperature, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the

retention time of cefotaxime beginning after the solvent peak.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase A to make exactly 100 mL. Pipet 2 mL of this solution, and add the mobile phase A to make exactly 20 mL. Confirm that the peak area of cefotaxime obtained from 10 μ L of this solution is equivalent to 0.15 to 0.25% of that obtained from 10 μ L of the standard solution.

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately an amount of Cefotaxime Sodium and Cefotaxime Reference Standard, equivalent to about 40 mg (potency), dissolve each in the mobile phase A to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of cefotaxime of these solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefotaxime (C}_{16}\text{H}_{17}\text{N}_5\text{O}_7\text{S}_2) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefotaxime Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase A: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 860 mL of this solution add 140 mL of methanol.

Mobile phase B: To 0.05 mol/L disodium hydrogen phosphate TS add phosphoric acid to adjust the pH to 6.25. To 600 mL of this solution add 400 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 7	100	0
7 – 9	100 → 80	0 → 20
9 – 16	80	20
16 – 45	80 → 0	20 → 100
45 – 50	0	100

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 14 minutes (about 1.3 mL/min).

System suitability—

System performance: To 1 mL of the standard solution add 7.0 mL of water and 2.0 mL of methanol, mix, then add

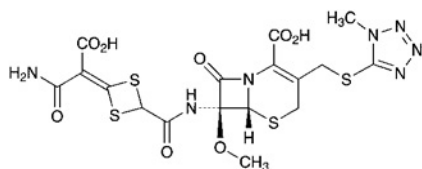
25 mg of sodium carbonate decahydrate, and shake. After allowing to stand for 10 minutes, add 3 drops of acetic acid (100) and 1 mL of the standard solution, and mix. When the procedure is run with 10 μ L of this solution under the above operating conditions, desacetyl cefotaxime with the relative retention time being about 0.3 to cefotaxime and cefotaxime are eluted in this order with the resolution between these peaks being not less than 20, and the symmetry factor of the peak of cefotaxime is not more than 2.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotaxime is not more than 2.0%.

Containers and storage Containers—Tight containers.

Cefotetan

セフォテタン



$C_{17}H_{17}N_7O_8S_4$: 575.62

(6*R*,7*R*)-7-[4-(Carbamoylcarboxymethylidene)-1,3-dithietane-2-carbonyl]amino}-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid [69712-56-7]

Cefotetan contains not less than 960 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefotetan is expressed as mass (potency) of cefotetan ($C_{17}H_{17}N_7O_8S_4$).

Description Cefotetan occurs as white to light yellowish white powder.

It is sparingly soluble in methanol, and slightly soluble in water and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Cefotetan in phosphate buffer solution for antibiotics, pH 6.5 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotetan Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotetan as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotetan Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve 50 mg of Cefotetan in 0.5 mL of a solution of sodium hydrogen carbonate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25). Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium

3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B, C and D, at around δ 3.6 ppm, at around δ 4.0 ppm, at around δ 5.1 ppm and at around δ 5.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C:D, is about 3:3:1:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +112 – +124° (0.5 g calculated on the anhydrous basis, a solution of sodium hydrogen carbonate (1 in 200), 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefotetan in 10 mL of a solution of sodium hydrogen carbonate (1 in 30): the solution is clear, and colorless or light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefotetan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Cefotetan, dissolve in a suitable amount of methanol, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and about 2 mg of Cefotetan Reference Standard, calculated on the anhydrous basis, dissolve in methanol to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and methanol to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{Ta} , Q_{Tb} , Q_{Tc} , Q_{Td} , Q_{Te} and Q_{Tf} , of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone having the relative retention time of about 0.5 with respect to cefotetan, Δ_2 -cefotetan having the relative retention time of about 1.2 with respect to cefotetan, isothiazole substance having the relative retention time of about 1.3 with respect to cefotetan, each of other related substances and the total of other related substances, to the peak area of the internal standard, respectively, obtained from the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak areas of 1-methyl-1*H*-tetrazole-5-thiol and cefotetan, to the peak area of the internal standard, respectively, obtained from the standard solution. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, cefotetan lactone, Δ_2 -cefotetan, isothiazole substance, each of other related substances and the total of other related substances from the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol is not more than 0.3%, cefotetan lactone is not more than 0.3%, Δ_2 -cefotetan is not more than 0.5%, isothiazole substance is not more than 0.5%, each of other related substances is not more than 0.2% and the total of other related substances is not more than 0.4%.

$$\begin{aligned} & \text{1-Methyl-1H-tetrazole-5-thiol (\%)} \\ & = (W_{Sa}/W_T) \times (Q_{Ta}/Q_{Sa}) \times (1/100) \end{aligned}$$

$$\begin{aligned} & \text{Cefotetan lactone (\%)} \\ & = (W_{Sb}/W_T) \times (Q_{Tb}/Q_{Sb}) \times (1/100) \end{aligned}$$

$$\begin{aligned} & \Delta_2\text{-Cefotetan (\%)} \\ & = (W_{Sb}/W_T) \times (Q_{Tc}/Q_{Sb}) \times (1/100) \end{aligned}$$

$$\text{Isothiazole substance (\%)}$$

$$= (W_{\text{sb}}/W_{\text{T}}) \times (Q_{\text{Td}}/Q_{\text{Sb}}) \times (1/100)$$

Each of other related substances (%)

$$= (W_{\text{sb}}/W_{\text{T}}) \times (Q_{\text{Te}}/Q_{\text{Sb}}) \times (1/100)$$

Total of other related substances (%)

$$= (W_{\text{sb}}/W_{\text{T}}) \times (Q_{\text{Tf}}/Q_{\text{Sb}}) \times (1/100)$$

W_{sa} : Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol

W_{sb} : Amount (mg) of Cefotetan Reference Standard, calculated on the anhydrous basis

W_{T} : Amount (g) of the sample

Internal standard solution—A solution of anhydrous caffeine in methanol (3 in 10,000).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3.5 times as long as the retention time of cefotetan.

System suitability—

Test for required detectability: Measure exactly 15 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of cefotetan obtained from 5 μL of this solution is equivalent to 12 to 18% of that from 5 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 2.0%.

Water <2.48> Not more than 2.5% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Dissolve 10 mg of Cefotetan in 20 mL of methanol, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the adjacent two peaks appeared at around the retention time of 40 minutes, one having shorter retention time is *l*-substance and another having longer retention time is *d*-substance, by the area percentage method: the amount of *l*-substance is not less than 35% and not more than 45%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, water and a solution of tetrabutylammonium hydrogensulfate in acetonitrile (1 in 150) (9:9:2).

Flow rate: Adjust the flow rate so that the retention time of *l*-substance is about 40 minutes.

System suitability—

System performance: When the procedure is run with 5 μL

of the sample solution under the above operating conditions, *l*-substance and *d*-substance are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: To exactly 1 mL of the sample solution add methanol to make exactly 10 mL. When the test is repeated 6 times with 5 μL of this solution under the above operating conditions, the relative standard deviation of the peak area of *l*-substance is not more than 5.0%.

Assay Weigh accurately an amount of Cefotetan and Cefotetan Reference Standard, equivalent to about 50 mg (potency), and dissolve each in phosphate buffer solution for antibiotics, pH 6.5 to make exactly 50 mL. Pipet 15 mL each of these solutions, add exactly 10 mL of the internal standard solution and phosphate buffer solution for antibiotics, pH 6.5 to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of the peak area of cefotetan to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{17}\text{H}_{17}\text{N}_7\text{O}_8\text{S}_4 \\ &= W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Cefotetan Reference Standard

Internal standard solution—A solution of anhydrous caffeine (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 11.53 g of phosphoric acid in 1000 mL of water. To 850 mL of this solution add 50 mL of acetonitrile, 50 mL of acetic acid (100) and 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of cefotetan is about 17 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and cefotetan are eluted in this order with the resolution between these peaks being not less than 8.

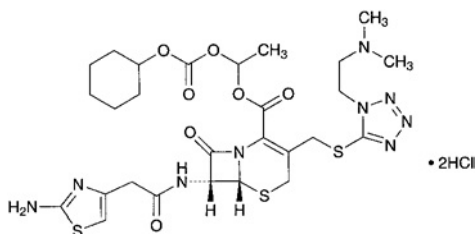
System repeatability: When the test is repeated 5 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefotetan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefotiam Hexetil Hydrochloride

セフォチアム ヘキセチル塩酸塩



$C_{27}H_{37}N_9O_7S_3 \cdot 2HCl$: 768.76

(1*RS*)-1-Cyclohexyloxycarbonyloxyethyl (6*R*,7*R*)-7-[2-(2-aminothiazol-4-yl)acetamino]-3-[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate dihydrochloride [95789-30-3]

Cefotiam Hexetil Hydrochloride contains not less than 615 μ g (potency) and not more than 690 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hexetil Hydrochloride is expressed as mass (potency) of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Description Cefotiam Hexetil Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, in methanol and in ethanol (95), freely soluble in dimethylsulfoxide, and slightly soluble in acetonitrile.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hexetil Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hexetil Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefotiam Hexetil Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits two single signals, A and B, at around δ 2.8 ppm and at around δ 6.6 ppm, and a multiple signal, C, at around δ 6.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 6:1:1.

(3) To a solution of Cefotiam Hexetil Hydrochloride (1 in 200) add 2 mL of dilute nitric acid and 1 mL of silver nitrate TS, and mix: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +52 – +60° (0.1 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of

Cefotiam Hexetil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Cefotiam Hexetil Hydrochloride according to Method 3, and perform the test, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 1 ppm).

(3) Related substance 1—Weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 10 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Cefotiam Hexetil Hydrochloride Reference Standard, and dissolve in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Pipet 1 mL of this solution, add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amount of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, is not more than 2.0%, and each amount of the other related substances is not more than 0.5%. For this calculation, use the value of the peak area obtained by the automatic integration method of the related substance having the relative retention time of about 1.2 to one of the peaks of cefotiam hexetil, which has the larger retention time, after multiplying by its relative response factor, 0.78.

$$\begin{aligned} \text{Amount (\%)} \text{ of each related substance} \\ = (W_S/W_T) \times (A_T/A_S) \times 5 \end{aligned}$$

W_S : Amount (g) of Cefotiam Hexetil Hydrochloride Reference Standard

W_T : Amount (g) of sample

A_S : Total of two peak areas of cefotiam hexetil from the standard solution

A_T : Each peak area of related substance from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Mobile phase B: A mixture of acetonitrile, diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2) and acetic acid (100) (60:40:1).

Flowing of the mobile phase: Adjust so that the mixing rate of the mobile phase A and the mobile phase B is changed linearly from 1:0 to 0:1 for 30 minutes.

Flow rate: 0.7 mL per minute.

Time span of measurement: As long as about 3 times of the retention time of one of the cefotiam hexetil peaks, which appears first, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Confirm that each area of the two peaks of cefotiam hexetil obtained from 10 μ L of this solution is equivalent to 1.6 to 2.4% of that from 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the resolution between the two peaks of cefotiam hexetil is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the total of the two peak areas of cefotiam hexetil is not more than 2.0%.

(4) Related substance 2—Weigh accurately about 20 mg of Cefotiam Hexetil Hydrochloride, dissolve in exactly 2 mL of methanol, add a mixture of a solution of diammonium hydrogen phosphate (79 in 20,000) and acetic acid (100) (200:3) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Cefotiam Hydrochloride Reference Standard, and dissolve in the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of the related substances by the following equation: the amounts of the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam are not more than 1.0%, respectively, and each amount of the related substances other than the related substances having the relative retention time of about 0.1 and 0.9 to cefotiam is not more than 0.5%. For this calculation, use the value of the peak area of the related substance having the relative retention time of about 0.9 to cefotiam after multiplying by its sensitivity coefficient, 0.76.

$$\begin{aligned} &\text{Amount (\%)} \text{ of each related substance} \\ &= (W_S/W_T) \times (A_T/A_S) \times 4 \end{aligned}$$

W_S : Amount (g) of Cefotiam Hydrochloride Reference Standard

W_T : Amount (g) of the sample

A_S : Peak area of cefotiam from the standard solution

A_T : Each peak area from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of diammonium hydrogen phosphate (79 in 20,000), methanol and acetic acid (100) (200:10:3).

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 15 minutes.

Time span of measurement: As long as about 2 times of the retention time of cefotiam beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of cefotiam obtained from 10 μ L of this solution is equivalent to 1.6 to 2.4% of that from 10 μ L of the standard solution.

System performance: To 1 mL of a solution of acetaminophen in the mobile phase (1 in 50,000) add 3 mL of the standard solution, and mix well. When the procedure is run with 10 μ L of this solution under the above operating conditions, acetaminophen and cefotiam are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefotiam is not more than 2.0%.

(5) Total amount of related substances—The total of the amount of related substances obtained in the Related substance 1 and the Related substance 2 is not more than 6.5%.

Water <2.48> Not more than 3.5% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Proceed the test with 20 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the conditions directed in the Assay, and determine the areas of the two peaks, A_a for the faster peak and A_b for the later peak, closely appeared each other at the retention time of around 10 minutes: $A_a/(A_a + A_b)$ is not less than 0.45 and not more than 0.55.

Assay Weigh accurately an amount of Cefotiam Hexetil Hydrochloride and Cefotiam Hexetil Hydrochloride Reference Standard, equivalent to about 30 mg (potency), and dissolve each in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL. Measure exactly 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefotiam hexetil to that of the internal standard. For this calculation, the total of the areas of the two peaks appeared closely each other at the retention time of around 10 minutes is used as the peak area of cefotiam hexetil.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefotiam (C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefotiam Hexetil Hydrochloride Reference Standard

Internal standard solution—A solution of benzoic acid in a mixture of diluted phosphoric acid (1 in 100) and acetonitrile (4:1) (7 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 2), acetonitrile and acetic acid (100) (72:28:1).

Flow rate: Adjust the flow rate so that the retention time of the faster peak of cefotiam hexetil is about 9 minutes.

System suitability—

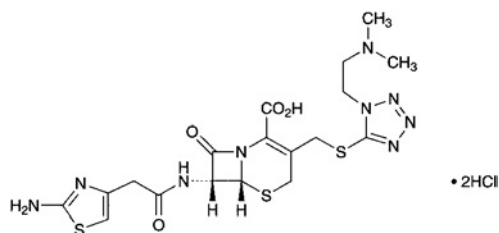
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and cefotiam hexetil are eluted in this order with the resolution between the two peaks of cefotiam hexetil being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefotiam hexetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefotiam Hydrochloride

セフォチアム塩酸塩



$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$: 598.55

(6*R*,7*R*)-7-[2-(2-Aminothiazol-4-yl)acetyl]amino-3-[1-(2-dimethylaminoethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrochloride [66309-69-1]

Cefotiam Hydrochloride contains not less than 810 μ g (potency) and not more than 890 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefotiam Hydrochloride is expressed as mass (potency) of cefotiam ($C_{18}H_{23}N_9O_4S_3$: 525.63).

Description Cefotiam Hydrochloride occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water, in methanol and in formamide, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotiam Hydrochloride Reference Standard prepared in the same manner as the sample solu-

tion: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotiam Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotiam Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefotiam Hydrochloride in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals at around δ 3.1 ppm and at around δ 6.7 ppm, respectively. The ratio of integrated intensity of each signal is about 6:1.

(4) Dissolve 0.1 g of Cefotiam Hydrochloride in 5 mL of dilute nitric acid, and immediately add 1 mL of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +60 – +72° (1 g calculated on the anhydrous bases, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the pH of the solution is between 1.2 and 1.7.

Purity (1) Clarity of solution—Dissolve 1.0 g of Cefotiam Hydrochloride in 10 mL of water: the solution is clear, and colorless to yellow.

(2) Heavy metals <1.07>—To 1.0 g of Cefotiam Hydrochloride add 1 mL of sulfuric acid, and heat gently to carbonize. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, then heat gradually to incinerate. If a carbonized residue still retains, moisten the residue with a little amount of sulfuric acid, and ignite again to incinerate. After cooling, add 2 mL of hydrochloric acid to the residue, heat on a water bath to dissolve, then heat to dryness. Add 10 mL of water, and heat to dissolve. After cooling, add ammonia TS dropwise to adjust to pH 3 – 4, if necessary, filter, wash the residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution in the same manner as for preparation of the test solution (not more than 20 ppm).

(3) Arsenic <1.11>—Incinerate 1.0 g of Cefotiam Hydrochloride according to Method 4. After cooling, add 10 mL of dilute hydrochloric acid to the residue, heat to dissolve on the water bath, and use this solution as the test solution. Perform the test (not more than 2 ppm).

Water <2.48> Not more than 7.0% (0.25 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefotiam Hydrochloride and Cefotiam Hydrochloride Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 10 μ L each of the sample

solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefotiam of these solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefotiam (C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefotiam Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 125 mm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 800 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L potassium dihydrogenphosphate TS to adjust the pH to 7.7. To 440 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefotiam is about 14 minutes.

System suitability—

System performance: Dissolve 0.04 g of orcine in 10 mL of the standard solution. When the procedure is run with 10 μL of the standard solution under the above operating conditions, orcine and cefotiam are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefotiam is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefotiam Hydrochloride for Injection

注射用セフトリアム塩酸塩

Cefotiam Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of cefotiam (C₁₈H₂₃N₉O₄S₃; 525.63).

Method of Preparation Prepare as directed under Injection, with Cefotiam Hydrochloride.

Description Cefotiam Hydrochloride for Injection occurs as a white to light yellow powder.

Identification (1) Determine the absorption spectrum of a solution of Cefotiam Hydrochloride for Injection (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

(2) Dissolve 50 mg of Cefotiam Hydrochloride for Injection in 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy, and determine the spectrum of this solution as

directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits a single signal A between δ 2.7 ppm and δ 3.0 ppm, and a single signal B at around δ 6.5 ppm. The ratio of the integrated intensity of each signal, A:B, is about 6:1.

pH <2.54> The pH of a solution prepared by dissolving an amount of Cefotiam Hydrochloride for Injection, equivalent to 0.5 g (potency) according to the labeled amount, in 5 mL of water is between 5.7 and 7.2.

Purity Clarity and color of solution—Dissolve an amount of Cefotiam Hydrochloride for Injection, equivalent to 1.0 g (potency) of Cefotiam Hydrochloride according to the labeled potency, in 10 mL of water: the solution is clear, and the absorbance of this solution, determined at 450 nm 10 minutes after dissolving as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.20.

Loss on drying <2.41> Not more than 6.0% (0.5 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.125 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the contents of not less than 10 Cefotiam Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 50 mg (potency) of Cefotiam Hydrochloride according to the labeled amount, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefotiam Hydrochloride Reference Standard, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefotiam Hydrochloride.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of cefotiam (C}_{18}\text{H}_{23}\text{N}_9\text{O}_4\text{S}_3) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefotiam Hydrochloride Reference Standard

Operating conditions—

Proceed as directed in the Assay under Cefotiam Hydrochloride.

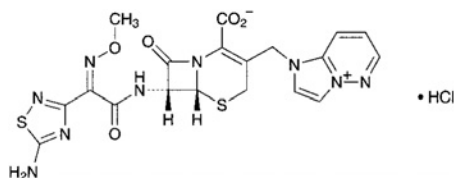
System Suitability—

Proceed as directed in the Assay under Cefotiam Hydrochloride.

Containers and storage Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injections may be used.

Cefozopran Hydrochloride

セフォゾプラン塩酸塩



$C_{19}H_{17}N_9O_5S_2 \cdot HCl$: 551.99

(6*R*,7*R*)-7-[(*Z*)-2-(5-Amino-1,2,4-thiadiazol-3-yl)-2-(methoxyimino)acetylamino]-3-(1*H*-imidazo[1,2-*b*]pyridazin-4-ium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monohydrochloride [113359-04-9, Cefozopran]

Cefozopran Hydrochloride contains not less than 860 μ g (potency) and not more than 960 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefozopran Hydrochloride is expressed as mass (potency) of cefozopran ($C_{19}H_{17}N_9O_5S_2$: 515.53).

Description Cefozopran Hydrochloride occurs as a white to pale yellow, crystals or crystalline powder.

It is freely soluble in dimethylsulfoxide and in formamide, slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in acetonitrile and diethylene.

Identification (1) Dissolve 0.02 g of Cefozopran Hydrochloride in 10 mL of water, add 1 mL of a solution of hydroxylammonium chloride (1 in 10) and 2 mL of sodium hydroxide TS, allow to stand for 5 minutes, then add 3 mL of 1 mol/L hydrochloric acid TS and 3 drops of iron (III) chloride TS, and mix: a red-purple color develops.

(2) Determine the absorption spectra of solutions of Cefozopran Hydrochloride and Cefozopran Hydrochloride Reference Standard in a mixture of sodium chloride TS and methanol (3:2) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the spectrum of a solution of Cefozopran Hydrochloride in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.9 ppm, a double signal B at around δ 5.2 ppm, and a quartet signal C at around δ 8.0 ppm, and the ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Dissolve 0.01 g of Cefozopran Hydrochloride in 1 mL of water and 2 mL of acetic acid (100), add 2 drops of silver nitrate TS, and mix: a white turbidity is formed.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (238 nm): 455 – 485 (50 mg calculated on the anhydrous basis, a mixture of sodium chloride TS and methanol (3:2), 5000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: -73 – -78° (0.1 g calculated on the anhydrous basis, a mixture of sodium chloride TS and

methanol (3:2), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefozopran Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic—Being specified separately.

(4) Related substances—Being specified separately.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 0.05 EU/mg (potency).

Assay Weigh accurately an amount of Cefozopran Hydrochloride and Cefozopran Hydrochloride Reference Standard, equivalent to about 50 mg (potency), and dissolve each in the mobile phase to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefozopran to that of the internal standard of these solutions.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefozopran } (C_{19}H_{17}N_9O_5S_2) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefozopran Hydrochloride Reference Standard

Internal standard solution—A solution of 2,4-dihydroxybenzoic acid in the mobile phase (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Mix 0.366 g of diethylamine with water to make 1000 mL, and add 60 mL of acetonitrile and 5 mL of acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of cefozopran is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cefozopran and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefozopran to that of the internal standard is not

more than 1.0%.

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Cefozopran Hydrochloride for Injection

注射用セフォゾプラン塩酸塩

Cefozopran Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled potency of cefozopran ($C_{19}H_{17}N_9O_5S_2$; 515.53).

Method of Preparation Prepare as directed under the Injections, with Cefozopran Hydrochloride.

Description Cefozopran Hydrochloride for Injection occurs as a white to light yellow, powder or masses.

Identification (1) Determine the absorption spectrum of a solution of Cefozopran Hydrochloride for Injection (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 236 nm and 241 nm.

(2) To 50 mg of Cefozopran Hydrochloride for Injection add 0.8 mL of deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy, and filter after shaking, and determine the spectrum of the filtrate as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 3.9 ppm, a double signal B at around δ 5.0 ppm, and a quartet signal C at around δ 8.0 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 3:1:1.

pH <2.54> Dissolve an amount of Cefozopran Hydrochloride for Injection, equivalent to 0.5 g (potency) of Cefozopran Hydrochloride according to the labeled amount, in 5 mL of water: the pH of this solution is between 7.5 and 9.0.

Purity (1) Clarity and color of solution—Dissolve an amount of Cefozopran Hydrochloride for Injection, equivalent to 1 g (potency) of Cefozopran Hydrochloride according to the labeled amount, in 10 mL of water: the solution is clear and has no more color than Matching Fluid N.

(2) Related substances—Being specified separately.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fischer method and methanol for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Bacterial endotoxins <4.01> Less than 0.05 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test accord-

ing to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Cefozopran Hydrochloride for Injection. Weigh accurately an amount of the contents, equivalent to about 0.5 g (potency) of Cefozopran Hydrochloride according to the labeled amount, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Cefozopran Hydrochloride Reference Standard, equivalent to about 50 mg (potency), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add the mobile phase to make 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Cefozopran Hydrochloride.

$$\text{Amount [mg (potency)] of cefozopran (C}_{19}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 10$$

W_S : Amount [mg (potency)] of Cefozopran Hydrochloride Reference Standard

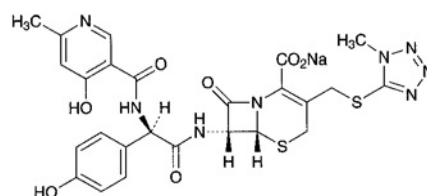
Internal standard solution—A solution 2,4-dihydroxybenzoic acid in the mobile phase (1 in 1250).

Containers and storage Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injections may be used.

Storage—Light-resistant.

Cefpiramide Sodium

セフピラミドナトリウム



$C_{25}H_{23}N_8NaO_7S_2$; 634.62

Monosodium (6*R*,7*R*)-7-[(2*R*)-2-[(4-hydroxy-6-methylpyridine-3-carbonyl)amino]-2-(4-hydroxyphenyl)acetyl]amino]-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [74849-93-7]

Cefpiramide Sodium contains not less than 900 μ g (potency) and not more than 990 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefpiramide Sodium is expressed as mass (potency) of cefpiramide ($C_{25}H_{24}N_8O_7S_2$; 612.64).

Description Cefpiramide Sodium occurs as white to yellowish white powder.

It is very soluble in dimethylsulfoxide, freely soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a

solution of Cefpiramide Sodium in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefpiramide Sodium in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A, B and C, at around δ2.3 ppm, at around δ3.9 ppm and at around δ8.2 ppm, respectively. The ratio of the integrated intensity of each signal, A:B:C, is about 3:3:1.

(3) Cefpiramide Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -33 - -40° (0.2 g calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 2.0 g of Cefpiramide Sodium in 20 mL of water is between 5.5 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefpiramide Sodium in 10 mL of 0.05 mol/L phosphate buffer solution, pH 7.0: the solution is clear, and colorless or light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefpiramide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately about 25 mg of Cefpiramide Sodium, dissolve in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of 1-methyl-1*H*-tetrazole-5-thiol for liquid chromatography, previously dried in a desiccator (in vacuum, silica gel) for 2 hours, and an amount of Cefpiramide Reference Standard, equivalent to about 75 mg (potency), dissolve them in 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL. Pipet 2 mL of this solution, add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances by the following equations: the amount of 1-methyl-1*H*-tetrazole-5-thiol, each of the other related substances and the total of the other related substances are not more than 1.0 %, not more than 1.5% and not more than 4.0%, respectively.

$$\begin{aligned} \text{Amount (\%)} & \text{ of 1-methyl-1H-tetrazole-5-thiol (C}_2\text{H}_4\text{N}_4\text{S)} \\ & = (W_{\text{Sa}}/W_{\text{T}}) \times (A_{\text{Ta}}/A_{\text{Sa}}) \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} & \text{ of each of other related substances} \\ & = (W_{\text{Sb}}/W_{\text{T}}) \times (A_{\text{Tc}}/A_{\text{Sb}}) \end{aligned}$$

W_{Sa} : Amount (mg) of 1-methyl-1*H*-tetrazole-5-thiol

W_{Sb} : Amount [mg (potency)] of Cefpiramide Reference Standard

W_{T} : Amount (mg) of the sample

A_{Sa} : Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the standard solution

A_{Sb} : Peak area of cefpiramide from the standard solution

A_{Ta} : Peak area of 1-methyl-1*H*-tetrazole-5-thiol from the sample solution

A_{Tc} : Area of each peak other than 1-methyl-1*H*-tetrazole-5-thiol and cefpiramide from the sample solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.03 mol/L phosphate buffer solution, pH 7.5 and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 11 minutes.

Time span of measurement: About 2 times as long as the retention time of cefpiramide.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add 0.03 mol/L phosphate buffer solution, pH 7.5 to make exactly 50 mL. Confirm that the peak area of 1-methyl-1*H*-tetrazole-5-thiol obtained from 5 μL of this solution is equivalent to 8 to 12% of that from 5 μL of the standard solution.

System performance: Dissolve 25 mg of Cefpiramide Reference Standard and 7 mg of cinnamic acid in the mobile phase to make 50 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, cinnamic acid and cefpiramide are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 1-methyl-1*H*-tetrazole-5-thiol is not more than 2.0%.

Water <2.48> Not more than 7.0% (0.35 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefpiramide Sodium and Cefpiramide Reference Standard, equivalent to about 50 mg (potency), add exactly 5 mL of the internal standard solution to dissolve, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of the peak area of cefpiramide to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] & \text{ of cefpiramide (C}_2\text{H}_4\text{N}_4\text{O}_7\text{S}_2) \\ & = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Cefpiramide Reference Standard

Internal standard solution—A solution of 4-dimethylaminoantipyrine (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octylsilylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.01 mol/L phosphate buffer solution, pH 6.8, acetonitrile, methanol and tetrahydrofuran (22:1:1:1).

Flow rate: Adjust the flow rate so that the retention time of cefpiramide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, cefpiramide and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

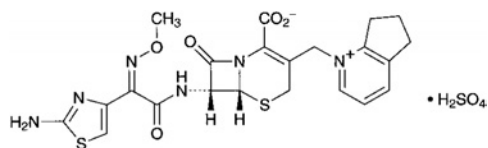
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cefpiramide to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature not exceeding 5°C.

Cefpirome Sulfate

セフピロム硫酸塩



$\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_5\text{S}_2 \cdot \text{H}_2\text{SO}_4$: 612.66

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(6,7-dihydro-5*H*-cyclopenta[*b*]pyridinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate monosulfate [98753-19-6]

Cefpirome Sulfate contains not less than 760 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpirome Sulfate is expressed as mass (potency) of cefpirome ($\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_5\text{S}_2$: 514.58).

Description Cefpirome Sulfate occurs as a white to pale yellowish white crystalline powder, and has a slight, characteristic odor.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 10 mg of Cefpirome Sulfate in 2 mL of water, add 3 mL of hydroxylammonium hydrochloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Dissolve 1 mg of Cefpirome Sulfate in 4 mL of water, add 1 mL of dilute hydrochloric acid while cooling in ice, add 1 mL of a freshly prepared solution of sodium nitrite (1 in

100), and allow to stand for 2 minutes. Add 1 mL of ammonium amidosulfuric acid TS while cooling in ice bath, allow to stand for 1 minute, and add 1 mL of a solution of *N*-1-naphthylethylene dihydrochloride (1 in 1000): a purple color develops.

(3) Take 5 mg of Cefpirome Sulfate, dissolve in 1 mL of ethanol (95) and 1 mL of water, add 100 mg of 1-chloro-2,4-dinitrobenzene, and heat on a water bath for 5 minutes. After cooling, add 2 or 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-brown color develops.

(4) Determine the absorption spectra of solutions of Cefpirome Sulfate and Cefpirome Sulfate Reference Standard in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the spectrum of a solution of Cefpirome Sulfate in heavy water for nuclear magnetic resonance spectroscopy (1 in 25) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.1 ppm, a double signal B at around δ 5.9 ppm, a single signal C at around δ 7.1 ppm, and a multiple signal D at around δ 7.8 ppm. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:1.

(6) A solution of Cefpirome Sulfate (1 in 250) responds to the Qualitative Tests <1.09> (1) for sulfate salt.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (270 nm): 405 – 435 (50 mg calculated on the anhydrous basis, 0.01 mol/L hydrochloric acid TS, 2500 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: –27 – –33° (50 mg calculated on the anhydrous basis, a solution prepared by addition of water to 25 mL of acetonitrile to make 50 mL, 20 mL, 100 mm).

pH <2.54> Dissolve 0.1 g of Cefpirome Sulfate in 10 mL of water: the pH of the solution is between 1.6 and 2.6.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefpirome Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic—Being specified separately.

(4) Related substances—Being specified separately.

(5) Residual solvents—Being specified separately.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 0.10 EU/mg (potency).

Assay Weigh accurately an amount of Cefpirome Sulfate and Cefpirome Sulfate Reference Standard, equivalent to about 50 mg (potency), dissolve each in water to make exactly 100 mL. Pipet 5 mL of these solutions, add each in water to make exactly 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20

μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of cefpirome of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefpirome (C}_{22}\text{H}_{22}\text{N}_6\text{O}_5\text{S}_2) \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefpirome Sulfate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.45 g of ammonium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.3 with phosphoric acid. To 800 mL of this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefpirome is about 7.5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates of the peak of cefpirome is not less than 3600.

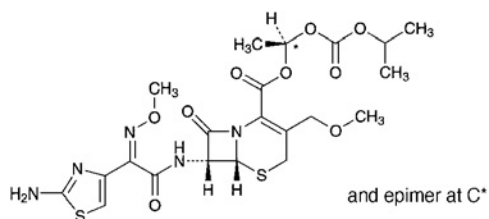
System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefpirome is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—At a temperature between 2 and 8°C.

Cefpodoxime Proxetil

セフポドキシム プロキシセチル



$\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_9\text{S}_2$; 557.60

(1*R*,5*R*)-1-[(1-Methylethyl)carboxyloxy]ethyl
(6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[87239-81-4]

Cefpodoxime Proxetil contains not less than 706 μg (potency) and not more than 774 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefpodoxime Proxetil is expressed as mass (potency) of cefpodoxime ($\text{C}_{15}\text{H}_{17}\text{N}_5\text{O}_6\text{S}_2$; 427.46).

Description Cefpodoxime Proxetil occurs as a white to light

brownish white powder.

It is very soluble in acetonitrile, in methanol and in chloroform, freely soluble in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefpodoxime Proxetil in acetonitrile (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefpodoxime Proxetil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefpodoxime Proxetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefpodoxime Proxetil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefpodoxime Proxetil in deuteriochloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits double signals, A and B, at around δ 1.3 ppm and at around δ 1.6 ppm, and single signals, C and D, at around δ 3.3 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of these signals, A:B:C:D, is about 2:1:1:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +24.0 – +31.4° (0.1 g calculated on the anhydrous basis, acetonitrile, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefpodoxime Proxetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Cefpodoxime Proxetil in 50 mL of a mixture of water, acetonitrile and acetic acid (100) (99:99:2), and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. If necessary, perform the test in the same manner with 20 μL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to compensate for the base line. Determine each peak area by the automatic integration method, and calculate the amounts of them by the area percentage method: the peak, having the relative retention time of about 0.8 with respect to the isomer B of cefpodoxime proxetil, is not more than 2.0%, the peak other than cefpodoxime proxetil is not more than 1.0%, and the sum of the peaks other than cefpodoxime proxetil is not more than 6.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 22°C.

Mobile phase A: A mixture of water, methanol and a solu-

tion of formic acid (1 in 50) (11:8:1).

Mobile phase B: A mixture of methanol and a solution of formic acid (1 in 50) (19:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 65	95	5
65 – 145	95 → 15	5 → 85
145 – 155	15	85

Flow rate: Adjust the flow rate so that the retention time of the isomer B of cefpodoxime proxetil is about 60 minutes.

Time span of measurement: About 2.5 times as long as the retention time of the isomer B of cefpodoxime proxetil beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 200 mL, and use this solution as the solution for required detectability test. Pipet 2 mL of the solution for required detectability test, and add the mixture of water, acetonitrile and acetic acid (100) (99:99:2) to make exactly 100 mL. Confirm that the peak areas of the isomer A and the isomer B of cefpodoxime proxetil obtained from 20 μ L of this solution are equivalent to 1.4 to 2.6% of them from 20 μ L of the solution for required detectability test, respectively.

System performance: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2). When the procedure is run with 20 μ L of this solution under the above operating conditions, the isomer A and the isomer B of cefpodoxime proxetil are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: Dissolve 1 mg of Cefpodoxime Proxetil in 100 mL of the mixture of water, acetonitrile and acetic acid (100) (99:99:2). When the test is repeated 5 times with 20 μ L of this solution under the above operating conditions, the relative standard deviations of the peak areas of the isomer A and the isomer B are not more than 2.0%, respectively.

Water <2.48> Not more than 2.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Isomer ratio Perform the test with 5 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_a and A_b , of the two isomers of cefpodoxime proxetil, having the smaller and larger retention times, respectively, by the automatic integration method: $A_b/(A_a + A_b)$ is between 0.50 and 0.60.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately an amount of Cefpodoxime Prox-

etil and Cefpodoxime Proxetil Reference Standard, equivalent to about 60 mg (potency), dissolve in 80 mL of acetonitrile, add exactly 4 mL of the internal standard solution, add acetonitrile to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T1} , Q_{S1} , Q_{T2} and Q_{S2} , of the areas of the two peaks of the isomers of cefpodoxime proxetil to the peak area of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of cefpodoxime (C}_{15}\text{H}_{17}\text{N}_5\text{O}_6\text{S}_2) \\ = W_S \times \{(Q_{T1} + Q_{T2}) / (Q_{S1} + Q_{S2})\} \times 1000$$

W_S : Amount [mg (potency)] of Cefpodoxime Proxetil Reference Standard

Internal standard solution—Dissolve 0.3 g of ethyl parahydroxybenzoate in a solution of citric acid in acetonitrile (1 in 2000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (11:9).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 11 minutes.

System suitability—

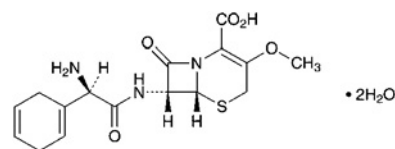
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the internal standard, the isomer A and the isomer B are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of the isomer B of cefpodoxime proxetil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefroxadine Hydrate

セフロキサジン水和物



$\text{C}_{16}\text{H}_{19}\text{N}_5\text{O}_5\text{S} \cdot 2\text{H}_2\text{O}$: 401.43

(6*R*,7*R*)-7-[(2*R*)-2-Amino-2-cyclohexa-1,4-dienylacetyl-amino]-3-methoxy-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [51762-05-1, anhydride]

Cefroxadine Hydrate contains not less than 930 μ g

(potency) and not more than 1020 μg (potency) per mg, calculated on the dehydrated basis. The potency of Cefroxadine Hydrate is expressed as mass (potency) of cefroxadine ($\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$: 365.40).

Description Cefroxadine Hydrate occurs as pale yellowish white to light yellow, crystalline particles or powder.

It is very soluble in formic acid, slightly soluble in water and in methanol, and very slightly soluble in acetonitrile and in ethanol (95).

It dissolves in 0.001 mol/L hydrochloric acid TS and in dilute acetic acid.

Identification (1) Determine the absorption spectrum of a solution of Cefroxadine Hydrate in 0.001 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefroxadine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefroxadine Hydrate in deuterated formic acid for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits three sharp single signals, A, B and C, at around $\delta 2.8$ ppm, at around $\delta 4.1$ ppm and at around $\delta 6.3$ ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 4:3:1.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +95 – +108° (0.1 g calculated on the dehydrated basis, diluted acetic acid (100) (3 in 25), 100 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Weigh 1.0 g of Cefroxadine Hydrate in a porcelain crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), mix, burn the ethanol, and carbonize by gently heating. After cooling, add 2 mL of nitric acid, heat carefully, and incinerate by ignition at 500 – 600°C. If a carbonized substance is still remained, moisten it with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Moisten the residue with 3 drops of hydrochloric acid, and add 10 mL of hot water to dissolve the residue by heating on a water bath. After cooling, adjust the pH between 3 and 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, transfer to a Nessler tube, wash the crucible with 10 mL of water, and add the washing and water to the tube to make 50 mL. Perform the test with this solution. Prepare the control solution as follows: Put 2.0 mL of Standard Lead Solution and 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) in a porcelain crucible, and proceed as directed for the preparation of the test solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefroxadine Hydrate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 10 mg of Cefroxadine Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 40 μL each of the sample solution and standard solution

as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: the areas of the peaks appeared at the relative retention times of 0.07, 0.6 and 0.8 against the peak of cefroxadine from the sample solution are not more than 2 times, 4 times and 1 time of the peak area of cefroxadine from the standard solution, respectively, and any peak area other than cefroxadine and other than the peaks mentioned above from the sample solution is not more than 1/2 of the peak area of cefroxadine from the standard solution, and the total area of the peaks other than cefroxadine from the sample solution is not more than 6 times of the peak area of cefroxadine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.4 g of sodium perchlorate in 1000 mL of a mixture of water and acetonitrile (489:11).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 20 minutes.

Time span of measurement: About 2 times as long as the retention time of cefroxadine.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of cefroxadine obtained from 40 μL of this solution is equivalent to 7 to 13% of that obtained from 40 μL of the standard solution.

System performance: Dissolve 3 mg of Cefroxadine Hydrate and 15 mg of orcin in 100 mL of the mobile phase. When the procedure is run with 40 μL of this solution under the above operating conditions, orcin and cefroxadine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 40 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefroxadine is not more than 2.0%.

Water <2.48> Not less than 8.5% and not more than 12.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefroxadine Hydrate and Cefroxadine Reference Standard, equivalent to about 50 mg (potency), dissolve each in a suitable amount of a mixture of dilute acetic acid and phosphoric acid (500:1), add exactly 5 mL of the internal standard solution and a mixture of dilute acetic acid and phosphoric acid (500:1) to make 200 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of the peak area of cefroxadine to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefroxadine } (\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}) \\ = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_s : Amount [mg (potency)] of Cefroxadine Reference Standard

Internal standard solution—Dissolve 1.6 g of vanillin in 5 mL of methanol, and add a mixture of dilute acetic acid and phosphoric acid (500:1) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 50) and acetonitrile (97:3).

Flow rate: Adjust the flow rate so that the retention time of cefroxadine is about 10 minutes.

System suitability—

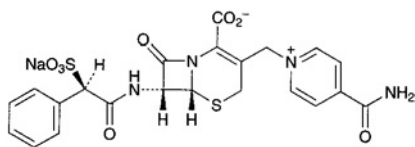
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cefroxadine and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of cefroxadine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cefsulodin Sodium

セフスロジンナトリウム



$C_{22}H_{19}N_4NaO_8S_2$: 554.53

Monosodium (6*R*,7*R*)-3-(4-carbamoylpyridinium-1-ylmethyl)-8-oxo-7-[(2*R*)-2-phenyl-2-sulfonatoacetyl-amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[52152-93-9]

Cefsulodin Sodium contains not less than 900 μ g (potency) and not more than 970 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefsulodin Sodium is expressed as mass (potency) of cefsulodin ($C_{22}H_{20}N_4O_8S_2$: 532.55).

Description Cefsulodin Sodium occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water and in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Cefsulodin Sodium (1 in 50,000) as directed under

Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefsulodin Sodium Reference Standard prepared in the same manner as sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefsulodin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefsulodin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefsulodin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a multiple signal A between δ 7.3 ppm and δ 7.7 ppm, and double signals, B and C, at around δ 8.4 ppm and at around δ 9.1 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C, is about 5:2:2.

(4) Cefsulodin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +16.5 – +20.0° (0.1 g calculated on the anhydrous basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the pH of the solution is not less than 3.3 and not more than 4.8.

Purity (1) Clarity of solution—Dissolve 1.0 g of Cefsulodin Sodium in 10 mL of water: the solution is clear.

(2) Heavy metals <1.07>—To 1.0 g of Cefsulodin Sodium add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), mix, fire the ethanol to burn, then heat gradually to carbonize. After cooling, add 2 mL of nitric acid, heat carefully, then heat at 500 – 600°C to incinerate. If a carbonized residue still retains, add a little amount of nitric acid, and heat again to incinerate. After cooling, add 6 mL of hydrochloric acid to the residue, heat to dryness on a water bath, then moisten the residue with 3 drops of hydrochloric acid, add 10 mL of hot water, and heat on a water bath to dissolve. Add ammonia TS dropwise to adjust to pH 3 – 4, and add 2 mL of dilute acetic acid. If necessary, filter, wash the crucible and residue on the filter with 10 mL of water, transfer the filtrate and washings into a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: To 2.0 mL of Standard Lead Solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5), fire the ethanol to burn. After cooling, add 2 mL of nitric acid, heat carefully, then heat at 500 – 600°C. After cooling, add 6 mL of hydrochloric acid, then proceed in the same manner as for the preparation of the test solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefsulodin Sodium according to Method 3, using a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) and 15 mL of dilute hydrochloric acid instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50) and 3 mL of hydrochloric acid, and perform the test (not more than 2 ppm).

(4) Related substances—Weigh accurately about 0.10 g of Cefsulodin Sodium, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of isonicotinic acid amide and about 20 mg of Cefsulodin Sodium Reference Standard (separately determine the water <2.48> in the same manner as Cefsulodin Sodium), and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of isonicotinic acid amide is not more than 1.0%, and the total of other related substances is not more than 1.2%.

$$\begin{aligned} \text{Amount (\%)} \text{ of isonicotinic acid amide} \\ = (A/B_1) \times (W_1/W_T) \times 5 \end{aligned}$$

$$\begin{aligned} \text{Total amount (\%)} \text{ of the other related substances} \\ = (B/B_S) \times (W_S/W_T) \times 5 \end{aligned}$$

A: Peak area of isonicotinic acid amide from the sample solution

B: Total peak area other than cefsulodin and other than isonicotinic acid amide from the sample solution

*B*₁: Peak area of isonicotinic acid amide from the standard solution

*B*_S: Peak area of cefsulodin from the standard solution

*W*_T: Amount (g) of the sample

*W*_S: Amount (g) of Cefsulodin Sodium Reference Standard

*W*₁: Amount (g) of isonicotinic acid amide

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Mobile phase B: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (92:8).

Flowing of the mobile phase: Change the mobile phase A to B at 14 minutes after the injection of sample.

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

Time span of measurement: About 4 times as long as the retention time of cefsulodin.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, add water to make exactly 10 mL. Confirm that the peak areas of isonicotinic acid amide and cefsulodin obtained from 10 μ L of this solution are equivalent to 7 to 13% of those of isonicotinic acid amide and cefsulodin obtained from 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less

than 5.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Water <2.48> Not more than 5.0% (1 g, volumetric titration, direct titration, avoiding moisture absorption of the sample, using a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Weigh accurately an amount of Cefsulodin Sodium and Cefsulodin Sodium Reference Standard, equivalent to about 0.1 g (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, *A*_T and *A*_S, of cefsulodin of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefsulodin (C}_{22}\text{H}_{20}\text{N}_4\text{O}_8\text{S}_2) \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

*W*_S: Amount [mg (potency)] of Cefsulodin Sodium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium sulfate (1 in 100) and acetonitrile (97:3).

Flow rate: Adjust the flow rate so that the retention time of cefsulodin is about 9 minutes.

System suitability—

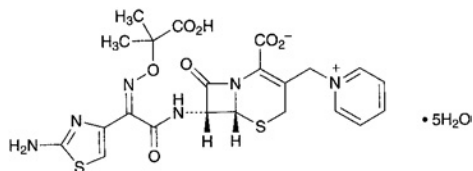
System performance: Dissolve 40 mg of isonicotinic acid amide in 25 mL of the standard solution. When the procedure is run with 10 μ L of this solution under the above operating conditions, isonicotinic acid amide and cefsulodin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefsulodin is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Cefotaxime Hydrate

セフトキシム水和物



$C_{22}H_{22}N_6O_7S_2 \cdot 5H_2O$: 636.65

(6*R*,7*R*)-7-[(*Z*)-2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methylethoxyimino)acetyl-amino]-3-(pyridinium-1-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate pentahydrate [78439-06-2]

Cefotaxime Hydrate contains not less than 950 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the dried basis. The potency of Cefotaxime Hydrate is expressed as mass (potency) of cefotaxime ($C_{22}H_{22}N_6O_7S_2$: 546.58).

Description Cefotaxime Hydrate occurs as a white to light yellowish white crystalline powder.

It is slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefotaxime Hydrate in phosphate buffer solution, pH 6.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefotaxime Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefotaxime Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefotaxime Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.05 g of Cefotaxime Hydrate add 5 mg of dried sodium carbonate, and add 0.5 mL of heavy water for nuclear magnetic resonance spectroscopy to dissolve. Determine the spectrum of this solution as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 1.5 ppm and at around δ 6.9 ppm, and a multiple signal C between δ 7.9 ppm and δ 9.2 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 6:1:5.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-28 - -34^\circ$ (0.5 g calculated on the dried bases, phosphate buffer solution, pH 6.0, 100 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of Cefotaxime Hydrate in 100 mL of water: the pH of the solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Cefotaxime Hydrate in 10 mL of a solution obtained by dissolving 5 g of anhydrous disodium hydrogenphosphate and 1 g of potassium dihydrogenphosphate in water to make 100 mL: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefotaxime Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefotaxime Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances (i) Trityl-*t*-butyl substance and *t*-butyl substance—Dissolve 0.10 g of Cefotaxime Hydrate in 2 mL of diluted disodium hydrogenphosphate TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted disodium hydrogenphosphate TS (1 in 3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of *n*-butyl acetate, acetic acid (100), acetate buffer solution, pH 4.5 and 1-butanol (16:16:13:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots which appear upper in position than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(ii) Other related substances—Dissolve 20 mg of Cefotaxime Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: each peak area other than cefotaxime from the sample solution is not more than that of cefotaxime from the standard solution, and the total of peak areas other than cefotaxime from the sample solution is not more than 5 times of the peak area of cefotaxime from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.0 g of ammonium dihydrogenphosphate in 750 mL of water, adjust to pH 3.5 with phosphoric acid, and add water to make 870 mL. To this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 4 minutes.

Time span of measurement: About 3 times as long as the retention time of cefotaxime beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the standard so-

lution, add the mobile phase to make exactly 5 mL, and confirm that the peak area of cefotaxime obtained from 5 μ L of this solution is equivalent to 15 to 25% of that of cefotaxime obtained from 5 μ L of the standard solution.

System performance: Dissolve about 0.01 g each of Cefotaxime Hydrate and acetanilide in 20 mL of the mobile phase. When the procedure is run with 5 μ L of this solution under the above operating conditions, cefotaxime and acetanilide are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefotaxime is not more than 2.0%.

(5) Free pyridine—Weigh accurately about 50 mg of Cefotaxime Hydrate, dissolve in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak height, H_T and H_S , of pyridine of each solution: the amount of free pyridine is not more than 0.3%.

$$\begin{aligned} & \text{Amount (mg) of free pyridine} \\ & = W_S \times (H_T/H_S) \times (1/1000) \end{aligned}$$

W_S : Amount (mg) of pyridine

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.88 g of ammonium dihydrogenphosphate in 500 mL of water, add 300 mL of acetonitrile and water to make 1000 mL, and adjust to pH 7.0 with ammonia solution (28).

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 4 minutes.

System suitability—

Test for required detection: Confirm that the peak height of pyridine obtained from 10 μ L of the standard solution is equivalent to 50% of the full scale.

System performance: Dissolve 5 mg of Cefotaxime Hydrate in 100 mL of a solution of pyridine in the mobile phase (1 in 20,000). When the procedure is run with 10 μ L of this solution under the above operating conditions, cefotaxime and pyridine are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the heights of pyridine is not more than 5.0%.

Loss on dryness <2.41> Not less than 13.0% and not more than 15.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Cefotaxime Hydrate and Cefotaxime Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cefotaxime to that of the internal standard of each solution.

$$\begin{aligned} & \text{Amount } [\mu\text{g (potency)}] \text{ of cefotaxime (C}_{22}\text{H}_{22}\text{N}_6\text{O}_7\text{S}_2) \\ & = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefotaxime Reference Standard

Internal standard solution—A solution of dimedon in 0.05 mol/L phosphate buffer solution, pH 7.0 (11 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with hexasilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.26 g of anhydrous disodium hydrogenphosphate and 2.72 g of potassium dihydrogenphosphate in 980 mL of water, and add 20 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefotaxime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the internal standard and cefotaxime are eluted in this order with the resolution between these peaks being not less than 3.

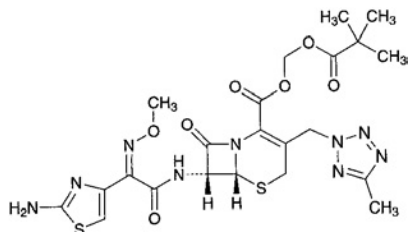
System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefotaxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefteram Pivoxil

セフテラム ピボキシル



$C_{22}H_{27}N_9O_7S_2$: 593.64

2,2-Dimethylpropanoyloxymethyl (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-3-(5-methyl-2*H*-tetrazol-2-ylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate
[82547-58-8, Cefteram]

Cefteram Pivoxil contains not less than 743 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefteram Pivoxil is expressed as mass (potency) of cefteram ($C_{16}H_{17}N_9O_5S_2$: 479.49).

Description Cefteram Pivoxil occurs as a white to pale yellowish white powder.

It is very soluble in acetonitrile, freely soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefteram Pivoxil in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Cefteram Pivoxil in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals A, B and C, at around δ 1.2 ppm, at around δ 2.5 ppm and at around δ 4.0 ppm, respectively. The ratio of the integrated intensity of these signals, A:B:C, is about 3:1:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +35 – +43° (0.4 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cefteram Pivoxil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefteram Pivoxil according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Cefteram Pivoxilin in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution

as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil from the sample solution is not more than 1.25 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1 with respect to cefteram pivoxil from the sample solution is not more than 0.25 times the peak area of cefteram pivoxil from the standard solution, and the total area of the peaks other than cefteram pivoxil from the sample solution is not more than 2.75 times the peak area of cefteram pivoxil from the standard solution. For the above calculation, use the area of the peak, having the relative retention time of about 0.1, after multiplying by its relative response factor, 0.74.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of cefteram pivoxil.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cefteram pivoxil obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the standard solution.

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefteram pivoxil are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cefteram pivoxil is not more than 3.0%.

Water <2.48> Not more than 3.0% (0.3 g, coulometric titration).

Assay Weigh accurately an amount of Cefteram Pivoxil and Cefteram Pivoxil Mesitylenesulfonate Reference Standard, equivalent to about 40 mg (potency), dissolve each in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefteram pivoxil to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefteram } (C_{16}H_{17}N_9O_5S_2) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefteram Pivoxil Mesitylenesulfonate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0 add 375 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cefteram pivoxil is about 14 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefteram pivoxil are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cefteram pivoxil to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Cefteram Pivoxil Fine Granules

セフテラム ピボキシル細粒

Cefteram Pivoxil Fine Granules contain not less than 90.0% and not more than 110.0% of the labeled amount of cefteram ($\text{C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_2$; 479.49).

Method of preparation Prepare to finely granulated form as directed under Powders, with Cefteram Pivoxil.

Identification Powder Cefteram Pivoxil Fine Granules. To a portion of the powder, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add 20 mL of methanol, shake well, and filter. To 1 mL of the filtrate add 0.05 mol/L hydrochloric acid-methanol TS to make 500 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 262 nm and 266 nm.

Purity Related substances—Powder Cefteram Pivoxil Fine Granules, if necessary. To a portion, equivalent to 0.1 g (potency) of Cefteram Pivoxil according to the labeled amount, add diluted acetonitrile (1 in 2) to make 100 mL, disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.9 with respect to cefteram pivoxil, is not larger than 1.75 times the peak area of cefteram pivoxil from the standard solution, the area of the peak, having the relative retention time of about 0.1, is not larger than 0.68 times the peak area of cefteram pivoxil from the standard solution, and the total

area of the peaks other than cefteram pivoxil is not larger than 3.7 times the peak area of cefteram pivoxil from the standard solution. For this calculation, use the peak area for the peak having the relative retention time of about 0.1 after multiplying by its relative response factor, 0.74.

Operating conditions—

Proceed as directed in the Purity (3) under Cefteram Pivoxil.

System suitability—

Proceed as directed in the Purity (3) under Cefteram Pivoxil.

Water <2.48> Not more than 0.3% (0.1 g (potency), coulometric titration).

Uniformity of dosage units <6.02> The Granules in single-unit container meet the requirement of the Mass variation test.

Dissolution Being specified separately.

Particle size <6.03> It meets the requirement of fine granules of the Powders.

Assay Powder Cefteram Pivoxil Fine Granules, if necessary, and use as the sample. Weigh accurately an amount of the sample, equivalent to about 0.3 g (potency) of Cefteram Pivoxil according to the labeled amount, add exactly 30 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 300 mL. Disperse the particle with the aid of ultrasonic waves, then filter, and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg (potency) of Cefteram Pivoxil Mesitylenesulfonate Reference Standard, dissolve in 20 mL of diluted acetonitrile (1 in 2), add exactly 5 mL of the internal standard solution and diluted acetonitrile (1 in 2) to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cefteram pivoxil to that of the internal standard.

$$\begin{aligned} &\text{Amount [mg (potency)] of cefteram (C}_{16}\text{H}_{17}\text{N}_9\text{O}_5\text{S}_2) \\ &= W_S \times (Q_T/Q_S) \times 6 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefteram Pivoxil Mesitylenesulfonate Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in diluted acetonitrile (1:2) (1 in 1000).

Operating conditions—

Proceed as directed in the Assay under Cefteram Pivoxil.

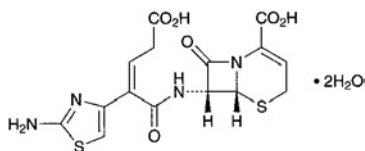
System suitability—

Proceed as directed in the Assay under Cefteram Pivoxil.

Containers and storage Containers—Tight containers.

Ceftibuten Hydrate

セフチブテン水和物



$C_{15}H_{14}N_4O_6S_2 \cdot 2H_2O$: 446.46
(6*R*,7*R*)-7-[(2*Z*)-2-Aminothiazol-4-yl]-4-carboxybut-2-enoylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid dihydrate [118081-34-8]

Ceftibuten Hydrate contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Ceftibuten Hydrate is expressed as mass (potency) of ceftibuten ($C_{15}H_{14}N_4O_6S_2$: 410.42).

Description Ceftibuten Hydrate occurs as a white to pale yellowish white crystalline powder and has a slight, characteristic odor.

It is freely soluble in *N,N*-dimethylformamide and in dimethyl sulfoxide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Ceftibuten Hydrate in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

(2) Determine the infrared absorption spectrum of Ceftibuten Hydrate as directed in the paste method under the Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3249 cm^{-1} , 1772 cm^{-1} , 1700 cm^{-1} , 1651 cm^{-1} and 1544 cm^{-1} .

(3) Determine the spectrum of a solution of Ceftibuten Hydrate in deuterated dimethyl sulfoxide for nuclear magnetic resonance spectroscopy (1 in 30), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits double signals A and B, at around δ 3.2 ppm and at around δ 5.1 ppm, a quartet signal C, at around δ 5.8 ppm, and a single signal D, at around δ 6.3 ppm. The ratio of integrated intensity of each signal except the signal at around δ 3.2 ppm, B:C:D is about 1:1:1.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (263 nm): 320 – 345 (20 mg calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, 1000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: +135 – +155° (0.3 g calculated on the anhydrous basis, 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ceftibuten Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Being specified separately.

Water <2.48> Not less than 8.0% and not more than 13.0% (0.2 g, volumetric titration, direct titration. Use a mixture of pyridine for water determination and ethylene glycol for water determination (5:1) instead of methanol for water determination).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Ceftibuten Hydrate and Ceftibuten Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in 36 mL of 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0, add exactly 4 mL each of the internal standard solution, shake, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ceftibuten to that of the internal standard. Keep the sample solution and the standard solution at 5°C or below and use within 2 hours.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of ceftibuten } (C_{15}H_{14}N_4O_6S_2) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Ceftibuten Hydrochloride Reference Standard

Internal standard solution—A solution of methyl *p*-hydroxybenzoate in acetonitrile (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.005 mol/L *n*-decyl trimethylammonium bromide TS and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ceftibuten is about 10 minutes.

System suitability—

System performance: Dissolve 5 mg of Ceftibuten Hydrate in 1 mol/L Hydrochloric acid TS to make 50 mL, and allow to stand for 4 hours at room temperature. To 10 mL of this solution add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make 25 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, trans-isomer and ceftibuten are eluted in this order with the resolution between these peaks being not less than 1.5.

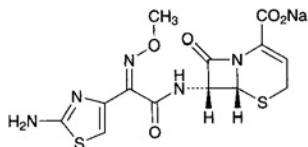
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftibuten to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 5°C.

Cefprozime Sodium

セフチゾキシムナトリウム



$C_{13}H_{12}N_5NaO_5S_2$: 405.38

Monosodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [68401-82-1]

Cefprozime Sodium contains not less than 925 μ g (potency) and not more than 965 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cefprozime Sodium is expressed as mass (potency) of cefprozime ($C_{13}H_{13}N_5O_5S_2$: 383.40).

Description Cefprozime Sodium occurs as a white to light yellow, crystals or crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefprozime Sodium (1 in 63,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefprozime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefprozime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.0 ppm, a multiple signal B around δ 6.3 ppm, and a single signal C at around δ 7.0 ppm. The ratio of integrated intensity of each signal, A:B:C, is about 3:1:1.

(4) Cefprozime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +125 – +145° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefprozime Sodium in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefprozime Sodium in 10 mL of water: the solution is clear, and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cefprozime Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard

Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Cefprozime Sodium according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.11 g of Cefprozime Sodium in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: each peak area other than cefprozime is not more than 0.5% of the peak area of cefprozime, and the total of peak areas other than cefprozime is not more than 1.0% of that of cefprozime.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 2.31 g of disodium hydrogenphosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 200 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefprozime is about 12 minutes.

Time span of measurement: About 5 times as long as the retention time of cefprozime beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the solution for test for required detection. Pipet 1 mL of the solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and confirm that the peak area of cefprozime obtained from 5 μ L of this solution is equivalent to 7 to 13% of that of cefprozime obtained from 5 μ L of the solution for test for required detection.

System performance: Dissolve about 10 mg of Cefprozime Reference Standard in 100 mL of 0.1 mol/L phosphate buffer solution, pH 7.0, and use this solution as the solution for system suitability test. When the procedure is run with 5 μ L of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cefprozime are not less than 4000 steps and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of cefprozime is not more than 2.0%.

Water <2.48> Not more than 8.5% (0.4 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefprozime Sodium and Cefprozime Reference Standard, equivalent to about 0.1 g (potency), and dissolve each in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 10 mL of the internal standard solution, then add 0.1 mol/L phosphate buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of

ceftizoxime to that of the internal standard of each solution.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of ceftizoxime (C}_{13}\text{H}_{13}\text{N}_5\text{O}_5\text{S}_2) \\ = W_s \times (Q_T/Q_S) \times 1000$$

W_s : Amount [mg (potency)] of Ceftriaxone Reference Standard

Internal standard solution—A solution of 3-hydroxybenzoic acid in 0.1 mol/L phosphate buffer solution, pH 7.0 (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2.31 g of disodium hydrogenphosphate dodecahydrate and 1.42 g of citric acid monohydrate in 1000 mL of water, and adjust to pH 3.6 with diluted phosphoric acid (1 in 10) or dilute sodium hydroxide TS. To 450 mL of this solution add 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ceftizoxime is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, ceftizoxime and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.0 and the symmetry factor of each peak is not more than 2.

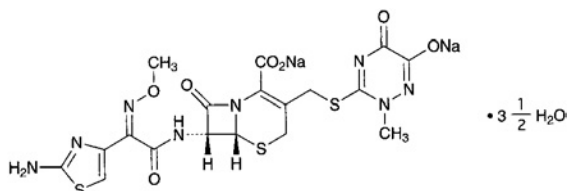
System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftizoxime to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ceftriaxone Sodium Hydrate

セフトリアキソンナトリウム水和物



$\text{C}_{18}\text{H}_{16}\text{N}_8\text{Na}_2\text{O}_7\text{S}_3 \cdot 3\frac{1}{2}\text{H}_2\text{O}$: 661.60

Disodium (6*R*,7*R*)-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino)acetyl-amino]-3-(6-hydroxy-2-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-ylsulfanylmethyl)-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate hemiheptahydrate [104376-79-6]

Ceftriaxone Sodium Hydrate contains not less than 905 μg (potency) and not more than 935 μg (potency)

per mg, calculated on the anhydrous basis. The potency of Ceftriaxone Sodium Hydrate is expressed as mass (potency) of ceftriaxone ($\text{C}_{18}\text{H}_{18}\text{N}_8\text{O}_7\text{S}_3$: 554.58).

Description Ceftriaxone Sodium Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in water and in dimethylsulfoxide, sparingly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Ceftriaxone Sodium Hydrate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ceftriaxone Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Ceftriaxone Sodium Hydrate in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 10), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H): it exhibits single signals, A, B, C and D, at around δ 3.5 ppm, at around δ 3.8 ppm, at around δ 6.7 ppm and at around δ 7.2 ppm, respectively. The ratio of integrated intensity of each signal, A: B: C: D, is about 3:3:1:2. When the signal at around δ 3.5 ppm overlaps with the signal of water, perform the measurement in the probe kept at about 50°C.

(3) Ceftriaxone Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -153 – -170° (50 mg calculated on the anhydrous basis, water, 2.5 mL, 20 mm).

pH <2.54> Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 0.6 g of Ceftriaxone Sodium Hydrate in 5 mL of water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ceftriaxone Sodium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances 1—Dissolve 20 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of the impurity 1 having the relative retention time of about 0.5 and the impurity 2 having the relative retention time of about 1.3 to ceftriaxone from the sample solution are not more than the peak area of ceftriaxone from the standard solution. In this case, these peak areas for the impurity 1 and the impurity 2 are used after multiplying by 0.9 and 1.2, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, add 55 mL of the solution A, 5 mL of the solution B and 490 mL of water.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 200 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μ L of this solution is equivalent to 0.9 to 1.1% of that from 10 μ L of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of water and acetonitrile for liquid chromatography (11:9) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order, with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

(5) Related substances 2—Dissolve 10 mg of Ceftriaxone Sodium Hydrate in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the each peak area of the impurities which appear after the peak of ceftriaxone from the sample solution is not more than the peak area of ceftriaxone from the standard solution, and the total peak area of these impurities is not more than 2.5 times

of the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as the solution A. Separately, dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as the solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 55 mL of the solution A, 5 mL of the solution B, 490 mL of water and 700 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 3 minutes.

Time span of measurement: About 10 times as long as the retention time of ceftriaxone.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL, and use this solution as the solution for system suitability test. Measure exactly 1 mL of the solution for system suitability test, and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make exactly 100 mL. Confirm that the peak area of ceftriaxone obtained from 10 μ L of this solution is equivalent to 0.9 to 1.1% of that from 10 μ L of the solution for system suitability test.

System performance: Dissolve 10 mg of Ceftriaxone Sodium Hydrate in a mixture of acetonitrile for liquid chromatography and water (23:11) to make 5 mL, add 5 mL of a solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000), and add a mixture of acetonitrile for liquid chromatography and water (23:11) to make 200 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, ceftriaxone and diethyl terephthalate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of ceftriaxone is not more than 1.0%.

Water <2.48> Not less than 8.0% and not more than 11.0% (0.15 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ceftriaxone Sodium Hydrate and Ceftriaxone Sodium Reference Standard, equivalent to about 0.1 g (potency), dissolve each in a mixture of water and acetonitrile for liquid chromatography (11:9) to make exactly 50 mL. Pipet 5 mL of each solution, add exactly 5 mL of the internal standard solution and a mixture of water and acetonitrile for liquid chromatography (11:9) to make 200 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with

10 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ceftriaxone to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)] of ceftriaxone (C}_{18}\text{H}_{18}\text{N}_8\text{O}_7\text{S}_3) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Ceftriaxone Sodium Reference Standard

Internal standard solution—A solution of diethyl terephthalate in a mixture of water and acetonitrile for liquid chromatography (11:9) (9 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 5.796 g of anhydrous disodium hydrogen phosphate and 3.522 g of potassium dihydrogen phosphate in water to make exactly 1000 mL, and use this solution as solution A. Dissolve 20.256 g of citric acid monohydrate and 7.840 g of sodium hydroxide in water to make exactly 1000 mL, and use this solution as solution B. Dissolve 4.00 g of tetra-*n*-heptylammonium bromide in 450 mL of acetonitrile for liquid chromatography, and add 490 mL of water, 55 mL of solution A, and 5 mL of solution B.

Flow rate: Adjust the flow rate so that the retention time of ceftriaxone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ceftriaxone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

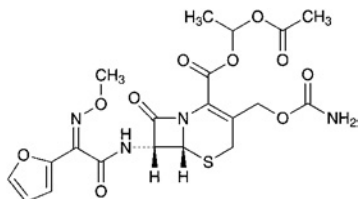
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ceftriaxone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefuroxime Axetil

セフロキシム アキセチル



$\text{C}_{20}\text{H}_{22}\text{N}_4\text{O}_{10}\text{S}$: 510.47

(1*R*,5*S*)-1-Acetoxyethyl (6*R*,7*R*)-3-carbamoyloxymethyl-7-(*Z*)-2-furan-2-yl-2-(methoxyimino)acetylamin]-8-oxo-5-

thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [64544-07-6]

Cefuroxime Axetil contains not less than 800 μg (potency) and not more than 850 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of acetone. The potency of Cefuroxime Axetil is expressed as mass (potency) of cefuroxime ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$: 424.39).

Description Cefuroxime Axetil occurs as white to yellowish white, non-crystalline powder.

It is freely soluble in dimethylsulfoxide, soluble in methanol, sparingly soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cefuroxime Axetil in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefuroxime Axetil Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefuroxime Axetil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefuroxime Axetil Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Cefuroxime Axetil in deuterated dimethylsulfoxide for nuclear magnetic resonance spectroscopy (1 in 20) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal or a pair of double signals A at around δ 1.5 ppm, a pair of single signals B at around δ 2.1 ppm, and a single signal C at around δ 3.9 ppm. The ratio of the integrated intensity of each signal, A:B:C, is about 1:1:1.

Optical rotation <2.49> $[\alpha]_D^{20}$: +41 – +47° (0.5 g, methanol, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cefuroxime Axetil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Put 1.0 g of Cefuroxime Axetil in a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, then heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substance—Dissolve 25 mg of Cefuroxime Axetil in 4 mL of methanol, add a solution of ammonium dihydrogen phosphate (23 in 1000) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 40 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the

test with exactly 2 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cefuroxime axetil obtained from the sample solution is not more than 1.5 times the sum area of two peaks of cefuroxime axetil obtained from the standard solution, and the sum area of the peaks other than cefuroxime axetil from the sample solution is not more than 4 times the sum area of two peaks of cefuroxime axetil from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of the peak having the larger retention time of the two peaks of cefuroxime axetil beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 4 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make exactly 10 mL. Confirm that the sum area of the two peaks of cefuroxime axetil obtained from 2 μL of this solution is equivalent to 7 to 13% of that obtained from 2 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the sum area of the two peaks of cefuroxime axetil is not more than 2.0%.

(4) Acetone—Weigh accurately about 1 g of Cefuroxime Axetil, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetone, and add dimethylsulfoxide to make exactly 100 mL. Pipet 0.2 mL of this solution, add exactly 0.2 mL of the internal standard solution and dimethylsulfoxide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratios, Q_T and Q_S , of the peak area of acetone to that of the internal standard: the amount of acetone is not more than 1.3%.

$$\text{Amount (\%)} \text{ of acetone} = (W_S/W_T) \times (Q_T/Q_S) \times 0.2$$

W_S : Amount (g) of acetone

W_T : Amount (g) of the sample

Internal standard solution—A solution of 1-propanol in dimethylsulfoxide (1 in 200).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography coated with a mixture of polyethylene glycol 600 for gas chromatography and polyethylene glycol 1500 for gas chromatography (1:1) in the ratio of 20% (125 – 150 μm in particle diameter).

Column temperature: A constant temperature of about 90°C.

Temperature of injection port: A constant temperature of about 115°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 4 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, acetone and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of acetone to that of the internal standard is not more than 5.0%.

Water <2.48> Not more than 2.0% (0.4 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Isomer ratio Perform the test with 10 μL of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area, A_a , of the peak having the smaller retention time and the area, A_b , of the peak having the bigger retention time of the two peaks of cefuroxime axetil: $A_b/(A_a + A_b)$ is between 0.48 and 0.55.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately an amount of Cefuroxime Axetil and Cefuroxime Axetil Reference Standard, equivalent to about 50 mg (potency), and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, 5 mL of methanol and a solution of ammonium dihydrogen phosphate (23 in 1000) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefuroxime (C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefuroxime Axetil Reference Standard

Internal standard solution—A solution of acetanilide in methanol (27 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 278 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with trimethylsilanized

silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of ammonium dihydrogen phosphate (23 in 1000) and methanol (5:3).

Flow rate: Adjust the flow rate so that the retention time of the peak having the smaller retention time of the two peaks of cefuroxime axetil is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and cefuroxime axetil are eluted in this order with the resolution between the two peaks of cefuroxime axetil being not less than 1.5.

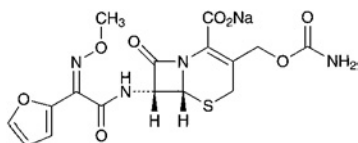
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the sum area of the two peaks of cefuroxime axetil to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cefuroxime Sodium

セフロキシムナトリウム



$\text{C}_{16}\text{H}_{15}\text{N}_4\text{NaO}_8\text{S}$: 446.37

Monosodium (6*R*,7*R*)-3-carbamoyloxymethyl-7-[(*Z*)-2-furan-2-yl-2-(methoxyimino)acetylamino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [56238-63-2]

Cefuroxime Sodium contains not less than 875 μg (potency) per mg, calculated on the anhydrous basis. The potency of Cefuroxime Sodium is expressed as mass (potency) of cefuroxime ($\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S}$: 424.39).

Description Cefuroxime Sodium occurs as a white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, and very slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cefuroxime Sodium (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cefuroxime Sodium Reference Standard prepared in the same manner as sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cefuroxime Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cefuroxime Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same

wave numbers.

(3) Determine the spectrum of a solution of Cefuroxime Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (^1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ 4.0 ppm, a quartet signal B at around δ 6.6 ppm, and double signals, C and D, at around δ 6.9 ppm and around δ 7.7 ppm, respectively. The ratio of integrated intensity of each signal, A:B:C:D, is about 3:1:1:1.

(4) Cefuroxime Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +59 – +66° (0.5 g calculated on the anhydrous bases, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cefuroxime Sodium in 10 mL of water: the pH of the solution is between 6.0 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cefuroxime Sodium in 10 mL of water: the solution is clear, and its absorbance <2.24> at 450 nm is not more than 0.25.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cefuroxime Sodium according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cefuroxime Sodium according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 25 mg of Cefuroxime Sodium in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: each peak area other than cefuroxime from the sample solution is not more than the peak area of cefuroxime from the standard solution, and the total of the peak areas other than cefuroxime from the sample solution is not more than 3 times of the peak area of cefuroxime from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of cefuroxime beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, add water to make exactly 10 mL, and confirm that the peak area of cefuroxime obtained from 20 μL of this solution is equivalent to 7 to 13% of that of cefuroxime obtained from 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefuroxime is not more than 2.0%.

Water <2.48> Not more than 4.0% (0.4 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Cefuroxime Sodium and Cefuroxime Sodium Reference Standard, equivalent to about 25 mg (potency), and dissolve each in water to make exactly 25 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak area, A_T and A_S , of cefuroxime of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of cefuroxime (C}_{16}\text{H}_{16}\text{N}_4\text{O}_8\text{S)} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Cefuroxime Sodium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 125 mm in length, packed with hexasilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.68 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 3.4 with acetic acid (100), and add water to make 1000 mL. To 990 mL of this solution add 10 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of cefuroxime is about 8 minutes.

System suitability—

System performance: Allow the sample solution to stand at 60°C for 10 minutes. When the procedure is run with 20 μ L of this solution soon after cooling under the above operating conditions, the resolution between the peak of cefuroxime and the peak corresponding to the retention time of about 0.7 to the peak of cefuroxime is being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of cefuroxime is not more than 1.0%.

Containers and storage Containers—Tight containers.

Microcrystalline Cellulose

結晶セルロース

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Microcrystalline Cellulose is purified, partially depolymerized α -cellulose, obtained as a pulp from fibrous plant material, with mineral acids.

♦The label indicates the degree of polymerization, loss on drying, and bulk density values with the range.◆

♦**Description** Microcrystalline Cellulose occurs as a white crystalline powder having fluidity.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It swells with sodium hydroxide TS on heating.◆

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Microcrystalline Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

♦(2) Sieve 20 g of Microcrystalline Cellulose for 5 minutes on an air-jet sieve equipped with a screen (No.391, 200 mm in inside diameter) having 38- μ m openings. If more than 5% is retained on the screen, mix 30 g of Microcrystalline Cellulose with 270 mL of water; otherwise, mix 45 g with 255 mL of water. Perform the mixing for 5 minutes in a high-speed (18,000 revolutions per minute or more) power blender. Transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 3 hours: a white, opaque, bubble-free dispersion, which does not form a supernatant liquid at the surface, is obtained.◆

(3) Transfer 1.3 g of Microcrystalline Cellulose, accurately weighed, to a 125-mL conical flask, and add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS. Immediately purge the solution with nitrogen, insert the stopper, and shake on a suitable mechanical shaker to dissolve. Perform the test with a suitable amount of this solution, taken exactly, according to Method 1 under Viscosity Determination <2.53> using a capillary viscometer having the viscosity constant (K) of approximately 0.03, at $25 \pm 0.1^\circ\text{C}$, and determine the kinematic viscosity, ν . Separately, perform the test with a mixture of exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS in the same manner as above, using a capillary viscometer having K of approximately 0.01, and determine the kinematic viscosity, ν_0 .

Calculate the relative viscosity, η_{rel} , of Microcrystalline Cellulose by the formula:

$$\eta_{\text{rel}} = \nu / \nu_0$$

Obtain the product, $[\eta]C$, of intrinsic viscosity $[\eta]$ (mL/g) and concentration C (g/100 mL) from the value η_{rel} of the Table. When calculate the degree of polymerization, P , by the following formula, P is not more than 350 ♦and within the labeled range.◆

$$P = (95)[\eta]C / W_T$$

W_T : Amount (g) of the sample, calculated on the dried basis

pH <2.54> Shake 5.0 g of Microcrystalline Cellulose with 40 mL of water for 20 minutes, and centrifuge: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity ♦(1) Heavy metals <1.07>—Proceed with 2.0 g of Microcrystalline Cellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Water-soluble substances—Shake 5.0 g of Microcrystalline Cellulose with 80 mL of water for 10 minutes, filter with the aid of vacuum through a filter paper for quantitative analysis (5C) into a vacuum flask. Evaporate the clear filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, cool in a desiccator, and weigh: the difference between the mass of the residue

and the mass obtained from a blank determination does not exceed 12.5 mg.

(3) Diethyl ether-soluble substances—Place 10.0 g of Microcrystalline Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free diethyl ether through the column. Evaporate the eluate to dryness in a previously dried and tared evaporation dish. Dry the residue at 105°C for 30 minutes, allow to cool in a desiccator, and weigh: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 5.0 mg.

ator, and weigh: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 5.0 mg.

Conductivity <2.51> Perform the test as directed in the Conductivity Measurement with the supernatant liquid obtained in the pH as the sample solution, and determine the conductivity ♦ at 25 ± 0.1°C. ♦ Determine in the same way the con-

Table for Conversion of Relative Viscosity (η_{rel}) into the Product of Limiting Viscosity and Concentration ($[\eta]C$)

η_{rel}	$[\eta]C$									
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.1	0.098	0.106	0.115	0.125	0.134	0.143	0.152	0.161	0.170	0.180
1.2	0.189	0.198	0.207	0.216	0.225	0.233	0.242	0.250	0.259	0.268
1.3	0.276	0.285	0.293	0.302	0.310	0.318	0.326	0.334	0.342	0.350
1.4	0.358	0.367	0.375	0.383	0.391	0.399	0.407	0.414	0.422	0.430
1.5	0.437	0.445	0.453	0.460	0.468	0.476	0.484	0.491	0.499	0.507
1.6	0.515	0.522	0.529	0.536	0.544	0.551	0.558	0.566	0.573	0.580
1.7	0.587	0.595	0.602	0.608	0.615	0.622	0.629	0.636	0.642	0.649
1.8	0.656	0.663	0.670	0.677	0.683	0.690	0.697	0.704	0.710	0.717
1.9	0.723	0.730	0.736	0.743	0.749	0.756	0.762	0.769	0.775	0.782
2.0	0.788	0.795	0.802	0.809	0.815	0.821	0.827	0.833	0.840	0.846
2.1	0.852	0.858	0.864	0.870	0.876	0.882	0.888	0.894	0.900	0.906
2.2	0.912	0.918	0.924	0.929	0.935	0.941	0.948	0.953	0.959	0.965
2.3	0.971	0.976	0.983	0.988	0.994	1.000	1.006	1.011	1.017	1.022
2.4	1.028	1.033	1.039	1.044	1.050	1.056	1.061	1.067	1.072	1.078
2.5	1.083	1.089	1.094	1.100	1.105	1.111	1.116	1.121	1.126	1.131
2.6	1.137	1.142	1.147	1.153	1.158	1.163	1.169	1.174	1.179	1.184
2.7	1.190	1.195	1.200	1.205	1.210	1.215	1.220	1.225	1.230	1.235
2.8	1.240	1.245	1.250	1.255	1.260	1.265	1.270	1.275	1.280	1.285
2.9	1.290	1.295	1.300	1.305	1.310	1.314	1.319	1.324	1.329	1.333
3.0	1.338	1.343	1.348	1.352	1.357	1.362	1.367	1.371	1.376	1.381
3.1	1.386	1.390	1.395	1.400	1.405	1.409	1.414	1.418	1.423	1.427
3.2	1.432	1.436	1.441	1.446	1.450	1.455	1.459	1.464	1.468	1.473
3.3	1.477	1.482	1.486	1.491	1.496	1.500	1.504	1.508	1.513	1.517
3.4	1.521	1.525	1.529	1.533	1.537	1.542	1.546	1.550	1.554	1.558
3.5	1.562	1.566	1.570	1.575	1.579	1.583	1.587	1.591	1.595	1.600
3.6	1.604	1.608	1.612	1.617	1.621	1.625	1.629	1.633	1.637	1.642
3.7	1.646	1.650	1.654	1.658	1.662	1.666	1.671	1.675	1.679	1.683
3.8	1.687	1.691	1.695	1.700	1.704	1.708	1.712	1.715	1.719	1.723
3.9	1.727	1.731	1.735	1.739	1.742	1.746	1.750	1.754	1.758	1.762
4.0	1.765	1.769	1.773	1.777	1.781	1.785	1.789	1.792	1.796	1.800
4.1	1.804	1.808	1.811	1.815	1.819	1.822	1.826	1.830	1.833	1.837
4.2	1.841	1.845	1.848	1.852	1.856	1.859	1.863	1.867	1.870	1.874
4.3	1.878	1.882	1.885	1.889	1.893	1.896	1.900	1.904	1.907	1.911
4.4	1.914	1.918	1.921	1.925	1.929	1.932	1.936	1.939	1.943	1.946
4.5	1.950	1.954	1.957	1.961	1.964	1.968	1.971	1.975	1.979	1.982
4.6	1.986	1.989	1.993	1.996	2.000	2.003	2.007	2.010	2.013	2.017
4.7	2.020	2.023	2.027	2.030	2.033	2.037	2.040	2.043	2.047	2.050
4.8	2.053	2.057	2.060	2.063	2.067	2.070	2.073	2.077	2.080	2.083
4.9	2.087	2.090	2.093	2.097	2.100	2.103	2.107	2.110	2.113	2.116
5.0	2.119	2.122	2.125	2.129	2.132	2.135	2.139	2.142	2.145	2.148
5.1	2.151	2.154	2.158	2.160	2.164	2.167	2.170	2.173	2.176	2.180
5.2	2.183	2.186	2.190	2.192	2.195	2.197	2.200	2.203	2.206	2.209
5.3	2.212	2.215	2.218	2.221	2.224	2.227	2.230	2.233	2.236	2.240
5.4	2.243	2.246	2.249	2.252	2.255	2.258	2.261	2.264	2.267	2.270
5.5	2.273	2.276	2.279	2.282	2.285	2.288	2.291	2.294	2.297	2.300
5.6	2.303	2.306	2.309	2.312	2.315	2.318	2.320	2.324	2.326	2.329
5.7	2.332	2.335	2.338	2.341	2.344	2.347	2.350	2.353	2.355	2.358
5.8	2.361	2.364	2.367	2.370	2.373	2.376	2.379	2.382	2.384	2.387
5.9	2.390	2.393	2.396	2.400	2.403	2.405	2.408	2.411	2.414	2.417
6.0	2.419	2.422	2.425	2.428	2.431	2.433	2.436	2.439	2.442	2.444

ductivity of water used for the preparation of the sample solution: the difference between these conductivities is not more than $75 \mu\text{S}\cdot\text{cm}^{-1}$

Loss on drying <2.41> Not more than 7.0% ♦ and within a range as specified on the label ♦ (1 g, 105°C, 3 hours).

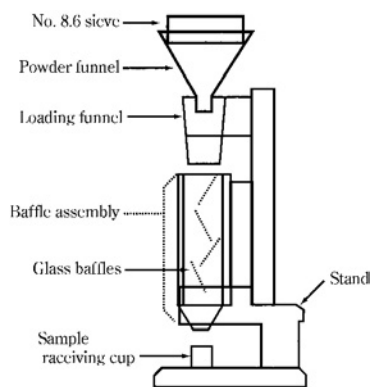
Residue on ignition <2.44> Not more than 0.1% ♦ (2 g) ♦.

Bulk density (i) Apparatus—Use a volumeter shown in

the figure. Put a No.8.6 sieve (2000 μm) on the top of the volumeter. A funnel is mounted over a baffle box, having four glass baffle plates inside which the sample powder slides as it passes. At the bottom of the baffle box is a funnel that collect the powder, and allows it to pour into a sample receiving cup mounted directly below it.

(ii) Procedure—Weigh accurately the mass of a brass or stainless steel cup, which has a capacity of $25.0 \pm 0.05 \text{ mL}$

η_{rel}	[η]C									
	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
6.1	2.447	2.450	2.453	2.456	2.458	2.461	2.464	2.467	2.470	2.472
6.2	2.475	2.478	2.481	2.483	2.486	2.489	2.492	2.494	2.497	2.500
6.3	2.503	2.505	2.508	2.511	2.513	2.516	2.518	2.521	2.524	2.526
6.4	2.529	2.532	2.534	2.537	2.540	2.542	2.545	2.547	2.550	2.553
6.5	2.555	2.558	2.561	2.563	2.566	2.568	2.571	2.574	2.576	2.579
6.6	2.581	2.584	2.587	2.590	2.592	2.595	2.597	2.600	2.603	2.605
6.7	2.608	2.610	2.613	2.615	2.618	2.620	2.623	2.625	2.627	2.630
6.8	2.633	2.635	2.637	2.640	2.643	2.645	2.648	2.650	2.653	2.655
6.9	2.658	2.660	2.663	2.665	2.668	2.670	2.673	2.675	2.678	2.680
7.0	2.683	2.685	2.687	2.690	2.693	2.695	2.698	2.700	2.702	2.705
7.1	2.707	2.710	2.712	2.714	2.717	2.719	2.721	2.724	2.726	2.729
7.2	2.731	2.733	2.736	2.738	2.740	2.743	2.745	2.748	2.750	2.752
7.3	2.755	2.757	2.760	2.762	2.764	2.767	2.769	2.771	2.774	2.776
7.4	2.779	2.781	2.783	2.786	2.788	2.790	2.793	2.795	2.798	2.800
7.5	2.802	2.805	2.807	2.809	2.812	2.814	2.816	2.819	2.821	2.823
7.6	2.826	2.828	2.830	2.833	2.835	2.837	2.840	2.842	2.844	2.847
7.7	2.849	2.851	2.854	2.856	2.858	2.860	2.863	2.865	2.868	2.870
7.8	2.873	2.875	2.877	2.879	2.881	2.884	2.887	2.889	2.891	2.893
7.9	2.895	2.898	2.900	2.902	2.905	2.907	2.909	2.911	2.913	2.915
8.0	2.918	2.920	2.922	2.924	2.926	2.928	2.931	2.933	2.935	2.937
8.1	2.939	2.942	2.944	2.946	2.948	2.950	2.952	2.955	2.957	2.959
8.2	2.961	2.963	2.966	2.968	2.970	2.972	2.974	2.976	2.979	2.981
8.3	2.983	2.985	2.987	2.990	2.992	2.994	2.996	2.998	3.000	3.002
8.4	3.004	3.006	3.008	3.010	3.012	3.015	3.017	3.019	3.021	3.023
8.5	3.025	3.027	3.029	3.031	3.033	3.035	3.037	3.040	3.042	3.044
8.6	3.046	3.048	3.050	3.052	3.054	3.056	3.058	3.060	3.062	3.064
8.7	3.067	3.069	3.071	3.073	3.075	3.077	3.079	3.081	3.083	3.085
8.8	3.087	3.089	3.092	3.094	3.096	3.098	3.100	3.102	3.104	3.106
8.9	3.108	3.110	3.112	3.114	3.116	3.118	3.120	3.122	3.124	3.126
9.0	3.128	3.130	3.132	3.134	3.136	3.138	3.140	3.142	3.144	3.146
9.1	3.148	3.150	3.152	3.154	3.156	3.158	3.160	3.162	3.164	3.166
9.2	3.168	3.170	3.172	3.174	3.176	3.178	3.180	3.182	3.184	3.186
9.3	3.188	3.190	3.192	3.194	3.196	3.198	3.200	3.202	3.204	3.206
9.4	3.208	3.210	3.212	3.214	3.215	3.217	3.219	3.221	3.223	3.225
9.5	3.227	3.229	3.231	3.233	3.235	3.237	3.239	3.241	3.242	3.244
9.6	3.246	3.248	3.250	3.252	3.254	3.256	3.258	3.260	3.262	3.264
9.7	3.266	3.268	3.269	3.271	3.273	3.275	3.277	3.279	3.281	3.283
9.8	3.285	3.287	3.289	3.291	3.293	3.295	3.297	3.298	3.300	3.302
9.9	3.304	3.305	3.307	3.309	3.311	3.313	3.316	3.318	3.320	3.321
10	3.32	3.34	3.36	3.37	3.39	3.41	3.43	3.45	3.46	3.48
11	3.50	3.52	3.53	3.55	3.56	3.58	3.60	3.61	3.63	3.64
12	3.66	3.68	3.69	3.71	3.72	3.74	3.76	3.77	3.79	3.80
13	3.80	3.83	3.85	3.86	3.88	3.89	3.90	3.92	3.93	3.95
14	3.96	3.97	3.99	4.00	4.02	4.03	4.04	4.06	4.07	4.09
15	4.10	4.11	4.13	4.14	4.15	4.17	4.18	4.19	4.20	4.22
16	4.23	4.24	4.25	4.27	4.28	4.29	4.30	4.31	4.33	4.34
17	4.35	4.36	4.37	4.38	4.39	4.41	4.42	4.43	4.44	4.45
18	4.46	4.47	4.48	4.49	4.50	4.52	4.53	4.54	4.55	4.56
19	4.57	4.58	4.59	4.60	4.61	4.62	4.63	4.64	4.65	4.66



and an inside diameter of 30.0 ± 2.0 mm, and put the cup directly below the funnel of the volumeter. Slowly pour Microcrystalline Cellulose 5.1 cm height from the upper part of the powder funnel through the sieve, at a rate suitable to prevent clogging, until the cup overflows. If the clogging occurs, take out the sieve. Level the excess powder with the aid of a slide glass, weigh the filled cup, and weigh accurately the content of the cup, and then calculate the bulk density by the following expression: the bulk density is within the labeled specification.

$$\text{Bulk density (g/cm}^3\text{)} = A/25$$

A: Measured mass (g) of the content of the cup

◆**Microbial limit** <4.05> The total aerobic microbial count is not more than 1000 per g, the total count of fungi and yeast is not more than 100 per g, and yeast is not more than 100 per g, and *Escherichia coli*, *Salmonella species*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.◆

◆**Containers and storage** Containers—Tight containers.◆

Powdered Cellulose

粉末セルロース

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (◆ ◆).

Powdered Cellulose is a purified, mechanically disintegrated alpha cellulose ◆ obtained as a pulp, after partial hydrolysis as occasion demands◆, from fibrous plant materials.

The label indicates the mean degree of polymerization value with a range.

◆**Description** Powdered Cellulose occurs as a white powder.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.◆

Identification (1) Dissolve 20 g of zinc chloride and 6.5 g of potassium iodide in 10.5 mL of water, add 0.5 g of iodine, and shake for 15 minutes. Place about 10 mg of Powdered Cellulose on a watch glass, and disperse in 2 mL of this solution: the substance develops a blue-violet color.

◆(2) Mix 30 g of Powdered Cellulose with 270 mL of

water in a high-speed (18,000 revolutions per minute or more) blender for 5 minutes, transfer 100 mL of the dispersion to a 100-mL graduated cylinder, and allow to stand for 1 hour: a supernatant liquid appears above the layer of the cellulose.◆

(3) Transfer 0.25 g of Powdered Cellulose, accurately weighed, to a 125-mL conical flask, add exactly 25 mL each of water and 1 mol/L cupriethylenediamine TS, and proceed as directed in the Identification (3) under Microcrystalline Cellulose. The mean degree of polymerization, P, is not less than 440 and is within the labeled specification.

pH <2.54> Mix 10 g of Powdered Cellulose with 90 mL of water, and allow to stand for 1 hour with occasional stirring: the pH of the supernatant liquid is between 5.0 and 7.5.

Purity ◆(1) Heavy metals <1.07>—Proceed with 2.0 g of Powdered Cellulose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(2) Water-soluble substances—Shake 6.0 g of Powdered Cellulose with 90 mL of recently boiled and cooled water, and allow to stand for 10 minutes with occasional shaking. Filter, with the aid of vacuum through a filter paper, discard the first 10 mL of the filtrate, and pass the subsequent filtrate through the same filter, if necessary, to obtain a clear filtrate. Evaporate a 15.0-mL portion of the filtrate in a tared evaporating dish to dryness without charring, dry at 105°C for 1 hour, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg.

(3) Diethyl ether-soluble substances—Place 10.0 g of Powdered Cellulose in a column having an internal diameter of about 20 mm, and pass 50 mL of peroxide-free diethyl ether through the column. Evaporate the eluate to dryness in a previously dried and tared evaporation dish. Dry the residue at 105°C for 30 minutes, and weigh after allowing to cool in a desiccator: the difference between the mass of the residue and the mass obtained from a blank determination does not exceed 15.0 mg.

Loss on drying <2.41> Not more than 6.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g calculated on the dried basis).

◆**Microbial limit** <4.05> The total aerobic microbial count does not exceed 1000 per g, the total combined fungus and yeast count does not exceed 100 per g, and *Escherichia coli*, *Salmonella species*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not observed.◆

◆**Containers and storage** Containers—Tight containers.◆

Cellacefate

Cellulose Acetate Phthalate

セラセフェート

Cellulose acetate benzene-1,2-dicarboxylate
[9004-38-0]

This monograph is harmonized with the European

Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Cellacefate is a reaction product of phthalic anhydride and partially acetylated cellulose.

Cellacefate, calculated on the anhydrous and free acid-free basis, contains not less than 21.5% and not more than 26.0% of acetyl group (-COCH₃: 43.04), and not less than 30.0% and not more than 36.0% of carboxybenzoyl group (-COC₆H₄COOH: 149.12).

♦**Description** Cellacefate occurs as a white powder or grain.

It is freely soluble in acetone, and practically insoluble in water, in methanol and in ethanol (99.5).♦

Identification Determine the infrared absorption spectrum of Cellacefate directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with ♦the Reference Spectrum or ♦ spectrum of Cellacefate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Viscosity <2.53> Weigh accurately a quantity of Cellacefate, equivalent to 15 g calculated on the anhydrous basis, dissolve in 85 g of a mixture of acetone and water (249: 1 in mass), and perform the test with this solution at 25 ± 0.2°C as directed in Method 1 to obtain the kinematic viscosity ν . Separately, determine the density, ρ , of Cellacefate as directed under Determination of Specific Gravity and Density <2.56>, and calculate the viscosity, η , as $\eta = \rho\nu$: not less than 45 mPa·s and not more than 90 mPa·s.

Purity (1) ♦Heavy metals <1.07>—Proceed with 2.0 g of Cellacefate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).♦

(2) Free acids—Weigh accurately about 3 g of Cellacefate, put in a glass-stoppered conical flask, add 100 mL of diluted methanol (1 in 2), stopper tightly, and filter after shaking for 2 hours. Wash both the flask and residue with two 10-mL portions each of diluted methanol (1 in 2), combine the washes to the filtrate, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform the blank determination with 120 mL of diluted methanol (1 in 2), and make any necessary correction.

$$\text{Amount (\%)} \text{ of free acids} = (0.8306 \times A) / W$$

A: amount (mL) of 0.1 mol/L sodium hydroxide consumed

W: amount (g) of the test sample, calculated on the anhydrous basis

The amount of free acids is not more than 3.0%, calculated as phthalic acid (C₈H₆O₄: 166.13).

Water <2.48> Not more than 5.0% (1 g, volumetric titration, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for Karl Fischer method).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Carboxybenzoyl group—Weigh accurately about 1 g of Cellacefate, dissolve in 50 mL of a mixture of ethanol (95) and acetone (3: 2), and titrate <2.50> with 0.1

mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Content (%) of carboxybenzoyl group (C₈H₅O₃)

$$= \frac{\frac{1.491 \times A}{W} - (1.795 \times B)}{100 - B} \times 100$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide consumed

B: Amount (%) of free acids obtained in the Purity (2) Free acids

W: Amount (g) of the test sample, calculated on the anhydrous basis

Assay (2) Acetyl group—Weigh accurately about 0.1 g of Cellacefate, put in a glass-stoppered conical flask, add exactly 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 30 minutes under a reflux condenser. After cooling, add 5 drops of phenolphthalein TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS. Perform a blank determination.

Content (%) of free acids and bound acetyl group (C₂H₃O)

$$= 0.4305A / W$$

A: Amount (mL) of 0.1 mol/L sodium hydroxide consumed, corrected by the blank determination

W: Amount (g) of the test sample, calculated on the anhydrous basis

Content (%) of acetyl group (C₂H₃O)

$$= [\{100 \times (P - 0.5182B)\} / (100 - B)] - 0.5772C$$

B: Amount (%) of free acids obtained in the Purity (2) Free acids

C: Content (%) of carboxybenzoyl group

P: Content (%) of free acids and bound acetyl group (C₂H₃O)

Containers and storage Containers—Tight containers.

Celmo-leukin (Genetical Recombination)

セルモロイキン(遺伝子組換え)

Ala-Pro-Thr-Ser-Ser-Ser-Thr-Lys-Lys-Thr-Gln-Leu-Gln-Leu-Glu-His-Leu-Leu-Leu-Asp-Gln-Gln-Met-Ile-Leu-Asn-Gly-Ile-Asn-Asn-Tyr-Lys-Asn-Pro-Lys-Leu-Thr-Arg-Met-Leu-Thr-Phe-Lys-Phe-Tyr-Met-Pro-Lys-Lys-Ala-Thr-Glu-Leu-Lys-His-Leu-Gln-Cys-Leu-Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu-Ala-Gln-Ser-Lys-Asn-Phe-His-Leu-Arg-Pro-Arg-Asp-Leu-Ile-Ser-Asn-Ile-Asn-Val-Ile-Val-Leu-Glu-Leu-Lys-Gly-Ser-Glu-Thr-Thr-Phe-Met-Cys-Glu-Tyr-Ala-Asp-Glu-Thr-Ala-Thr-Ile-Val-Glu-Phe-Leu-Asn-Arg-Trip-Ile-Thr-Phe-Cys-Gln-Ser-Ile-Ile-Ser-Thr-Leu-Thr

C₆₉₃H₁₁₁₈N₁₇₈O₂₀₃S₇: 15415.82
[94218-72-I]

The desired product of Celmo-leukin (Genetical Recombination) is a protein consisting of 133 amino

acid residues manufactured by *E. coli* through expression of human interleukin-2 cDNA.

It is a solution having a T-lymphocyte activating effect.

It contains not less than 0.5 and not more than 1.5 mg of protein per mL, and 1 mg of this protein contains potency not less than 8.0×10^6 units.

Description Celmoleukin (Genetical Recombination) occurs as a colorless, clear liquid.

Identification (1) To 1 mL of Celmoleukin (Genetical Recombination) add 0.05 mL of diluted copper (II) sulfate TS (1 in 10), shake, add 0.9 g of potassium hydroxide, and shake. When 0.3 mL of ethanol (99.5) is added to this solution and shaken, the ethanol layer exhibits a violet color.

(2) Based on the results of the Assay (1), place an amount of Celmoleukin (Genetical Recombination) equivalent to about 50 μ g of protein in two hydrolysis tubes, and evaporate to dryness under vacuum. To one of the tubes add 100 μ L of a mixture of diluted hydrochloric acid (59→125), mercaptoacetic acid and phenol (100:10:1), and shake. Place this hydrolysis tube in a vial and humidify the inside of the vial with 200 μ L of the mixture of diluted hydrochloric acid (59→125), mercaptoacetic acid and phenol (100:10:1). Replace the vial interior with inert gas or reduce the pressure, and heat for 24 hours at about 115°C. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (1). To the other hydrolysis tube, add 100 μ L of ice cold performic acid, oxidize for 1.5 hours on ice, add 50 μ L of hydrobromic acid, and dry under vacuum. Add 200 μ L of water, repeat the dry under vacuum procedure two more times, place the hydrolysis tube in a vial, and humidify the inside of the vial with 200 μ L of diluted hydrochloric acid (59→125). Replace the vial interior with inert gas or reduce the pressure (vacuum), and heat for 24 hours at about 115°C. After drying under vacuum, dissolve in 0.5 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution (2). Separately, accurately weigh 60 mg of L-aspartic acid, 100 mg of L-glutamic acid, 17 mg of L-alanine, 23 mg of L-methionine, 21 mg of L-tyrosine, 24 mg of L-histidine hydrochloride monohydrate, 58 mg of L-threonine, 22 mg of L-proline, 14 mg of L-cystine, 45 mg of L-isoleucine, 37 mg of L-phenylalanine, 32 mg of L-arginine hydrochloride, 32 mg of L-serine, 6 mg of glycine, 18 mg of L-valine, 109 mg of L-leucine, 76 mg of L-lysine hydrochloride, and 8 mg of L-tryptophan, add 0.1 mol/L hydrochloric acid TS to dissolve to make 500 mL, add 40 μ L of this solution to two hydrolysis tubes, and evaporate to dryness under vacuum to make the standard solutions (1) and (2) processed in the same way for each respective sample solution. Perform the test with 250 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> and from the peak areas for each amino acid obtained from the sample solutions and standard solutions determine the molar number of the amino acids contained in 1 mL of the sample solutions. Furthermore, when calculating the number of amino acids assuming there are 22 leucine residues in one mole of Celmoleukin (Genetical Recombination), there are 17 or 18 glutamic acid (or glutamine), 11 to 13 threonine, 11 or 12 aspartic acid (or asparagine), 11 lysine, 7 or 8 isoleucine, 6 to 9 serine, 6 phenylalanine, 5 alanine, 5 or 6 proline, 4 arginine and 4 methionine, 3 or 4 cysteine, 3 or 4 valine, 3 tyrosine, 3 histi-

dine, 2 glycine, and 1 tryptophan.

(3) Molecular mass Based on the results of the Assay (1), add buffer for celmoleukin and dilute to prepare a sample solution so that there is about 0.5 mg of protein per mL. To vertical uncontinuous buffer SDS-polyacrylamide gel prepared from resolving gel for celmoleukin and stacking gel for celmoleukin add 20 μ L of the sample solution or 20 μ L of molecular weight marker for celmoleukin to each stacking gel well, and perform the electrophoresis. The molecular weight of the main electrophoretic band is between the range of 12500 and 13800 when the band is stained by immersion in Coomassie staining TS.

(4) Add 100 μ L of protein digestive enzyme TS to 100 μ L of Celmoleukin (Genetical Recombination), shake, leave standing for 18 to 24 hours at 37°C, and then add 2 μ L of 2-mercaptoethanol. Leave for a further 30 minutes at 37°C, and add 5 μ L of trifluoroacetic acid solution (1 in 10). This is the sample solution. Separately, process celmoleukin for liquid chromatography using the same method. This is the standard solution. Perform the test using 50 μ L of each both the sample and standard solutions as directed under Liquid Chromatography <2.01> according to the following conditions. When comparing the chromatograms obtained from the sample and standard solutions, the retention times for the sample and standard solutions are identical, and the peak heights are similar.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (particle size: 5 μ m).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A solution of trifluoroacetic acid (1 in 1000).

Mobile phase B: A solution of trifluoroacetic acid in a mixture of acetonitrile and water (17:3) (1 in 1000).

Mobile phase flow: The concentration gradient is controlled by changing the ratio of mobile phases A and B as shown in the table below.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	100	0
5 – 45	100→60	0→40
45 – 75	60→0	40→100
75 – 85	0	100

Flow: Adjust so that the retention time of celmoleukin is about 70 minutes.

System suitability—

System performance: Add 2 μ L of 2-mercaptoethanol to 100 μ L of celmoleukin for liquid chromatography, leave for 2 hours at 37°C, and then run this solution under the above conditions. Under these conditions, celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 1.5.

(5) Accurately measure an appropriate amount of Celmoleukin (Genetical Recombination), dilute by adding culture medium for celmoleukin, and prepare a sample solution

containing 800 units per mL. Add 25 μL of this sample solution to 2 holes (A and B) of a flat-bottomed microtest plate for tissue culture, and then add 25 μL of reference anti-interleukin-2 antiserum solution diluted with culture medium for celmoleukin to hole A and 25 μL of culture medium for celmoleukin to hole B. Add 50 μL of culture medium for celmoleukin to another hole (hole C). After shaking the microtest plate, warm for 30 minutes to 2 hours at 37°C in air containing 5% carbon dioxide. Next, add to each hole 50 μL of culture medium for celmoleukin containing the interleukin-2 dependent mouse natural killer cells NKC3 and culture for 16 to 24 hours at 37°C. Add 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide TS, culture for 4 to 6 hours at 37°C, and add sodium lauryl sulfate TS and leave for 24 to 48 hours. When the absorbance at 590 nm of the solution in each hole is measured as directed under Ultraviolet-visible Spectrophotometry <2.24>, the difference in absorbance between the solutions from holes A and C is not more than 3% of the difference in absorbance between the solutions from holes B and C.

pH <2.54> 4.5 – 5.5

Purity (1) Host cell-derived protein—Prepare a sample solution by the accurate two times stepwise dilution of Celmoleukin (Genetical Recombination) with phosphate buffer-sodium chloride TS (hereinafter referred to as PBS) containing bovine serum. Prepare a series of 5 standard solutions by accurately diluting *E. coli* protein (hereinafter referred to as ECP) over a range of 0.25 to 6 ng per mL with PBS containing bovine serum. Pipet 100 μL of goat anti-ECP antibody TS into each hole of a flat-bottomed microtest plate, leave for 16 to 24 hours at 4°C, and then remove the liquid. Wash three times with PBS, add 200 μL of PBS containing bovine serum albumin, leave for at least 3 hours at room temperature, and then wash three more times with PBS. Pipet 100 μL of the sample solution and each standard solution into each hole, leave for 16 to 24 hours at 4°C, and then wash 5 times with PBS. Add 100 μL of peroxidase-labeled rabbit anti-ECP antibody Fab' TS, leave for at least 4 hours at room temperature, and wash 5 times with PBS. Next, add 100 μL of substrate buffer for celmoleukin, allow to react for 5 to 25 minutes at room temperature in a dark place, and then add 100 μL of diluted sulfuric acid (3 in 25). Measure the absorbances of these flat-bottomed microtest plates by Ultraviolet-visible Spectrophotometry <2.24> at a wavelength of 492 nm. Separately, using 100 μL of PBS containing bovine serum, perform a blank test using the same method and correct. Determine the absorbance of each standard solution, prepare a calibration curve, determine the amount of ECP per mL of the sample solution, and multiply by the sample solution dilution factor. When determining the concentration of ECP per unit of protein in the sample solution, there is not more than 0.02% (0.2 $\mu\text{g}/\text{mg}$ of protein).

(2) Polymers—Dilute (at least 4 steps) the sample solution prepared in the Identification (3) with buffer solution for celmoleukin so that the protein content is within the range of about 2 to 32 μg per mL to prepare a series of standard solutions. Pipet 20 μL of the sample solution or each of the standard solutions into the stacking gel well, and perform vertical uncoupled buffer SDS-polyacrylamide gel electrophoresis followed by immersion in Coomassie staining TS. Each electrophoretic band is stained blue. Next, determine the peak area of the electrophoretic bands obtained from each stan-

dard solution using a densitometer and calculate the protein content using the calibration curve mentioned above. When determining the polymer proteins derived from celmoleukin, other than celmoleukin monomer, the amount is not more than 2% in relation to the total protein.

(3) Related substances—Perform the test with 10 μL each of Celmoleukin (Genetical Recombination) and 0.01 mol/L acetic acid buffer solution, pH 5.0, as directed under Liquid Chromatography <2.01> under the following conditions, and measure the area of each peak by an automatic integration method. When the amount of related substances other than celmoleukin is determined by the area percent method, the total amount is not more than 5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm)

Column: Stainless steel tube with an inside diameter of 4 mm and a length of 30 cm packed with octadecylsilanized silica gel for liquid chromatography (particle size: 5 μm).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of acetic acid and water (3:2) (1 in 1000)

Mobile phase B: A solution of trifluoroacetic acid in a mixture of acetic acid and water (13:7) (1 in 1000)

Mobile phase flow: The concentration gradient is controlled by changing the ratio of mobile phases A and B as shown in the table below.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 ~ 60	70 → 10	30 → 90

Flow: Adjust so that the retention time of celmoleukin is about 50 minutes.

Time span of measurement: About 1.3 times as long as the retention time of celmoleukin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 0.5 mL of Celmoleukin (Genetical Recombination), and add 0.01 mol/L acetic acid buffer solution, pH 5.0, to make exactly 50 mL. Confirm that the celmoleukin peak area obtained from 10 μL of this solution is 0.9 to 1.1% of the peak area obtained from 10 μL of Celmoleukin (Genetical Recombination).

System performance: Add 2 μL of 2-mercaptoethanol to 100 μL of Celmoleukin (Genetical Recombination), leave for 2 hours at 37°C, and then run this solution under the above conditions. Under these conditions, celmoleukin and its reduced form are eluted in this order with the resolution between these peaks being not less than 3.0.

Ammonium acetate Measure exactly 0.1 mL of Celmoleukin (Genetical Recombination), and add water to make exactly 10 mL. This is the sample solution. Separately, accurately weigh about 0.1 g of ammonium chloride, and add water to make exactly 100 mL. Measure exactly 5 mL of this solution, and add water to make exactly 100 mL. This is the standard stock solution. Measure exactly 3 mL of the standard stock solution, and add water to make exactly 50 mL.

This is the standard solution. Perform the test with exactly 25 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. When determining the area of the ammonium ion peak A_T and A_S , Celmo-leukin (Genetical Recombination) contains from 0.28 to 0.49 mg of ammonium acetate per mL.

Amount (mg) of ammonium acetate ($\text{CH}_3\text{COONH}_4$) per mL
 $= A_T/A_S \times W_S \times 0.003 \times 1.4410$

W_S : Amount (mg) of ammonium chloride

0.003: Dilution correction coefficient

1.4410: Molecular weight conversion coefficient for converting ammonium chloride to ammonium acetate

Operating conditions—

Detector: Electronic conductivity detector

Column: Resin column with an inside diameter of 5 mm and a length of 25 cm packed with weakly acidic ion exchange resin for liquid chromatography (particle size: 5.5 μm). As a guard column, connect to a column with an inside diameter of 5 mm and a length of 5 cm packed with weakly acidic ion exchange resin for liquid chromatography.

Column temperature: A constant temperature of about 40°C.

Mobile phase: Diluted 0.1 mol/L methanesulfonic acid TS (3 in 10).

Flow: Adjust the flow so that the retention time of ammonium is about 8 minutes.

System suitability—

System performance: Measure exactly 1 mL of Standard Sodium Stock Solution and 0.2 mL of Standard Potassium Stock Solution, and then add water to make exactly 100 mL. Measure exactly 5 mL of this solution and 3 mL of the standard stock solution, and then add water to make exactly 5 mL. When 25 μL of this solution is run under the above conditions, sodium, ammonium and potassium are eluted in this order with the resolution between the peaks of sodium and ammonium being not less than 3.0.

System repeatability: When the test is repeated 5 times with 25 μL of the standard solution under the above conditions, the relative standard deviation of the ammonium peak area is not more than 10%.

Bacterial endotoxins <4.01> Less than 100 EU/mL

Sterility <4.06> Perform the test according to the Direct method: it meets the requirement. In the test, add 0.5 mL of Celmo-leukin (Genetical Recombination) to 8 test tubes and 1.0 mL of Celmo-leukin (Genetical Recombination) to 8 test tubes containing 15 mL of thioglycol acid I for sterility test, as well as 1.0 mL of Celmo-leukin (Genetical Recombination) to 8 test tubes containing 15 mL of soybean-casein digest medium.

Assay (1) Total protein content—Measure accurately 1 mL of Celmo-leukin (Genetical Recombination) and add water to make exactly 10 mL. This is the sample solution. Separately, weigh accurately about 50 mg of bovine serum albumin for assay in water to prepare standard dilution solutions of 50, 100, and 150 $\mu\text{g}/\text{mL}$. Measure exactly 1 mL of the sample solution and each standard dilution solution, add exactly 2.5 mL of alkaline copper TS for protein content determination, shake, and leave for 15 minutes. Next, add exactly 2.5 mL of water and 0.5 mL of dilute Folin's TS, and

leave for 30 minutes at 37°C. Measure the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 1 mL of water processed in the same way as control. Using the calibration curve prepared from the absorbance of the standard dilution solution, determine the protein content of Celmo-leukin (Genetical Recombination).

(2) Specific activity—Measure exactly 0.1 mL of Celmo-leukin (Genetical Recombination) and add exactly 0.9 mL of culture medium for celmo-leukin to make the sample solution. Separately, take one Interleukin-2 Reference Standard and add exactly 1 mL of water to dissolve. This is the standard solution. Accurately serially dilute the sample and standard solutions in two-fold steps with culture medium for celmo-leukin, and add equal volumes of interleukin-2 dependent mouse natural killer NKC3 cells to the serially diluted solutions. The control solution is a mixture of equal volumes of NKC3 and culture medium for celmo-leukin. Incubate these solutions for 16 to 24 hours at 37°C. Following this, add a volume of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide TS that is 1/5 that of the volume of culture medium for celmo-leukin, incubate for 4 to 6 hours at 37°C, add a volume of sodium lauryl sulfate TS equivalent to the volume of the culture medium for celmo-leukin, and leave for 24 to 48 hours. After eluting the blue-colored pigment generated, perform the test on these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, and measure the absorbance at 590 nm. Taking the absorbance obtained when 1000 to 2000 units of celmo-leukin per mL are added as 100% and the absorbance of the control solution as 0%, determine the dilution factor (A) of the Interleukin-2 Reference Standard that shows an absorbance of 50% and dilution factor of Celmo-leukin (Genetical Recombination) (B). Multiply the B/A value by the unit number of the Interleukin-2 Reference Standard to determine the biological activity of 1 mL of Celmo-leukin (Genetical Recombination). Calculate the ratio of biological activity in relation to protein content determined in the total protein content test.

Containers and storage Containers—Sterilized, tight containers.

Storage—Store at -20°C or lower.

Cetanol

セタノール

Cetanol is a mixture of solid alcohols, and consists chiefly of $\text{C}_{16}\text{H}_{34}\text{O}$: 242.44.

Description Cetanol occurs as unctuous, white flakes, granules, or masses. It has a faint, characteristic odor. It is tasteless.

It is very soluble in pyridine, freely soluble in ethanol (95), in ethanol (99.5) and in diethyl ether, very slightly soluble in acetic anhydride, and practically insoluble in water.

Melting point <1.13> 47 – 53°C Prepare the sample according to Method 2, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer.

Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

Acid value <1.13> Not more than 1.0.

Ester value <1.13> Not more than 2.0.

Hydroxyl value <1.13> 210 – 232

Iodine value <1.13> Not more than 2.0.

Purity (1) Clarity of solution—Dissolve 3.0 g of Cetanol in 25 mL of ethanol (99.5) by warming; the solution is clear.

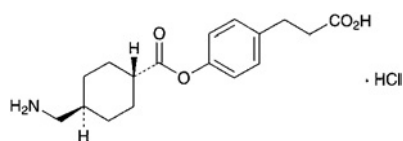
(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Well-closed containers.

Cetraxate Hydrochloride

セトラキサート塩酸塩



$C_{17}H_{23}NO_4 \cdot HCl$: 341.83

3-{4-[*trans*-4-(Aminomethyl)cyclohexylcarbonyloxy]-phenyl}propanoic acid monohydrochloride [27724-96-5]

Cetraxate Hydrochloride, when dried, contains not less than 98.5% of $C_{17}H_{23}NO_4 \cdot HCl$.

Description Cetraxate Hydrochloride occurs as white crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 236°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Cetraxate Hydrochloride in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 0.5 g of Cetraxate Hydrochloride in 5 mL of a mixture of water and 2-propanol (1:1) by warming, cool to below 25°C. Filter, dry the formed crystals in vacuum for 4 hours, and further dry at 105°C for 1 hour. Determine the infrared absorption spectrum of the dried matter as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cetraxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cetraxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cetraxate Hydrochloride according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

(3) *cis* Isomer—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of water, and use this solution as the sample solution. To exactly 5 mL of the sample solution add water to make exactly 100 mL. To exactly 2 mL of this solution add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak which has a retention time 1.3 to 1.6 times that of cetraxate from the sample solution is not larger than the peak area of cetraxate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:10:4) to 6.0 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of cetraxate is about 10 minutes.

System suitability—

System performance: Dissolve 0.02 g of Cetraxate Hydrochloride and 0.01 g of phenol in 100 mL of water. To 2 mL of this solution add water to make 20 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, cetraxate and phenol are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cetraxate is not more than 2.0%.

(4) 3-(*p*-Hydroxyphenyl)propionic acid—To 0.10 g of Cetraxate Hydrochloride add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of 3-(*p*-hydroxyphenyl)propionic acid in methanol to make exactly 100 mL. To exactly 2 mL of this solution add exactly 2 mL of the internal standard solution and methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard: Q_T is not larger than Q_S .

Internal standard solution—A solution of caffeine in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of a mixture of water, methanol and 0.5 mol/L ammonium acetate TS (15:5:2) to 5.5 with acetic acid (31).

Flow rate: Adjust the flow rate so that the retention time of 3-(*p*-hydroxyphenyl)propionic acid is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, 3-(*p*-hydroxyphenyl)propionic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 3-(*p*-hydroxyphenyl)propionic acid to that of the internal standard is not more than 1.0%.

(5) **Related substances**—Dissolve 0.10 g of Cetraxate Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and acetic acid (100) (20:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

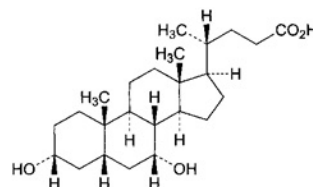
Assay Weigh accurately about 0.5 g of Cetraxate Hydrochloride, previously dried, dissolve in 100 mL of water, and adjust the pH of this solution to between 7.0 and 7.5 with dilute sodium hydroxide TS. To this solution add 10 mL of formaldehyde solution, stir for about 5 minutes, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS by taking over about 20 minutes (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 34.18 mg of C₁₇H₂₃NO₄·HCl

Containers and storage Containers—Tight containers.

Chenodeoxycholic Acid

ケノデオキシコール酸



C₂₄H₄₀O₄: 392.57

3 α ,7 α -Dihydroxy-5 β -cholan-24-oic acid
[474-25-9]

Chenodeoxycholic Acid, when dried, contains not less than 98.0% and not more than 101.0% of C₂₄H₄₀O₄.

Description Chenodeoxycholic Acid occurs as white, crystals, crystalline powder or powder.

It is freely soluble in methanol and in ethanol (99.5), soluble in acetone, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Chenodeoxycholic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +11.0 – +13.0° (after drying, 0.4 g, ethanol (99.5), 20 mL, 100 mm).

Melting point <2.60> 164 – 169°C

Purity (1) Chloride <1.03>—Dissolve 0.36 g of Chenodeoxycholic Acid in 30 mL of methanol, add 10 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of methanol, 10 mL of dilute nitric acid and water to make 50 mL (not more than 0.1%).

(2) **Heavy metals** <1.07>—Proceed with 1.0 g of Chenodeoxycholic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) **Barium**—To 2.0 g of Chenodeoxycholic Acid add 100 mL of water, and boil for 2 minutes. To this solution add 2 mL of hydrochloric acid, boil for 2 minutes, filter after cooling, and wash the filter with water until to get 100 mL of the filtrate. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is appeared.

(4) **Related substances**—Dissolve 0.20 g of Chenodeoxycholic Acid in a mixture of acetone and water (9:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of lithocholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 10 mL. Pipet 2 mL of this solution, add the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of ursodeoxycholic acid in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this so-

lution as the standard solution (2). Separately, dissolve 10 mg of cholic acid for thin-layer chromatography in the mixture of acetone and water (9:1) to make exactly 100 mL, and use this solution as the standard solution (3). Pipet 1 mL of the sample solution, and add the mixture of acetone and water (9:1) to make exactly 20 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 5 mL of this solution, add the mixture of acetone and water (9:1) to each of them to make exactly 50 mL, and designate these solutions as standard solution A, standard solution B, standard solution C, standard solution D and standard solution E, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution, standard solutions (1), (2), (3) and standard solutions A, B, C, D and E on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, toluene and formic acid (16:6:1) to a distance of about 15 cm, air-dry the plate, and further dry at 120°C for 30 minutes. Immediately, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) on the plate, and heat at 120°C for 2 to 3 minutes: the spot corresponding to the spot with the standard solution (1) is not more intense than the spot with the standard solution (1), the spot corresponding to the spot with the standard solution (2) is not more intense than the spot with the standard solution (2), and the spot corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

As compared to the spots with the standard solutions A, B, C, D and E, the spots other than the principal spot and other than the spots mentioned above are not more intense than the spot with the standard solution E, and the total amount of them is not more than 1.5%.

Loss on drying <2.41> Not more than 1.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

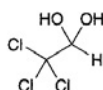
Assay Weigh accurately about 0.5 g of Chenodeoxycholic Acid, previously dried, dissolve in 40 mL of ethanol (95) and 20 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of $C_{24}H_{40}O_4$

Containers and storage Containers—Tight containers.

Chloral Hydrate

抱水クロラール



$C_2H_3Cl_3O_2$: 165.40
2,2,2-Trichloroethane-1,1-diol [302-17-0]

Chloral Hydrate contains not less than 99.5% of $C_2H_3Cl_3O_2$.

Description Chloral Hydrate occurs as colorless crystals. It

has a pungent odor and an acrid, slightly bitter taste.

It is very soluble in water, and freely soluble in ethanol (95) and in diethyl ether.

It slowly volatilizes in air.

Identification (1) Dissolve 0.2 g of Chloral Hydrate in 2 mL of water, and add 2 mL of sodium hydroxide TS: the turbidity is produced, and it separates into two clear layers by warming.

(2) Heat 0.2 g of Chloral Hydrate with 3 drops of aniline and 3 drops of sodium hydroxide TS: the disagreeable odor of phenylisocyanide (poisonous) is perceptible.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Chloral Hydrate in 2 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.20 g of Chloral Hydrate in 2 mL of water, and add 1 drop of methyl orange TS: a yellow color develops.

(3) Chloride <1.03>—Perform the test with 1.0 g of Chloral Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(4) Chloral alcoholate—Warm 1.0 g of Chloral Hydrate with 10 mL of sodium hydroxide TS, filter the upper layer, add iodine TS to the filtrate until a yellow color develops, and allow the solution to stand for 1 hour: no yellow precipitate is produced.

(5) Benzene—Warm the solution obtained in (1) with 3 mL of water: no odor of benzene is perceptible.

Residue on ignition <2.44> Not more than 0.1% (1 g).

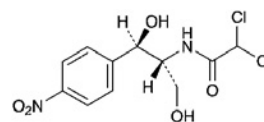
Assay Weigh accurately about 4 g of Chloral Hydrate in a glass-stoppered flask, add 10 mL of water and exactly 40 mL of 1 mol/L sodium hydroxide VS, and allow the mixture to stand for exactly 2 minutes. Titrate <2.50> the excess sodium hydroxide immediately with 0.5 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 1 mol/L sodium hydroxide VS
= 165.4 mg of $C_2H_3Cl_3O_2$

Containers and storage Containers—Tight containers.

Chloramphenicol

クロラムフェニコール



$C_{11}H_{12}Cl_2N_2O_5$: 323.13
2,2-Dichloro-*N*-[(1*R*,2*R*)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide [56-75-7]

Chloramphenicol contains not less than 980 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$).

Description Chloramphenicol occurs as white to yellowish

white, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and slightly soluble in water.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Chloramphenicol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +18.5 – +21.5° (1.25 g, ethanol (99.5), 25 mL, 100 mm).

Melting point <2.60> 150 – 155°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Chloramphenicol according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Chloramphenicol in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add methanol to make exactly 20 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of chloroform, methanol and acetic acid (100) (79:14:7) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot on the original obtained from the sample solution are not more intense than the spot from the standard solution (1), and the total amount of these spots is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Chloramphenicol and Chloramphenicol Reference Standard, equivalent to about 0.1 g (potency), dissolve each in 20 mL of methanol, and add water to make exactly 100 mL. Pipet 20 mL each of these solutions, and add water to make exactly 100 mL. Pipet 10 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S , at 278 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

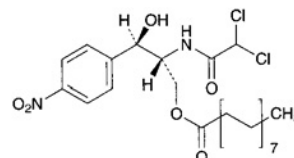
$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } C_{11}H_{12}Cl_2N_2O_5 \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Chloramphenicol Reference Standard

Containers and storage Containers—Tight containers.

Chloramphenicol Palmitate

クロラムフェニコールパルミチン酸エステル



$C_{27}H_{42}Cl_2N_2O_6$: 561.54

(2*R*,3*R*)-2-(Dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl palmitate [530-43-8]

Chloramphenicol Palmitate contains not less than 558 μ g (potency) and not more than 587 μ g (potency) per mg, calculated on the dried basis. The potency of Chloramphenicol Palmitate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Palmitate occurs as a white to grayish white, crystalline powder.

It is freely soluble in acetone, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Chloramphenicol Palmitate in ethanol (99.5) (1 in 33,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chloramphenicol Palmitate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Chloramphenicol Palmitate and Chloramphenicol Palmitate Reference Standard in 1 mL of acetone, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution has the same R_f value as the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{25}$: +21 – +25° (1 g calculated on the dried basis, ethanol (99.5), 20 mL, 100 mm).

Melting point <2.60> 91 – 96°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol Palmitate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Chloramphenicol Palmitate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. The test should be performed within 30 minutes after the sample solution and standard solution are prepared. Determine each peak area by the automatic integration method: the total area of the peaks other than the peak of chloramphenicol palmitate from the sample solution is not more than 3.5 times the peak area of chloramphenicol palmitate from the standard solution. For this calculation, use the peak areas for chloramphenicol, having the relative retention time of about 0.5 with respect to chloramphenicol palmitate, and for chloramphenicol dipalmitate, having the relative retention time of about 5.0 with respect to chloramphenicol palmitate, after multiplying by their relative response factors, 0.5 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of chloramphenicol palmitate.

System suitability—

Test for required detectability: Dissolve 50 mg of Chloramphenicol Palmitate in 50 mL of methanol. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add methanol to make exactly 50 mL. Confirm that the peak area of chloramphenicol palmitate obtained from 20 μ L of this solution is equivalent to 7 to 13% of that obtained from 20 μ L of the solution for system suitability test.

System performance: When the procedure is run with 20 μ L of the solution for system suitability test under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 5000.

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Chloramphenicol Palmitate and Chloramphenicol Palmitate Reference Standard, equivalent to about 37 mg (potency), dissolve each in

40 mL of methanol and exactly 1 mL of acetic acid (100), and add methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mobile phase to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S .

Amount [μ g (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)
 $= W_S \times (A_T/A_S) \times 1000$

W_S : Amount [mg (potency)] of Chloramphenicol Palmitate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water and acetic acid (100) (172:27:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol palmitate is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of chloramphenicol palmitate is not less than 2400.

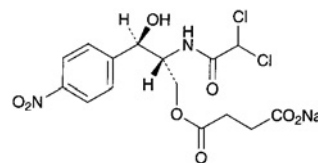
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chloramphenicol palmitate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chloramphenicol Sodium Succinate

クロラムフェニコールコハク酸エステルナトリウム



$C_{15}H_{15}Cl_2N_2NaO_8$: 445.18

Monosodium (2*R*,3*R*)-2-(dichloroacetyl)amino-3-hydroxy-3-(4-nitrophenyl)propan-1-yl succinate [982-57-0]

Chloramphenicol Sodium Succinate contains not less than 711 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Chloramphenicol Sodium Succinate is expressed as mass (potency) of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$: 323.13).

Description Chloramphenicol Sodium Succinate occurs as white to yellowish white, crystals or crystalline powder.

It is very soluble in water, and freely soluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Chloramphenicol Sodium Succinate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chloramphenicol Sodium Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Chloramphenicol Sodium Succinate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{25}$: +5 – +8° (1.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.4 g of Chloramphenicol Sodium Succinate in 5 mL of water is between 6.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Chloramphenicol Sodium Succinate in 10 mL of water: the solution is clear and colorless to yellowish.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Chloramphenicol Sodium Succinate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Chloramphenicol Sodium Succinate according to Method 1, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 2.0% (1.0 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Chloramphenicol Sodium Succinate, equivalent to about 20 mg (potency), dissolve in water to make exactly 1000 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Chloramphenicol Succinate Reference Standard, equivalent to about 20 mg (potency), add about 50 mL of water to make a suspension, and add gradually about 7 mL of 0.01 mol/L sodium hydroxide TS while stirring to adjust the pH to 7.0. To this solution add water to make exactly 1000 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 276 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

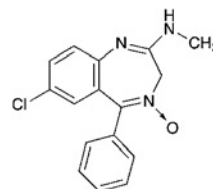
Amount [μ g (potency)] of chloramphenicol ($C_{11}H_{12}Cl_2N_2O_5$)
 $= W_S \times (A_T/A_S) \times 1000$

W_S : Amount [mg (potency)] of Chloramphenicol Succinate Reference Standard

Containers and storage Containers—Hermetic containers.

Chlordiazepoxide

クロルジアゼポキシド



$C_{16}H_{14}ClN_3O$: 299.75

7-Chloro-2-methylamino-5-phenyl-3H-1,4-benzodiazepin-4-oxide [58-25-3]

Chlordiazepoxide, when dried, contains not less than 98.5% of $C_{16}H_{14}ClN_3O$.

Description Chlordiazepoxide occurs as white to light yellow crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually affected by light.

Melting point: about 240°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Chlordiazepoxide in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chlordiazepoxide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Chlordiazepoxide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Chlordiazepoxide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Proceed with Chlordiazepoxide as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color develops.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chlordiazepoxide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.20 g of Chlordiazepoxide in exactly 10 mL of a mixture of methanol and ammonia TS (97:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and ammonia TS (97:3) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 10 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L of the

sample solution and 5 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (99.5) (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Spray evenly a solution of sodium nitrite in 1 mol/L hydrochloric acid TS (1 in 100) on the plate, allow to stand for 1 minute, and spray evenly *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS on the plate: the spots from the sample solution are not more intense than the spots from the standard solution (2).

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Chlordiazepoxide, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the supernatant liquid changes from purple through blue-purple to blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.98 mg of $\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlordiazepoxide Powder

クロルジアゼポキシド散

Chlordiazepoxide Powder contains not less than 93% and not more than 107% of the labeled amount of chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$: 299.75).

Method of preparation Prepare as directed under Powder, with Chlordiazepoxide.

Identification (1) Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of Chlordiazepoxide Powder, equivalent to 0.02 g of Chlordiazepoxide according to the labeled amount, add 10 mL of methanol, shake for 5 minutes, then filter by suction through a glass filter (G4), evaporate the filtrate with the aid of a current of air to dryness, and dry the residue in vacuum at 60°C for 1 hour. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1625 cm^{-1} , 1465 cm^{-1} , 1265

cm^{-1} , 850 cm^{-1} and 765 cm^{-1} .

Purity Conduct this procedure without exposure to daylight, using light-resistant vessels. To a portion of Chlordiazepoxide Powder, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide Reference Standard in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Powder, equivalent to about 0.1 g of Chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$), transfer to a glass-stoppered flask, wet with exactly 10 mL of water, add exactly 90 mL of methanol, stopper, shake vigorously for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Chlordiazepoxide Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, and dissolve in exactly 10 mL of water and 90 mL of methanol. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of chlordiazepoxide to that of the internal standard.

Amount (mg) of chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$)
= $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of Chlordiazepoxide Reference Standard

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with

10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlordiazepoxide Tablets

クロルジアゼポキシド錠

Chlordiazepoxide Tablets contain not less than 93% and not more than 107% of the labeled amount of chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$: 299.75).

Method of preparation Prepare as directed under Tablets, with Chlordiazepoxide.

Identification (1) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, shake, and filter. To 5 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 244 nm and 248 nm and between 306 nm and 310 nm, and a minimum between 288 nm and 292 nm.

(2) Weigh a portion of powdered Chlordiazepoxide Tablets, equivalent to 0.01 g of Chlordiazepoxide according to the labeled amount, add 10 mL of diethyl ether, shake vigorously, and centrifuge. Evaporate 5 mL of the supernatant liquid by warming on a water bath to dryness. Determine the infrared absorption spectrum of the residue as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1625 cm^{-1} , 1465 cm^{-1} , 1265 cm^{-1} , 850 cm^{-1} and 765 cm^{-1} .

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. To a portion of powdered Chlordiazepoxide Tablets, equivalent to 50 mg of Chlordiazepoxide according to the labeled amount, add exactly 5 mL of a mixture of methanol and ammonia TS (97:3), shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 50 mg of Chlordiazepoxide Reference Standard in a mixture of methanol and ammonia TS (97:3) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 5.0 mg of 2-amino-5-chlorobenzophenone for thin-layer chromatography in methanol to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μL of the sample solution and 10 μL each of the standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Purity (2) under Chlordiazepoxide.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Chlordiazepoxide Tablets at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 30 mL or more of the dissolved solution 60 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.8 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 3.7 μg of chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 12 mg of chlordiazepoxide for assay, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide, 60°C), and dissolve in 2nd fluid for dissolution test to make exactly 200 mL. Pipet 3 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Chlordiazepoxide Tablets in 60 minutes is not less than 70%.

$$\begin{aligned} &\text{Dissolution rate (\%)} \text{ with respect to the labeled} \\ &\text{amount of chlordiazepoxide } (\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}) \\ &= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 27 \end{aligned}$$

W_S : Amount (mg) of chlordiazepoxide for assay.

C : Labeled amount (mg) of chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$) in 1 tablet.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of Chlordiazepoxide Tablets, equivalent to about 0.1 g of Chlordiazepoxide ($\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}$), transfer to a glass-stoppered flask, add 10 mL of water, and shake well to disintegrate. Add 60 mL of methanol, shake well, add methanol to make exactly 100 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Chlordiazepoxide Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 4 hours, dissolve in 1 mL of water and a suitable amount of methanol, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of chlordiazepoxide to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of chlordiazepoxide } (\text{C}_{16}\text{H}_{14}\text{ClN}_3\text{O}) \\ &= W_S \times (Q_T/Q_S) \times 10 \end{aligned}$$

W_S : Amount (mg) of Chlordiazepoxide Reference Standard

Internal standard solution—A solution of isobutyl salicylate in methanol (1 in 20).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.02 mol/L ammonium dihydrogen phosphate TS (7:3).

Flow rate: Adjust the flow rate so that the retention time of chlordiazepoxide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, chlordiazepoxide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of chlordiazepoxide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Chlorhexidine Gluconate Solution

クロルヘキシジングルコン酸塩液

Chlorhexidine Gluconate Solution is a solution of digluconate of chlorhexidine.

It contains not less than 19.0 w/v% and not more than 21.0 w/v% of chlorhexidine gluconate ($\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}\cdot 2\text{C}_6\text{H}_{12}\text{O}_7$; 897.76).

Description Chlorhexidine Gluconate Solution is a clear, colorless or pale yellow liquid. It is odorless, and has a bitter taste.

It is miscible with water and with acetic acid (100). 1 mL of Chlorhexidine Gluconate Solution is miscible with not more than 5 mL of ethanol (99.5) and with not more than 3 mL of acetone. By further addition of each of these solvents, a white turbidity is formed.

It is gradually colored by light.

Specific gravity d_{20}^{20} : 1.06 – 1.07

Identification (1) To 0.05 mL of Chlorhexidine Gluconate Solution add 5 mL of methanol, 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) To 0.5 mL of Chlorhexidine Gluconate Solution add 10 mL of water and 0.5 mL of copper (II) sulfate TS: a white precipitate is formed. Heat to boiling: the precipitate changes to light purple.

(3) To 10 mL of Chlorhexidine Gluconate Solution add 5 mL of water, cool on ice, and add 5 mL of sodium hydroxide TS dropwise with stirring: a white precipitate is formed. Collect the precipitate by filtration, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals thus obtained melt <2.60> between 130°C and 134°C.

(4) Neutralize the filtrate obtained in (3) with 5 mol/L hydrochloric acid TS. To 5 mL of this solution add 0.65 mL of acetic acid (100) and 1 mL of freshly distilled phenylhydra-

zine, and heat on a water bath for 30 minutes. After cooling, scratch the inner wall of the vessel with a glass rod to induce crystallization. Collect the crystals, dissolve in 10 mL of hot water, add a small amount of activated charcoal, and filter. Cool the filtrate, scratch the inner side of the vessel, collect the formed crystals, and dry: the crystals thus obtained melt <2.60> at about 195°C (with decomposition).

pH <2.54> To 5.0 mL of Chlorhexidine Gluconate Solution add water to make 100 mL: the pH of the solution is between 5.5 and 7.0.

Purity *p*-Chloroaniline—To 2.0 mL of Chlorhexidine Gluconate Solution add water to make exactly 100 mL. Pipet 5 mL of the solution, and add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, add 1 mL of ethanol (95), and then add water to make 50 mL: the color of the solution is not more intense than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. Pipet 5 mL of the solution, add 20 mL of water and 5 mL of 1 mol/L hydrochloric acid TS, and proceed as directed for the preparation of the sample solution.

Residue on ignition <2.44> Not more than 0.1% (2 g, after evaporation).

Assay Pipet 2 mL of Chlorhexidine Gluconate Solution, evaporate to dryness on a water bath, dissolve the residue in 60 mL of acetic acid for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

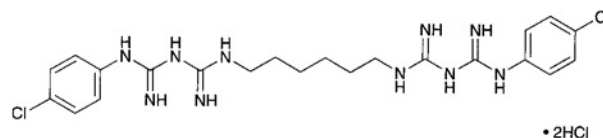
Each mL of 0.1 mol/L perchloric acid VS
= 22.44 mg of $\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}\cdot 2\text{C}_6\text{H}_{12}\text{O}_7$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorhexidine Hydrochloride

クロルヘキシジン塩酸塩



$\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}\cdot 2\text{HCl}$: 578.37

1,1'-Hexamethylenebis[5-(4-chlorophenyl)biguanide] dihydrochloride [3697-42-5]

Chlorhexidine Hydrochloride, when dried, contains not less than 98.0% of $\text{C}_{22}\text{H}_{30}\text{Cl}_2\text{N}_{10}\cdot 2\text{HCl}$.

Description Chlorhexidine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in formic acid, slightly soluble in methanol and in warm methanol, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Chlorhexidine Hydrochloride in 5 mL of methanol by warming, and add 1 mL of bromine TS and 1 mL of 8 mol/L sodium hydroxide TS: a deep red color is produced.

(2) Dissolve 0.3 g of Chlorhexidine Hydrochloride in 10 mL of 6 mol/L hydrochloric acid TS, cool in ice, and add 10 mL of 8 mol/L sodium hydroxide TS dropwise with stirring: a white precipitate is produced. Collect the precipitate, wash with water, recrystallize from diluted ethanol (95) (7 in 10), and dry at 105°C for 30 minutes: the crystals so obtained melt <2.60> between 130°C and 134°C.

(3) Dissolve 0.1 g of Chlorhexidine Hydrochloride in 50 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Chlorhexidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—To 1.0 g of Chlorhexidine Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol (95) to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

(3) *p*-Chloroaniline—Dissolve 0.10 g of Chlorhexidine Hydrochloride in 2 mL of formic acid, and add 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water immediately. Add 0.3 mL of sodium nitrite TS, shake, and allow to stand for 2 minutes. Add 4 mL of ammonium amidosulfate TS, and then allow to stand for 1 minute. Add 5 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate-acetone TS, allow to stand for 10 minutes, and add 1 mL of ethanol (95) and water to make 50 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 20 mg of 4-chloroaniline in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of the solution, and add water to make exactly 100 mL. To 2.0 mL of the solution add 2 mL of formic acid, 15 mL of 1 mol/L hydrochloric acid TS and 20 mL of water, and proceed in the same manner.

Loss on drying <2.41> Not more than 2.0% (1 g, 130°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Chlorhexidine Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.46 mg of C₂₂H₃₀Cl₂N₁₀·2HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorinated Lime

サラシ粉

Chlorinated Lime contains not less than 30.0% of available chlorine (Cl: 35.45).

Description Chlorinated Lime occurs as a white powder. It has a chlorine-like odor.

It dissolves partially in water. The solution changes red litmus paper to blue, then gradually decolorizes.

Identification (1) To Chlorinated Lime add dilute hydrochloric acid: a gas, which has the odor of chlorine, evolves, and the gas changes moistened starch-potassium iodide paper to blue.

(2) Shake 1 g of Chlorinated Lime with 10 mL of water, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt.

Assay Weigh accurately about 5 g of Chlorinated Lime, transfer to a mortar, and triturate thoroughly with 50 mL of water. Transfer to a 500-mL volumetric flask with the aid of water, and add water to make 500 mL. Mix well, immediately take exactly 50 mL of the mixture in an iodine flask, add 10 mL of potassium iodide TS and 10 mL of dilute hydrochloric acid, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

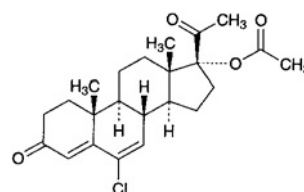
Each mL of 0.1 mol/L sodium thiosulfate VS
= 3.545 mg of Cl

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Chlormadinone Acetate

クロルマジノン酢酸エステル



C₂₃H₂₉ClO₄: 404.93

6-Chloro-3,20-dioxopregna-4,6-dien-17-yl acetate
[302-22-7]

Chlormadinone Acetate, when dried, contains not less than 98.0% of C₂₃H₂₉ClO₄.

Description Chlormadinone Acetate occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetonitrile, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Chlormadinone Acetate in 1 mL of ethanol (95), and add 1 mL of 1,3-dinitrobenzene TS and 1 mL of a solution of potassium hydroxide (1 in 5): a red-purple color develops.

(2) To 0.05 g of Chlormadinone Acetate add 2 mL of potassium hydroxide-ethanol TS, and boil on a water bath for 5 minutes. After cooling, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Determine the infrared absorption spectrum of Chlormadinone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlormadinone Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Chlormadinone Acetate as directed under Flame Coloration Test <1.04> (2): a green color appears.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-10.0 - -14.0^\circ$ (after drying, 0.2 g, acetonitrile, 10 mL, 100 mm).

Melting point <2.60> 211 – 215°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Chlormadinone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Chlormadinone Acetate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 20 mg of Chlormadinone Acetate in 10 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of peaks other than the peak of chlormadinone acetate from the sample solution is not larger than the peak area of chlormadinone acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 236 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of acetonitrile and water (13:7).

Flow rate: Adjust the flow rate so that the retention time of chlormadinone acetate is about 10 minutes.

Time span of measurement: About 1.5 times as long as the retention time of chlormadinone acetate beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of chlormadinone acetate obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of chlormadinone acetate obtained from 10 μ L of the stan-

dard solution.

System performance: Dissolve 8 mg of Chlormadinone Acetate and 2 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. When the procedure is run with 10 μ L of this solution under the above operating conditions, butyl parahydroxybenzoate and chlormadinone acetate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlormadinone acetate is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Chlormadinone Acetate and Chlormadinone Acetate Reference Standard, previously dried, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, to each add ethanol (95) to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S , at 285 nm.

$$\begin{aligned} \text{Amount (mg) of } C_{23}H_{29}ClO_4 \\ = W_S \times (A_T/A_S) \end{aligned}$$

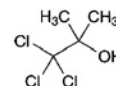
W_S : Amount (mg) of Chlormadinone Acetate Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorobutanol

クロロブタノール



$C_4H_7Cl_3O$: 177.46

1,1,1-Trichloro-2-methylpropan-2-ol [57-15-8]

Chlorobutanol contains not less than 98.0% of $C_4H_7Cl_3O$, calculated on the anhydrous basis.

Description Chlorobutanol occurs as colorless or white crystals. It has a camphoraceous odor.

It is very soluble in methanol, in ethanol (95) and in diethyl ether, and slightly soluble in water.

It slowly volatilizes in air.

Melting point: not lower than about 76°C.

Identification (1) To 5 mL of a solution of Chlorobutanol (1 in 200) add 1 mL of sodium hydroxide TS, then slowly add 3 mL of iodine TS: a yellow precipitate is produced and the odor of iodoform is perceptible.

(2) To 0.1 g of Chlorobutanol add 5 mL of sodium hydroxide TS, shake well the mixture, add 3 to 4 drops of aniline, and warm gently: the disagreeable odor of phenyl isocyanide (poisonous) is perceptible.

Purity (1) Acidity—Shake thoroughly 0.10 g of the powder of Chlorobutanol with 5 mL of water: the solution is neutral.

(2) Chloride <1.03>—Dissolve 0.5 g of Chlorobutanol in 25 mL of dilute ethanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS by adding 25 mL of dilute ethanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.071%).

Water <2.48> Not more than 6.0% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

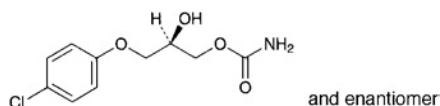
Assay Transfer about 0.1 g of Chlorobutanol, accurately weighed, to a 200-mL conical flask, and dissolve in 10 mL of ethanol (95). Add 10 mL of sodium hydroxide TS, boil under a reflux condenser for 10 minutes, cool, add 40 mL of dilute nitric acid and exactly 25 mL of 0.1 mol/L silver nitrate VS, and shake well. Add 3 mL of nitrobenzene, and shake vigorously until the precipitate is coagulated. Titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 5.915 mg of C₁₀H₁₂ClNO₄

Containers and storage Containers—Tight containers.

Chlorphenesin Carbamate

クロルフェネシンカルバミン酸エステル



C₁₀H₁₂ClNO₄; 245.66

(2*RS*)-3-(4-Chlorophenoxy)-2-hydroxypropyl carbamate
[886-74-8]

Chlorphenesin Carbamate, when dried, contains not less than 98.0% and not more than 102.0% of C₁₀H₁₂ClNO₄.

Description Chlorphenesin Carbamate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in methanol, in ethanol (95) and in pyridine, soluble in 2-propanol, sparingly soluble in diethyl ether, slightly soluble in water, and practically insoluble in hexane.

A solution of Chlorphenesin Carbamate in ethanol (95) (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Chlorphenesin Carbamate in ethanol (95) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorphenesin Carbamate, previously dried, as directed in the

potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Chlorphenesin Carbamate as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 88 – 91°C

Purity (1) Heavy metals <1.07>—Dissolve 2.0 g of Chlorphenesin Carbamate in 20 mL of ethanol (95), and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 20 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Chlorphenesin Carbamate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—(i) Chlorphenesin-2-carbamate: Dissolve 0.10 g of Chlorphenesin Carbamate in 20 mL of a mixture of hexane for liquid chromatography and 2-propanol (7:3), and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A_a, of chlorphenesin carbamate and the peak area, A_b, of chlorphenesin-2-carbamate by the automatic integration method: the ratio, A_b/(A_a + A_b), is not larger than 0.007.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of hexane for liquid chromatography, 2-propanol and acetic acid (100) (700:300:1).

Flow rate: Adjust the flow rate so that the retention time of chlorphenesin carbamate is about 9 minutes.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution, add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 100 mL, and use this solution as the solution for system suitability test. To exactly 5 mL of the solution for system suitability test add a mixture of hexane for liquid chromatography and 2-propanol (7:3) to make exactly 10 mL. Confirm that the peak area of chlorphenesin carbamate obtained from 10 μL of this solution is equivalent to 40 to 60% of that of chlorphenesin carbamate obtained from 10 μL of the solution for system suitability test.

System performance: Dissolve 0.1 g of Chlorphenesin Carbamate in 50 mL of methanol. To 25 mL of this solution add 25 mL of dilute sodium hydroxide TS, and warm at 60°C for 20 minutes. To 20 mL of this solution add 5 mL of 1 mol/L hydrochloric acid TS, shake well with 20 mL of ethyl acetate, and allow to stand to separate the ethyl acetate layer. When the procedure is run with 10 μL of this layer under the above operating conditions, chlorphenesin, chlorphenesin carbamate and chlorphenesin-2-carbamate are eluted in this order, with the ratios of the retention time of chlorphenesin and

chlorphenesin-2-carbamate with respect to chlorphenesin carbamate are about 0.7 and about 1.2, respectively, and with the resolution between the peaks of chlorphenesin and chlorphenesin carbamate being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of chlorphenesin carbamate is not more than 2.0%.

(ii) **Other related substances:** Dissolve 0.10 g of Chlorphenesin Carbamate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Chlorphenesin Carbamate, previously dried, dissolve in 20 mL of pyridine, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol TS, and warm at 70°C for 40 minutes. After cooling, add 100 mL of ethanol (95), and titrate <2.50> the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS until the color of the solution changes from blue through blue-green to yellow (indicator: 1 mL of thymol blue TS). Perform a blank determination.

Each mL of 0.1 mol/L potassium hydroxide-ethanol TS
= 24.57 mg of $C_{10}H_{12}ClNO_4$

Containers and storage Containers—Tight containers.

Chlorpheniramine and Calcium Powder

クロルフェニラミン・カルシウム散

Chlorpheniramine and Calcium Powder contains not less than 0.27% and not more than 0.33% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$; 390.86).

Method of preparation

Chlorpheniramine Maleate	3 g
Dibasic Calcium Phosphate Hydrate	800 g
Starch, Lactose Hydrate, or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Description Chlorpheniramine and Calcium Powder occurs as a white powder.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm (chlorpheniramine maleate).

(2) To 0.5 g of Chlorpheniramine and Calcium Powder add 10 mL of dilute hydrochloric acid, shake well, and filter: the filtrate responds to the Qualitative Tests <1.09> (3) for calcium salt.

(3) To 0.5 g of Chlorpheniramine and Calcium Powder add 10 mL of dilute nitric acid, shake well, and filter: the filtrate responds to the Qualitative tests <1.09> (2) for phosphate.

(4) Shake 1 g of Chlorpheniramine and Calcium Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of chlorpheniramine maleate in 17 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm. Air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and from the standard solution show the same *R_f* value. Spray evenly Dragendorff's TS for spraying upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal an orange color.

Assay Weigh accurately about 0.5 g of Chlorpheniramine and Calcium Powder, transfer to a 30-mL glass-stoppered centrifuge tube, add 20 mL of 0.05 mol/L sulfuric acid VS, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Add 20 mL of 0.05 mol/L sulfuric acid VS to the residue, and proceed twice in the same manner mentioned above. Transfer all the supernatant liquid to a 200-mL separator, add 30 mL of diethyl ether, shake, and allow to stand for 5 minutes. Filter the water layer through dry filter paper into another separator. Extract the diethyl ether layer with two 10-mL portions of 0.05 mol/L sulfuric acid VS, filter the extracts into the preceding separator containing the water layer. Wash the filter paper with 5 mL of 0.05 mol/L sulfuric acid VS, combine the washings with the water layer in the preceding separator, and add 10 mL of ammonia TS. Extract with two 50-mL portions of diethyl ether, combine the diethyl ether layer, wash with 20 mL of water, and extract the diethyl ether layer with two 20-mL and 5-mL portions of 0.25 mol/L sulfuric acid VS. Combine all the extracts, add 0.25 mol/L sulfuric acid VS to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve about 75 mg of chlorpheniramine maleate for assay, previously dried at 105°C for 3 hours and accurately weighed, in 10 mL of 0.05 mol/L sulfuric acid VS, and add 0.05 mol/L sulfuric acid VS to make exactly 100 mL. Pipet 2 mL of the solution into a 200-mL separator, add 58 mL of 0.05 mol/L sulfuric acid VS and 30 mL of diethyl ether, and shake. Proceed in the same manner as the sample solution, and use this solution as the standard solution. Determine the absorbances, A_T and

A_S , of the sample solution and the standard solution at 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.25 mol/L sulfuric acid VS as the blank.

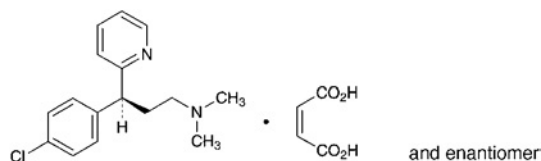
$$\begin{aligned} &\text{Amount (mg) of chlorpheniramine maleate} \\ &(\text{C}_{16}\text{H}_{19}\text{ClN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4) \\ &= W_S \times (A_T/A_S) \times (1/50) \end{aligned}$$

W_S : Amount (mg) of chlorpheniramine maleate for assay

Containers and storage Containers—Well-closed containers.

Chlorpheniramine Maleate

クロルフェニラミンマレイン酸塩



$\text{C}_{16}\text{H}_{19}\text{ClN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$: 390.86
(3*RS*)-3-(4-Chlorophenyl)-*N,N*-dimethyl-3-pyridin-2-ylpropylamine monomaleate [113-92-8]

Chlorpheniramine Maleate, when dried, contains not less than 98.0% and not more than 101.0% of *dl*-chlorpheniramine maleate ($\text{C}_{16}\text{H}_{19}\text{ClN}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$).

Description Chlorpheniramine Maleate occurs as white, fine crystals.

It is very soluble in acetic acid (100), freely soluble in water and in methanol, and soluble in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

A solution of Chlorpheniramine Maleate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Chlorpheniramine Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Chlorpheniramine Maleate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorpheniramine Maleate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Chlorpheniramine Maleate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.10 g of Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol, acetic acid (100) and water

(70:20:7:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained with the sample solution shows the same intense and R_f value with the spot with the standard solution.

pH <2.54> Dissolve 1.0 g of Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.5.

Melting point <2.60> 130 – 135°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Chlorpheniramine Maleate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Chlorpheniramine Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than maleic acid and chlorpheniramine is not larger than 2/3 times the peak area of chlorpheniramine obtained with the standard solution, and the total area of the peaks other than maleic acid and chlorpheniramine is not larger than the peak area of chlorpheniramine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 11 minutes.

Time span of measurement: About 4 times as long as the retention time of chlorpheniramine after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with

20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpheniramine is not more than 4.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Chlorpheniramine Maleate, previously dried and accurately weighed, in 20 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.54 mg of $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Chlorpheniramine Maleate Injection

クロルフェニラミンマレイン酸塩注射液

Chlorpheniramine Maleate Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of preparation Prepare as directed under Injections, with Chlorpheniramine Maleate.

Description Chlorpheniramine Maleate Injection is a clear, colorless liquid.
pH: 4.5 – 7.0

Identification Take a volume of Chlorpheniramine Maleate Injection, equivalent to 25 mg of Chlorpheniramine Maleate according to the labeled amount, add 5 mL of dilute sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 10 mL of water, shake with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940 cm^{-1} , 2810 cm^{-1} , 2770 cm^{-1} , 1589 cm^{-1} , 1491 cm^{-1} , 1470 cm^{-1} , 1434 cm^{-1} , 1091 cm^{-1} and 1015 cm^{-1} .

Bacterial endotoxins <4.01> Less than 8.8 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Transfer an exactly measured volume of Chlorpheniramine Maleate Injection, equivalent to about 3 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), to a 100-mL separator, add 20 mL of water and 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with 20 mL of water, and then extract with 20-mL, 20-mL and 5-mL portions of 0.25 mol/L sulfuric acid TS successively. Combine all acid extracts, and add 0.25 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 10 mL of this solution, add 0.25 mol/L sulfuric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Chlorpheniramine Maleate Reference Standard, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 200 mL. Pipet 20 mL of this solution, transfer to a 100-mL separator, add 2 mL of sodium hydroxide TS, and extract with two 50-mL portions of diethyl ether. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances A_T and A_S of the sample solution and standard solution at a wavelength of the maximum absorbance at about 265 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of chlorpheniramine maleate
($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$)
= $W_S \times (A_T/A_S) \times (1/10)$

W_S : Amount (mg) of Chlorpheniramine Maleate Reference Standard

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Chlorpheniramine Maleate Powder

クロルフェニラミンマレイン酸塩散

Chlorpheniramine Maleate Powder contains not less than 93.0% and not more than 107.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of preparation Prepare as directed under Powders, with Chlorpheniramine Maleate.

Identification Weigh a portion of Chlorpheniramine Maleate Powder, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath under reduced pressure, and determine the spectrum of the residue as directed in the liquid film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave number of about 2940 cm^{-1} , 2810 cm^{-1} , 2770 cm^{-1} , 1589 cm^{-1} , 1491 cm^{-1} , 1470 cm^{-1} , 1434 cm^{-1} , 1091 cm^{-1} and 1015 cm^{-1} .

Particle size <6.03> It meets the requirement.

Assay Weigh accurately an amount of Chlorpheniramine

Maleate Powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate Reference Standard, previously dried at 105°C for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of chlorpheniramine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of chlorpheniramine maleate} \\ & (C_{16}H_{19}ClN_2 \cdot C_4H_4O_4) \\ & = W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Chlorpheniramine Maleate Reference Standard

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 30 μ L of the standard solution under the above operating conditions, chlorpheniramine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 30 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Chlorpheniramine Maleate Tablets

クロルフェニラミンマレイン酸塩錠

Chlorpheniramine Maleate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of *dl*-chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$; 390.86).

Method of preparation Prepare as directed under Tablets, with Chlorpheniramine Maleate.

Identification Weigh a portion of powdered Chlorpheniramine Maleate Tablets, equivalent to 50 mg of Chlorpheniramine Maleate according to the labeled amount, shake with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. Transfer the filtrate to a separator, and wash with 40 mL of hexane. Add 10 mL of sodium hydroxide TS, and extract with 20 mL of hexane. Wash the hexane layer with 5 mL of water. Centrifuge, if necessary, shake the hexane extract with 0.5 g of anhydrous sodium sulfate for several minutes, and filter. Evaporate the filtrate in a water bath at about 50°C under a reduced pressure, and determine the infrared absorption spectrum of the residue as directed on the film method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2940 cm^{-1} , 2810 cm^{-1} , 2770 cm^{-1} , 1589 cm^{-1} , 1491 cm^{-1} , 1470 cm^{-1} , 1434 cm^{-1} , 1091 cm^{-1} and 1015 cm^{-1} .

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Chlorpheniramine Maleate Tablets add 10 mL of water, shake to disintegrate the tablet, then add water to make exactly V mL of a solution containing about 80 μ g of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$) per mL, and filter through a membrane filter with pore size of not more than 0.5 μ m. Pipet 5 mL of the filtrate, add exactly 2.5 mL of the internal standard solution, add water to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate Reference Standard, previously dried at 105°C for 3 hours, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 25 mL of the internal standard solution, add water to make 100 mL, and use this solution as the standard solution. Perform the test with 30 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay, and determine the ratios, Q_T and Q_S , of the peak area of chlorpheniramine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of chlorpheniramine maleate} \\ & (C_{16}H_{19}ClN_2 \cdot C_4H_4O_4) \\ & = W_S \times (Q_T/Q_S) \times (V/250) \end{aligned}$$

W_S : Amount (mg) of Chlorpheniramine Maleate Reference Standard

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate (1 in 250) add water to make 1000 mL.

Assay Weigh accurately the mass of not less than 20 Chlorpheniramine Maleate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 4 mg of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$), add 70 mL of the internal standard solution, shake for 15 minutes, then add the internal standard solution to make exactly 100 mL, filter through a membrane filter with pore size of not more than 0.5 μ m, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Chlorpheniramine Maleate Reference Standard, previously dried at 105°C for 3 hours, and add the internal standard to make exactly 100 mL. Pipet 20 mL of this solution, add the internal stan-

standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 30 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of chlorpheniramine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of chlorpheniramine maleate} \\ & (\text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_4\text{H}_4\text{O}_4) \\ & = W_S \times (A_T/A_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Chlorpheniramine Maleate Reference Standard

Internal standard solution—To 7 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 1000) add water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium 1-heptane sulfonate in 900 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine is about 8 minutes.

System suitability—

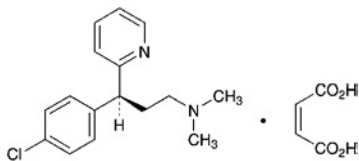
System performance: When the procedure is run with 30 μ L of the standard solution under the above operating conditions, the internal standard and chlorpheniramine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 30 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpheniramine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

d-Chlorpheniramine Maleate

d-クロルフェニラミンマレイン酸塩



$\text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_4\text{H}_4\text{O}_4$: 390.86
(3*S*)-3-(4-Chlorophenyl)-*N,N*-dimethyl-3-pyridin-2-ylpropylamine monomaleate [2438-32-6]

d-Chlorpheniramine Maleate, when dried, contains not less than 99.0% and not more than 101.0% of $\text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_4\text{H}_4\text{O}_4$.

Description *d*-Chlorpheniramine Maleate occurs as a white, crystalline powder.

It is very soluble in water, in methanol and in acetic acid (100), and freely soluble in *N,N*-dimethylformamide and in ethanol (99.5).

It dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectrum of a solution of *d*-Chlorpheniramine Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of *d*-Chlorpheniramine Maleate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.10 g of *d*-Chlorpheniramine Maleate in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 56 mg of maleic acid in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol, acetic acid (100) and water (70:20:7:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among two of the spots obtained with the sample solution shows the same intense to the spot with the standard solution, and its *R_f* value is about 0.4.

Optical rotation <2.49> $[\alpha]_D^{20}$: +39.5 – +43.0° (after drying, 0.5 g, *N,N*-dimethylformamide, 10 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.0 and 5.0.

Melting point <2.60> 111 – 115°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of *d*-Chlorpheniramine Maleate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of *d*-Chlorpheniramine Maleate according to Method 4 and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of *d*-Chlorpheniramine Maleate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than maleic acid and *d*-chlorpheniramine with the sample solution is not larger than 2/3 times the peak area of *d*-chlorpheniramine with the standard solution, and the total area of these peaks is not larger than the peak area of *d*-chlorpheniramine with the standard solu-

tion.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 8.57 g of ammonium dihydrogen phosphate and 1 mL of phosphoric acid in water to make 1000 mL. To 800 mL of this solution add 200 mL of acetic acid.

Flow rate: Adjust the flow rate so that the retention time of *d*-chlorpheniramine is about 11 minutes.

Time span of measurement: About 4 times as long as the retention time of *d*-chlorpheniramine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of *d*-chlorpheniramine obtained with 20 μL of this solution is equivalent to 7 to 13% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of *d*-chlorpheniramine are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of *d*-chlorpheniramine is not more than 4.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 65°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of *d*-Chlorpheniramine Maleate, previously dried, and dissolve in 20 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

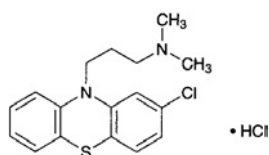
Each mL of 0.1 mol/L perchloric acid VS
= 19.54 mg of C₁₆H₁₉ClN₂·C₄H₄O₄

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorpromazine Hydrochloride

クロルプロマジン塩酸塩



C₁₇H₁₉ClN₂S·HCl: 355.33

3-(2-Chloro-10*H*-phenothiazin-10-yl)-*N,N*-dimethylpropylamine monohydrochloride [69-09-0]

Chlorpromazine Hydrochloride, when dried, contains not less than 99.0% of C₁₇H₁₉ClN₂S·HCl.

Description Chlorpromazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

Identification (1) To 5 mL of a solution of Chlorpromazine Hydrochloride (1 in 1000) add 1 drop of iron (III) chloride TS: a red color develops.

(2) Dissolve 0.1 g of Chlorpromazine Hydrochloride in 20 mL of water and 3 drops of dilute hydrochloric acid, add 10 mL of 2,4,6-trinitrophenol TS, and allow to stand for 5 hours. Collect the resulting precipitate, wash with water, recrystallize from a small portion of acetone, and dry at 105°C for 1 hour: the crystals so obtained melt <2.60> between 175°C and 179°C.

(3) Dissolve 0.5 g of Chlorpromazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. Cool, filter, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 194 – 198°C

pH <2.54> Dissolve 1.0 g of Chlorpromazine Hydrochloride in 20 mL of freshly boiled and cooled water, and measure within 10 minutes: the pH of this solution is between 4.0 and 5.0.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Chlorpromazine Hydrochloride in 20 mL of water, when observed within 10 minutes, is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Chlorpromazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Chlorpromazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.53 mg of C₁₇H₁₉ClN₂S·HCl

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorpromazine Hydrochloride Injection

クロルプロマジン塩酸塩注射液

Chlorpromazine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$: 355.33).

Method of preparation Prepare as directed under Injections, with Chlorpromazine Hydrochloride.

Description Chlorpromazine Hydrochloride Injection is a clear, colorless or pale yellow liquid.

pH: 4.0 – 6.5

Identification (1) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 5 mg of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (1) under Chlorpromazine Hydrochloride.

(2) Proceed with a volume of Chlorpromazine Hydrochloride Injection, equivalent to 0.1 g of Chlorpromazine Hydrochloride according to the labeled amount, as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Extractable volume <6.05> It meets the requirement.

Assay Transfer an exactly measured volume of Chlorpromazine Hydrochloride Injection, equivalent to about 0.15 g of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) to a separator, add 30 mL of water and 10 mL of a solution of sodium hydroxide (1 in 5), and extract with two 30-mL portions and three 20-mL portions of diethyl ether. Wash the combined diethyl ether extracts with successive 10-mL portions of water until the last washing shows no red color upon the addition of phenolphthalein TS. Concentrate the diethyl ether extracts on a water bath to 20 mL, add 5 g of anhydrous sodium sulfate, allow to stand for 20 minutes, and filter through a pledget of absorbent cotton. Wash with diethyl ether, combine the washings with the filtrate, and evaporate the diethyl ether on a water bath. Dissolve the residue in 50 mL of acetone and 5 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS until the color of the solution changes from red-purple to blue-purple (indicator: 3 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.77 mg of $C_{17}H_{19}ClN_2S \cdot HCl$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Chlorpromazine Hydrochloride Tablets

クロルプロマジン塩酸塩錠

Chlorpromazine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$: 355.33).

Method of preparation Prepare as directed under Tablets, with Chlorpromazine Hydrochloride.

Identification (1) Shake a quantity of powdered Chlorpromazine Hydrochloride Tablets, equivalent to 0.2 g of Chlorpromazine Hydrochloride according to the labeled amount, with 40 mL of 0.1 mol/L hydrochloric acid TS, and filter. To 1 mL of the filtrate add 4 mL of water and 1 drop of iron (III) chloride TS: a red color develops.

(2) To 20 mL of the filtrate obtained in (1) add 10 mL of 2,4,6-trinitrophenol TS dropwise, and proceed as directed in the Identification (2) under Chlorpromazine Hydrochloride.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Chlorpromazine Hydrochloride Tablet at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution after 60 minutes from the start of the dissolution test, and filter through a membrane filter with a pore size not exceeding 0.8 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add 2nd fluid for dissolution test to make exactly V' mL so that each mL of the filtrate contains about 5.6 μg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.09 g of chlorpromazine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in 2nd fluid for dissolution test to make exactly 200 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, further pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 254 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate of Chlorpromazine Hydrochloride Tablets in 60 minutes should be not less than 75%.

Dissolution rate (%) with respect to labeled amount of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (45/8)$

W_S : Amount (mg) of chlorpromazine hydrochloride for assay.

C : Labeled amount (mg) of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S \cdot HCl$) in 1 tablet.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately, and powder not less than 20 Chlorpromazine Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to

lent to about 50 mg of chlorpromazine hydrochloride ($C_{17}H_{19}ClN_2S.HCl$), add 60 mL of a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1), exposure to ultrasonic waves for 5 minutes, then shake vigorously for 20 minutes, and add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL. Filter the solution through a membrane filter with a pore size not exceeding $0.45 \mu m$, and discard the first 3 mL of the filtrate. To exactly 2.5 mL of the subsequent filtrate add exactly 5 mL of the internal standard solution, then add the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of chlorpromazine hydrochloride for assay, previously dried at $105^\circ C$ for 2 hours, and dissolve in the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) to make 25 mL, and use this solution as the standard solution. Perform the test with $10 \mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of chlorpromazine to that of the internal standard.

$$\text{Amount (mg) of chlorpromazine hydrochloride} \\ (C_{17}H_{19}ClN_2S.HCl) = W_S \times (Q_T/Q_S) \times 2$$

W_S : Amount (mg) of chlorpromazine hydrochloride for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of diluted phosphoric acid (1 in 500) and ethanol (99.5) (1:1) (1 in 4500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 256 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5 \mu m$ in particle diameter).

Column temperature: A constant temperature of about $25^\circ C$.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile (27:13).

Flow rate: Adjust the flow rate so that the retention time of chlorpromazine is about 15 minutes.

System suitability—

System performance: When the procedure is run with $10 \mu L$ of the standard solution under the above operating conditions, the internal standard and chlorpromazine are eluted in this order with the resolution between these peaks being not less than 10.

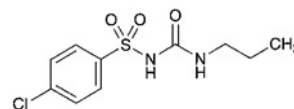
System repeatability: When the test is repeated 6 times with $10 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of chlorpromazine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Chlorpropamide

クロルプロパミド



$C_{10}H_{13}ClN_2O_3S$: 276.74

4-Chloro-*N*-(propylcarbamoyl)benzenesulfonamide
[94-20-2]

Chlorpropamide, when dried, contains not less than 98.0% of $C_{10}H_{13}ClN_2O_3S$.

Description Chlorpropamide occurs as white, crystals or crystalline powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 0.08 g of Chlorpropamide in 50 mL of methanol. To 1 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 200 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Chlorpropamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Chlorpropamide as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> $127 - 131^\circ C$

Purity (1) Acidity—To 3.0 g Chlorpropamide add 150 mL of water, and warm at $70^\circ C$ for 5 minutes. Allow to stand in ice water for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Chloride <1.03>—To 40 mL of the filtrate obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <1.14>—To 40 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Chlorpropamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.6 g of Chlorpropamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 300 mL, and use this solu-

tion as the standard solution (1). Separately, dissolve 60 mg of 4-chlorobenzene sulfonamide in acetone to make exactly 300 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, 3-methyl-1-butanol, methanol and ammonia solution (28) (15:10:5:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate at 100°C for 1 hour, spray evenly sodium hypochlorite TS on the plate, and air-dry for 15 minutes. Then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution equivalent to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than the spot mentioned above and other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Chlorpropamide, previously dried, dissolve in 30 mL of neutralized ethanol, and add 20 mL of water. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 27.67 \text{ mg of } C_{10}H_{13}ClN_2O_3S \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Chlorpropamide Tablets

クロルプロパミド錠

Chlorpropamide Tablets contain not less than 95% and not more than 105% of the labeled amount of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$: 276.74).

Method of preparation Prepare as directed under Tablets, with Chlorpropamide.

Identification Take a quantity of powdered Chlorpropamide Tablets, equivalent to 0.08 g of Chlorpropamide according to the labeled amount, add 50 mL of methanol, shake, and filter. To 1 mL of the filtrate add 0.01 mol/L hydrochloric acid TS to make 200 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 231 nm and 235 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Chlorpropamide Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution 45 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add 2nd fluid for dissolution test to make exactly V'

mL so that each mL contains about 10 μ g of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 232 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Chlorpropamide Tablets in 45 minutes should be not less than 70 %.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the} \\ \text{labeled amount of chlorpropamide } (C_{10}H_{13}ClN_2O_3S) \\ = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18 \end{aligned}$$

W_S : Amount (mg) of chlorpropamide for assay.

C : Labeled amount (mg) of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Chlorpropamide Tablets. Weigh accurately a quantity of the powder, equivalent to about 50 mg of chlorpropamide ($C_{10}H_{13}ClN_2O_3S$), add 75 mL of the mobile phase, shake for 10 minutes, and add the mobile phase to make exactly 100 mL. Centrifuge this solution, pipet 10 mL of the supernatant liquid, add the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of chlorpropamide for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak areas, A_T and A_S , of chlorpropamide of the sample solution and standard solution.

$$\begin{aligned} \text{Amount (mg) of chlorpropamide } (C_{10}H_{13}ClN_2O_3S) \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of chlorpropamide for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 100) and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of chlorpropamide is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of chlorpropamide are not less than 1500 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with

20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of chlorpropamide is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Cholera Vaccine

コレラワクチン

Cholera Vaccine is a liquid for injection containing inactivated *Vibrio cholerae* of the Ogawa and Inaba strains.

Monotypic products may be manufactured, if necessary.

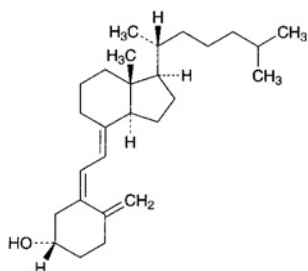
It conforms to the requirements of Cholera Vaccine in the Minimum Requirements for Biological Products.

Description Cholera Vaccine is a white-turbid liquid.

Cholecalciferol

Vitamin D₃

コレカルシフェロール



$C_{27}H_{44}O$: 384.64
(3*S*,5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3-ol
[67-97-0]

Cholecalciferol contains not less than 97.0% and not more than 103.0% of $C_{27}H_{44}O$.

Description Cholecalciferol occurs as white crystals. It is odorless.

It is freely soluble in ethanol (95), in chloroform, in diethyl ether and in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 84 – 88°C Transfer Cholecalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fireseal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Cholecalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Cholecalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cholecalciferol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (265 nm): 450 – 490 (10 mg, ethanol (95), 1000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$ +103 – +112° (50 mg, ethanol (95), 10 mL, 100 mm). Prepare the solution without delay, using Cholecalciferol from a container opened not longer than 30 minutes, previously, and determine the rotation within 30 minutes after the solution has been prepared.

Purity 7-Dehydrocholesterol—Dissolve 10 mg of Cholecalciferol in 2.0 mL of diluted ethanol (95) (9 in 10), add a solution prepared by dissolving 20 mg of digitonin in 2.0 mL of diluted ethanol (95) (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Proceed with the operation avoiding contact with air or other oxidizing agents and using light-resistant containers. Dissolve separately about 30 mg each of Cholecalciferol and Cholecalciferol Reference Standard, accurately weighed, in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cholecalciferol to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{27}H_{44}O \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Cholecalciferol Reference Standard

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 10 to 30 cm in length, packed with silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: Ordinary temperature.

Mobile phase: A mixture of hexane and *n*-amylalcohol (997:3).

Flow rate: Adjust the flow rate so that the retention time of cholecalciferol is about 25 minutes.

Selection of column: Dissolve 15 mg of Cholecalciferol Reference Standard in 25 mL of isooctane. Transfer this solution to a flask, heat under a reflux condenser in an oil bath for 2 hours, and cool to room temperature rapidly. Transfer this solution to a quartz test tube, and irradiate under a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To this solution add the mobile phase to make 50 mL. Proceed with 10 μ L of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D₃, transvitamin D₃ and tachysterol₃ to that of cholecalciferol being

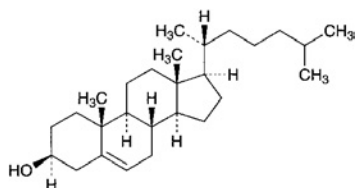
about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D₃ and trans-vitamin D₃, and that between cholecalciferol and tachysterol₃ being not less than 1.0.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Cholesterol

コレステロール



C₂₇H₄₆O: 386.65
Cholest-5-en-3β-ol [57-88-5]

Description Cholesterol occurs as white to pale yellow crystals or granules. It is odorless, or has a slight odor. It is tasteless.

It is freely soluble in chloroform and in diethyl ether, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5), and practically insoluble in water.

It gradually changes to a yellow to light yellow-brown color by light.

Identification (1) Dissolve 0.01 g of Cholesterol in 1 mL of chloroform, add 1 mL of sulfuric acid, and shake: a red color develops in the chloroform layer, and the sulfuric acid layer shows a green fluorescence.

(2) Dissolve 5 mg of Cholesterol in 2 mL of chloroform, add 1 mL of acetic anhydride and 1 drop of sulfuric acid, and shake: a red color is produced, and it changes to green through blue.

Optical rotation <2.49> [α]_D²⁵: -34 - -38° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 147 - 150°C

Purity (1) Clarity of solution—Place 0.5 g of Cholesterol in a glass-stoppered flask, dissolve in 50 mL of warm ethanol (95), and allow to stand at room temperature for 2 hours: no turbidity or deposit is produced.

(2) Acidity—Place 1.0 g of Cholesterol in a flask, dissolve in 10 mL of diethyl ether, add 10.0 mL of 0.1 mol/L sodium hydroxide VS, and shake for 1 minute. Expel the diethyl ether, and boil for 5 minutes. Cool, add 10 mL of water, and titrate <2.50> with 0.05 mol/L sulfuric acid VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

The volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.30 mL.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, 60°C, 4 hours).

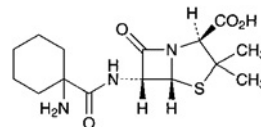
Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ciclacillin

シクラシリン



C₁₅H₂₃N₃O₄S: 341.43
(2*S*,5*R*,6*R*)-6-[(1-Aminocyclohexanecarbonyl)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid [3485-14-1]

Ciclacillin contains not less than 920 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dehydrated basis. The potency of Ciclacillin is expressed as mass (potency) of ciclacillin (C₁₅H₂₃N₃O₄S).

Description Ciclacillin occurs as white to light yellowish white crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, and practically insoluble in acetonitrile and in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Ciclacillin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ciclacillin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +300 - +315° (2 g, water, 100 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ciclacillin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ciclacillin according to Method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Ciclacillin and Ciclacillin Reference Standard, equivalent to about 50 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q*_T and *Q*_S, of the peak area of ciclacillin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

*W*_S: Amount [mg (potency)] of Ciclacillin Reference

Standard

Internal standard solution—A solution of orcin in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 4.0 with acetic acid (100), and add water to make 1000 mL. To 850 mL of this solution add 150 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ciclacillin is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ciclacillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

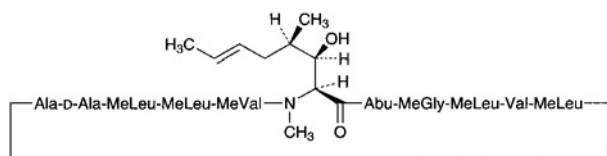
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak areas of ciclacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ciclosporin

Ciclosporin A

シクロスポリン



Abu = (2S)-2-Aminobutyric acid

MeGly = N-Methylglycine

MeLeu = N-Methylleucine

MeVal = N-Methylvaline

$C_{62}H_{111}N_{11}O_{12}$: 1202.61

cyclo{-[(2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-methylamino-oct-6-enyl]-L-2-aminobutanoyl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl-L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-} [59865-13-3]

Ciclosporin contains not less than 98.5% and not more than 101.5% of $C_{62}H_{111}N_{11}O_{12}$, calculated on the dried basis.

Description Ciclosporin occurs as a white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), freely soluble in diethyl ether, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Ciclosporin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -185 - -193° (0.1 g calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ciclosporin in 10 mL of ethanol (95); the solution is clear, and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To exactly 3.0 mL of Ferric Chloride Stock CS and exactly 0.8 mL of Cobaltous Chloride Stock CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (2): To exactly 3.0 mL of Ferric Chloride Stock CS, exactly 1.3 mL of Cobaltous Chloride Stock CS and exactly 0.5 mL of Cupric Sulfate Stock CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution (3): To exactly 0.5 mL of Iron (III) chloride Stock CS and exactly 1.0 mL of Cobaltous Chloride Stock CS add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ciclosporin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Use the sample solution obtained in the Assay as the sample solution. Pipet 2 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of the peak other than the ciclosporin is not more than 0.7 times of the peak area of ciclosporin from the standard solution, and the total area of all peaks other than the ciclosporin is not more than 1.5 times of the peak area of ciclosporin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of ciclosporin beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add a mixture of water and acetonitrile (1:1) to make exactly 20 mL. Confirm that the peak area of ciclosporin obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of ciclosporin obtained from 20 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of

ciclosporin is not more than 3.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 30 mg each of Ciclosporin and Ciclosporin Reference Standard (previously determine the loss on drying <2.41> in the same manner as Ciclosporin), and dissolve each in a mixture of water and acetonitrile (1:1) to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of ciclosporin.

$$\begin{aligned} \text{Amount (mg) of } C_{62}H_{111}N_{11}O_{12} \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ciclosporin Reference Standard, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter). Connect the sample injection port and the column with a stainless steel tube 0.3 mm in inside diameter and 1 m in length.

Column temperature: A constant temperature of about 80°C (including the sample injection port and the connecting tube).

Mobile phase: A mixture of water, acetonitrile, tert-butyl methyl ether and phosphoric acid (520:430:50:1).

Flow rate: Adjust the flow rate so that the retention time of ciclosporin is about 27 minutes.

System suitability—

System performance: Dissolve 3 mg of Ciclosporin U in 2.5 mL of a mixture of water and acetonitrile (1:1), and add 2.5 mL of the standard solution. When the procedure is run with 20 μ L of this solution under the above operating conditions, ciclosporin U and ciclosporin are eluted in this order with the resolution between these peaks being not less than 1.2.

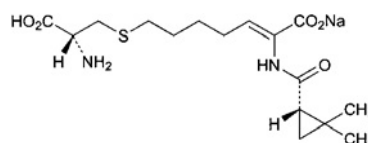
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ciclosporin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cilastatin Sodium

シラスタチンナトリウム



$C_{16}H_{25}N_2NaO_5S$: 380.43

Monosodium (2Z)-7-[[{(2R)-2-amino-2-carboxyethyl]sulfanyl]-2-({[(1S)-2,2-dimethylcyclopropyl]carbonyl} amino)hept-2-enoate [81129-83-1]

Cilastatin Sodium contains not less than 98.0% and not more than 101.0% of $C_{16}H_{25}N_2NaO_5S$, calculated on the anhydrous basis and corrected on the amount of the residual solvent.

Description Cilastatin Sodium occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Cilastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Cilastatin Sodium (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +41.5 – +44.5° (0.1 g, calculated on the anhydrous basis and corrected on the amount of the residual solvent, a solution of hydrochloric acid in methanol (9 in 1000), 10 mL, 100 mL).

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Cilastatin Sodium in 100 mL of water is between 6.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cilastatin Sodium in 100 mL of water: the solution is clear and the solution has no more color than the following control solution.

Control solution: To a mixture of 2.4 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.6 mL of Cobalt (II) Chloride Colorimetric Stock Solution add water to make 10 mL, pipet 5 mL of this solution, and add water to make exactly 100 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cilastatin Sodium according to Method 2, and perform the test. After carbonization, add 0.5 mL of sulfuric acid instead of nitric acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—To 2.0 g of Cilastatin Sodium add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, heat with two 2-mL portions of nitric acid, then heat with several 2-mL portions of hydrogen peroxide (30) until a colorless or pale yellow

low solution is obtained. After cooling, heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution: it shows no more color than the following color standard.

Color standard: Prepare a solution according to the above procedure without using Cilastatin Sodium, add exactly 2 mL of Standard Arsenic Solution, and perform the test in the same manner as the test solution (not more than 1 ppm).

(4) Related substances—Dissolve about 40 mg of Cilastatin Sodium in 25 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilastatin is not larger than 1/6 times the peak area of cilastatin from the standard solution, and the total area of the peaks other than the peak of cilastatin is not larger than the peak area of cilastatin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.5 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (7:3).

Mobile phase B: Diluted phosphoric acid (1 in 1000).

Flowing of the mobile phase: Control the gradient by mixing the mobile phase A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	15 → 100	85 → 0
30 – 40	100	0

Flow rate: 2.0 mL per minute.

Time span of measurement: 40 minutes.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 30 mL. Confirm that the peak area of cilastatin obtained with 20 μ L of this solution is equivalent to 2.3 to 4.5% of that obtained with 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the retention time of cilastatin is about 20 minutes, and the number of theoretical plates and the symmetry factor of the peak of cilastatin are not less than 10,000 and not more than 2.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilastatin is not more than 2.0%.

(5) Residual solvents <2.46>—Weigh accurately about 0.2

g of Cilastatin Sodium, add exactly 2 mL of the internal standard solution, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, measure exactly 2 mL of acetone, 0.5 mL of methanol and 0.5 mL of mesityl oxide, and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, add water to make 10 mL, and use this solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the ratios of the peak areas of acetone, methanol and mesityl oxide and to the peak area of the internal standard, Q_{Ta} and Q_{Sa} , Q_{Tb} and Q_{Sb} , Q_{Tc} and Q_{Sc} , and calculate the amounts of acetone, methanol and mesityl oxide by the following equation: they are not more than 1.0%, not more 0.5% and not more than 0.4%, respectively.

$$\begin{aligned} \text{Amount (\%)} \text{ of acetone (CH}_3\text{COCH}_3) \\ = (1/W_T) \times (Q_{Ta}/Q_{Sa}) \times 400 \times 0.79 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} \text{ of methanol (CH}_3\text{OH)} \\ = (1/W_T) \times (Q_{Tb}/Q_{Sb}) \times 100 \times 0.79 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} \text{ of mesityl oxide (CH}_3\text{COCH=C(CH}_3)_2) \\ = (1/W_T) \times (Q_{Tc}/Q_{Sc}) \times 100 \times 0.86 \end{aligned}$$

W_T : Amount (mg) of sample

0.79: Density (g/mL) of acetone and methanol

0.86: Density (g/mL) of mesityl oxide

Internal standard solution—To 0.5 mL of 1-propanol add water to make 1000 mL.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 2.1 m in length, packed with teflon for gas chromatography (250 – 420 μ m) coated with polyethylene glycol 20 M for gas chromatography at the ratio of 10%.

Column temperature: A constant temperature of about 70°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 5 minutes.

Time span of measurement: About 3 times as long as the retention time of the internal standard.

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, acetone, methanol, 1-propanol and mesityl oxide are eluted in this order, and these peaks completely separate each other.

System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviations of the ratio of the peak area of acetone, methanol and mesityl oxide to that of the internal standard are not more than 4.0%, respectively.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.3 g of Cilastatin Sodium, dissolve in 30 mL of methanol, add 5 mL of water, and adjust to pH 3.0 with 0.1 mol/L hydrochloric acid TS. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS from the first equivalence point to the third equivalence point (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS

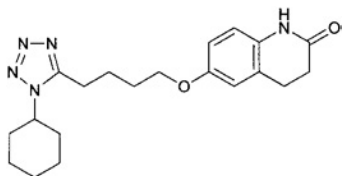
= 19.02 mg of $C_{16}H_{25}N_2NaO_5S$

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Cilostazol

シロスタゾール



$C_{20}H_{27}N_5O_2$: 369.46

6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)butyloxy]-
3,4-dihydroquinolin-2(1H)-one
[73963-72-1]

Cilostazol, when dried, contains not less than 98.5% and not more than 101.5% of $C_{20}H_{27}N_5O_2$.

Description Cilostazol occurs as white to pale yellowish white, crystals or crystalline powder.

It is slightly soluble in methanol, in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Cilostazol in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cilostazol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cilostazol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cilostazol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 158 – 162°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Cilostazol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Cilostazol in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cilostazol obtained with the sample solution is not larger than 0.7 times the peak area of cilostazol with the standard solution, and the total area of the peaks other than the peak of cilostazol with the sample solution is not larger than 1.2 times

the peak area of cilostazol with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, ethyl acetate and methanol (10:9:1).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 7 minutes.

Time span of measurement: About 3 times as long as the retention time of cilostazol beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of cilostazol obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the standard solution.

System performance: Pipet 1 mL of the sample solution, add 1 mL of a solution prepared by dissolving 5 mg of 3,4-dihydro-6-hydroxy-2(1H)-quinolinone in 10 mL of acetonitrile and acetonitrile to make exactly 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, 3,4-dihydro-6-hydroxy-2(1H)-quinolinone and cilostazol are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cilostazol is not more than 2.0%.

Loss on drying <2.41> Not more than 0.1% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Cilostazol and Cilostazol Reference Standard, previously dried, dissolve each in a suitable amount of methanol, add exactly 5 mL of the internal standard solution and methanol to make 50 mL. To 1 mL each of these solutions add methanol to make 10 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cilostazol to that of the internal standard.

$$\text{Amount (mg) of } C_{20}H_{27}N_5O_2 = W_S \times (Q_T / Q_S)$$

W_S : Amount (mg) of Cilostazol Reference Standard

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and methanol (10:7:3).

Flow rate: Adjust the flow rate so that the retention time of cilostazol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, cilostazol and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Cilostazol Tablets

シロスタゾール錠

Cilostazol Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$; 369.46).

Method of preparation Prepare as directed under Tablets, with Cilostazol.

Identification Mix well an amount of powdered Cilostazol Tablets, equivalent to 50 mg of Cilostazol according to the labeled amount, with 10 mL of acetone, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 25 mg of Cilostazol Reference Standard in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 6 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetonitrile, methanol and formic acid (75:25:5:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the principal spot with the sample solution and the spot with the standard solution are orange in color and have the same R_f value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Cilostazol Tablets add 2 mL of water to disintegrate the tablet, add the internal standard solution exactly 5 mL for a 50-mg tablet and exactly 10 mL for a 100-mg tablet, and add methanol to make 50 mL. Shake for 10 minutes for the 50-mg tablet and for 20 minutes for the 100-mg tablet. To 1 mL of the solution add methanol to make 10 mL for the 50-mg tablet and 20 mL for the 100-mg tablet, filter through a membrane filter with pore size of not more than 0.5 μm , and use the filtrate as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount (mg) of cilostazol } (\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2) \\ &= W_S \times (Q_T/Q_S) \times (C/50) \end{aligned}$$

W_S : Amount (mg) of Cilostazol Reference Standard

C : Labeled amount (mg) of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$) in 1 tablet

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Cilostazol Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of a solution of sodium lauryl sulfate (3 in 1000) as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 45 minutes after starting the test for a 50-mg tablet and 60 minutes after starting the test for a 100-mg tablet, and filter through a membrane filter with a pore size not exceeding 0.45 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the solution of sodium lauryl sulfate (3 in 1000) to make exactly V' mL so that each mL contains about 5.6 μg of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Cilostazol Reference Standard, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add the solution of sodium lauryl sulfate (3 in 1000) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry using the solution of sodium lauryl sulfate (3 in 1000) as the control: the dissolution rates of a 50-mg tablet in 45 minutes and a 100-mg tablet in 60 minutes are not less than 75% and not less than 70%, respectively.

Dissolution rate (%) with respect to the labeled amount of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S : Amount (mg) of Cilostazol Reference Standard

C : Labeled amount (mg) of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Cilostazol Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of cilostazol ($\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2$), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and shake well for 10 minutes. To 1 mL of this solution add methanol to make 10 mL, filter through a membrane filter with a pore size of not more than 0.5 μm , and use the filtrate as the sample solution. Separately, weigh accurately about 50 mg of Cilostazol Reference Standard, dissolve in a suitable amount of methanol, and add exactly 5 mL of the internal standard solution and methanol to make 50 mL. To 1 mL of this solution add methanol to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of cilostazol to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of cilostazol } (\text{C}_{20}\text{H}_{27}\text{N}_5\text{O}_2) \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Cilostazol Reference Standard

Internal standard solution—A solution of benzophenone in methanol (1 in 250).

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Cilostazol.

System suitability—

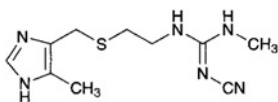
System performance: Proceed as directed in the system suitability in the Assay under Cilostazol.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of cilostazol to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Cimetidine

シメチジン



$C_{10}H_{16}N_6S$: 252.34

2-Cyano-1-methyl-3-[(5-methyl-1*H*-imidazol-4-yl)methylsulfanyl]ethylguanidine [51481-61-9]

Cimetidine, when dried, contains not less than 99.0% of $C_{10}H_{16}N_6S$.

Description Cimetidine occurs as a white crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) To 0.1 mL of a solution of Cimetidine in ethanol (95) (1 in 100) add 5 mL of citric acid-acetic anhydride TS, and heat in a water bath for 15 minutes: a red-purple color develops.

(2) Determine the infrared absorption spectrum of Cimetidine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.5 g of Cimetidine in 50 mL of freshly boiled and cooled water, shake for 5 minutes and filter: the pH of the filtrate is between 9.0 and 10.5.

Melting point <2.60> 140 – 144°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cimetidine in 10 mL of methanol: the solution is clear and colorless to pale yellow in color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cimetidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Dissolve 1.0 g of Cimetidine in 5 mL of dilute hydrochloric acid, and perform the test with this so-

lution (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Cimetidine in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 4 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (21:2:2) to a distance of about 15 cm, air-dry the plate, and then dry at 80°C for 30 minutes. Allow the plate to stand in iodine vapor for 45 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.24 g of Cimetidine, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

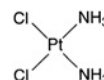
Each mL of 0.1 mol/L perchloric acid VS
= 25.23 mg of $C_{10}H_{16}N_6S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Cisplatin

シスプラチン



$Cl_2H_6N_2Pt$: 300.05

(*SP*-4-2)-Diamminedichloroplatinum [15663-27-1]

Cisplatin, when dried, contains not less than 98.0% and not more than 102.0% of $Cl_2H_6N_2Pt$.

Description Cisplatin occurs as a yellow crystalline powder.

It is sparingly soluble in *N,N*-dimethylformamide, slightly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 5 mL of a solution of Cisplatin (1 in 2000) add 2 to 3 drops of a solution of tin (II) chloride dihydrate (1 in 100): a brown precipitate is formed.

(2) Determine the absorption spectrum of a solution of Cisplatin in a solution of sodium chloride in 0.01 mol/L hydrochloric acid TS (9 in 1000) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cisplatin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Cisplatin as directed in the potassium bromide disk method

under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cisplatin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cisplatin (1 in 2000) responds to the Qualitative Test <1.09> (1) for chloride.

Purity Ammonium aminotrichloroplatinate—Conduct this procedure using light-resistant vessels. Dissolve 50 mg of Cisplatin in a solution of sodium chloride (9 in 1000) to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of ammonium aminotrichloroplatinate for liquid chromatography, previously dried at 80°C for 3 hours, in the solution of sodium chloride (9 in 1000) to make exactly 200 mL. Pipet 2 mL of this solution, add the solution of sodium chloride (9 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 40 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of ammonium aminotrichloroplatinate by the automatic integration method: the peak area from the sample solution is not more than that from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 209 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography having quaternary ammonium groups (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A solution of ammonium sulfate (1 in 800).

Flow rate: Adjust the flow rate so that the retention time of ammonium aminotrichloroplatinate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 40 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ammonium aminotrichloroplatinate are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ammonium aminotrichloroplatinate is not more than 3.0%.

Loss on drying <2.41> Not more than 0.1% (1 g, 105°C, 4 hours).

Assay Conduct this procedure using light-resistant vessels. Weigh accurately about 25 mg each of Cisplatin and Cisplatin Reference Standard, previously dried, dissolve in *N,N*-dimethylformamide to make exactly 25 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 40 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of cisplatin by the automatic integration method.

$$\text{Amount (mg) of Cl}_2\text{H}_6\text{N}_2\text{Pt} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Cisplatin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 310 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with aminopropyl-silanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ethyl acetate, methanol, water and *N,N*-dimethylformamide (25:16:5:5).

Flow rate: Adjust the flow rate so that the retention time of cisplatin is about 4 minutes.

System suitability—

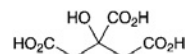
System performance: When the procedure is run with 40 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cisplatin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 40 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cisplatin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Anhydrous Citric Acid

無水クエン酸



$C_6H_8O_7$: 192.12

2-Hydroxypropane-1,2,3-tricarboxylic acid [77-92-9]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (♦ ◆).

Anhydrous Citric Acid contains not less than 99.5% and not more than 100.5% of $C_6H_8O_7$, calculated on the anhydrous basis.

♦**Description** Anhydrous Citric Acid occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).◆

♦**Identification** Determine the infrared absorption spectrum of Anhydrous Citric Acid, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 10 mL: the solution is clear and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution add water to make

1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 7.2 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.15 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 1.0 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

(2) Sulfates <1.14>—Dissolve 2.0 g of Anhydrous Citric Acid in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Anhydrous Citric Acid in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium hydrochloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time.

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

♦(4) Heavy metals <1.07>—Proceed with 2.0 g of Anhydrous Citric Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).♦

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Anhydrous Citric Acid, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water <2.48> Not more than 1.0% (2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

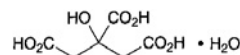
Assay Weigh accurately about 0.55 g of Anhydrous Citric Acid, dissolve in 50 mL of water, and titrate with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of C₆H₈O₇

♦**Containers and storage** Containers—Tight containers.♦

Citric Acid Hydrate

クエン酸水和物



C₆H₈O₇·H₂O: 210.14

2-Hydroxypropane-1,2,3-tricarboxylic acid monohydrate
[5949-29-1]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (♦ ♦).

Citric Acid Hydrate contains not less than 99.5% and not more than 100.5% of anhydrous citric acid (C₆H₈O₇: 192.12), calculated on the anhydrous basis.

♦**Description** Citric Acid Hydrate occurs as colorless crystals, white granules or crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It is efflorescent in dry air.♦

♦**Identification** Determine the infrared absorption spectrum of Citric Acid Hydrate, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.♦

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Citric Acid Hydrate in water to make 10 mL: the solution is clear and has no more color than the following control solutions (1), (2) or (3).

Control solution (1): To 1.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution add water to make 1000 mL.

Control solution (2): To 0.15 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 7.2 mL of Iron (III) Chloride Colorimetric Stock Solution and 0.15 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

Control solution (3): To 2.5 mL of Cobalt (II) Chloride Colorimetric Stock Solution, 6.0 mL of Iron (III) Chloride Colorimetric Stock Solution and 1.0 mL of Copper (II) Sulfate Colorimetric Stock Solution add water to make 1000 mL.

(2) Sulfates <1.14>—Dissolve 2.0 g of Citric Acid Hydrate in water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minute. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution,

add water to make exactly 100 mL, and proceed in the same manner as above using this solution instead of the sample solution.

(3) Oxalic acid—Dissolve 0.80 g of Citric Acid Hydrate in 4 mL of water, add 3 mL of hydrochloric acid and 1 g of zinc, and boil for 1 minute. After allowing to stand for 2 minutes, take the supernatant liquid, add 0.25 mL of a solution of phenylhydrazinium hydrochloride (1 in 100), heat to boil, and then cool quickly. To this solution add the equal volume of hydrochloric acid and 0.25 mL of a solution of potassium hexacyanoferrate (III) (1 in 20), mix, and allow to stand for 30 minutes: the solution has no more color than the following control solution prepared at the same time.

Control solution: To 4 mL of a solution of oxalic acid dihydrate (1 in 10,000) add 3 mL of hydrochloric acid and 1 g of zinc, and proceed in the same manner as the test solution.

♦(4) Heavy metals <1.07>—Proceed with 2.0 g of Citric Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).♦

(5) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Citric Acid Hydrate, provided that the solution is heated at 90°C for 1 hour and then cool quickly: the solution has no more color than Matching Fluid K.

Water <2.48> Not less than 7.5% and not more than 9.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

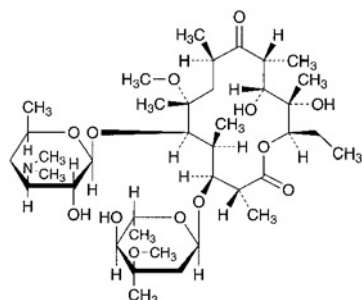
Assay Weigh accurately about 0.55 g of Citric Acid Hydrate, dissolve in 50 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

Each mL of 1 mol/L sodium hydroxide VS
= 64.04 mg of C₃₈H₆₉O₇

♦**Containers and storage** Containers—Tight containers.♦

Clarithromycin

クラリスロマイシン



C₃₈H₆₉NO₁₃: 747.95
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyloxy)-11,12-dihydroxy-6-methoxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide [81103-11-9]

Clarithromycin is a derivative of erythromycin.

It contains not less than 950 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clarithromycin is expressed as mass (potency) of clarithromycin (C₃₈H₆₉NO₁₃).

Description Clarithromycin occurs as a white crystalline powder and has a bitter taste.

It is soluble in acetone and in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) To 5 mg of Clarithromycin add 2 mL of sulfuric acid, and shake gently: a red-brown color develops.

(2) Dissolve 3 mg of Clarithromycin in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and changes immediately to red to deep purple.

(3) Determine the infrared absorption spectra of Clarithromycin and Clarithromycin Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 10 mg each of Clarithromycin and Clarithromycin Reference Standard in 4 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of chloroform, methanol and ammonia water (28) (100:5:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, and heat at 105°C for 10 minutes: the principal spot from the sample solution and the spot from the standard solution show a dark purple color and have the same R_f value.

Optical rotation <2.49> [α]_D²⁰: -87 - -97° (0.25 g calculated on the anhydrous basis, chloroform, 25 mL, 100 mm).

Melting point <2.60> 220 - 227°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Clarithromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic—Prepare the test solution with 1.0 g of Clarithromycin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Weigh accurately about 0.1 g of Clarithromycin, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Clarithromycin Reference Standard, dissolve in the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the each peak area by the automatic integration method: the amount of each related substance calculated on the anhydrous basis is not more than 2.0%, and the total of them is not more than 5.0%. Exclude any peak with an area of less than 0.05%.

Amount (%) of each related substance calculated on the anhydrous basis

$$= (W_S/W_T) \times (A_T/A_S) \times 100$$

Total amount (%) of the related substances calculated on the anhydrous basis

$$= (W_S/W_T) \times (\Sigma A_T/A_S) \times 100$$

W_S : Amount (mg) of Clarithromycin Reference Standard

W_T : Amount (mg) of the sample, calculated on the anhydrous basis

A_S : Peak area of clarithromycin obtained with the standard solution

A_T : Peak area of each related substance obtained with the sample solution

ΣA_T : Total area of the peaks other than clarithromycin obtained with the sample solution

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of the main peak after 2 minutes of sample injection.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL, and use this solution as the solution for system suitability test. Confirm that when the procedure is run with 10 μ L of the solution for system suitability test, the peak area of clarithromycin is equivalent to 14–26% of that obtained from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 3.0%.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately an amount of Clarithromycin and Clarithromycin Reference Standard, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 20 mL. Pipet 2 mL each of these solutions, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of clarithromycin to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } C_{38}H_{69}NO_{13} \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Clarithromycin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter

and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogenphosphate TS (1 in 3) and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of clarithromycin is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Clarithromycin Tablets

クラリスロマイシン錠

Clarithromycin Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of clarithromycin ($C_{38}H_{69}NO_{13}$: 747.95).

Method of preparation Prepare as directed under Tablets, with Clarithromycin.

Identification Shake a quantity of pulverized Clarithromycin Tablets, equivalent to 60 mg (potency) of Clarithromycin according to the labeled amount, with 40 mL of acetone for 10 minutes, and centrifuge at 4000 rpm for 5 minutes. Evaporate 30 mL of the supernatant liquid, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2980 cm^{-1} , 2940 cm^{-1} , 1734 cm^{-1} , 1693 cm^{-1} , 1459 cm^{-1} , 1379 cm^{-1} and 1171 cm^{-1} .

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Clarithromycin Tablets add exactly $V/20$ mL of the internal standard solution (1), then add the mobile phase so that each mL contains about 5 mg (potency) of clarithromycin ($C_{38}H_{69}NO_{13}$) to make V mL, and disperse to fine particles with the aid of ultrasonic waves for 20 minutes while occasional vigorous shaking. Centrifuge this solution at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size of not more than 0.45 μ m. Then, proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount [mg (potency)] of clarithromycin } (C_{38}H_{69}NO_{13}) \\ &= W_S \times (Q_T/Q_S) \times (V/10) \end{aligned}$$

W_S : Amount [mg (potency)] of Clarithromycin Reference Standard

Internal standard solution (1)—A solution of butyl para-

hydroxybenzoate in the mobile phase (1 in 1000).

Internal standard solution (2)—To exactly 1 mL of the internal standard solution (1) add the mobile phase to make exactly 20 mL.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Clarithromycin Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.45 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the mobile phase to make exactly V' mL so that each mL contains about 28 μg (potency) of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg (potency) of Clarithromycin Reference Standard, and dissolve in acetonitrile for liquid chromatography to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of clarithromycin. The dissolution rates in 30 minutes of a 50-mg tablet and a 200-mg tablet are not less than 80% and not less than 75%, respectively.

Dissolution rate (%) with respect to the labeled amount of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 90$$

W_S : Amount [mg (potency)] of Clarithromycin Reference Standard

C : Labeled amount [mg (potency)] of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 100 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clarithromycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 100 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clarithromycin is not more than 2.0%.

Assay To not less than 5 Clarithromycin Tablets add diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) so that each mL contains about 8 mg (potency) of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$), disperse to fine particles with the aid of ultrasonic waves, add exactly 1 mL of the internal standard solution (1) per 100 mg (potency) of clarithromycin according to the labeled amount, then add acetonitrile for liquid chromatography so that each mL contains about 5 mg (potency) of clarithromycin ($\text{C}_{38}\text{H}_{69}\text{NO}_{13}$), and disperse to fine particles with the aid of ultrasonic waves for 10 minutes while occasional vigorous shaking. Centrifuge of this solu-

tion at 4000 rpm for 15 minutes, and filter the supernatant liquid through a membrane filter with a pore size of not more than 0.45 μm . Discard the first 3 mL of the filtrate, to 2 mL of the subsequent filtrate add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg (potency) of Clarithromycin Reference Standard, and dissolve in the mobile phase to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution (2) and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of clarithromycin to that of the internal standard.

$$\begin{aligned} &\text{Amount [mg (potency)] of clarithromycin } (\text{C}_{38}\text{H}_{69}\text{NO}_{13}) \\ &= W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount [mg (potency)] of Clarithromycin Reference Standard

Internal standard solution (1)—A solution of butyl parahydroxybenzoate in the mobile phase (1 in 1000).

Internal standard solution (2)—To exactly 1 mL of the internal standard solution (1) add the mobile phase to make exactly 20 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted 0.2 mol/L potassium dihydrogen phosphate TS (1 in 3) and acetonitrile for liquid chromatography (13:7).

Flow rate: Adjust the flow rate so that the retention time of clarithromycin is about 8 minutes.

System suitability—

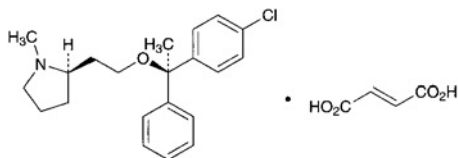
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, clarithromycin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of clarithromycin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Well-closed containers.

Clemastine Fumarate

クレマスチンフマル酸塩



$C_{21}H_{26}ClNO \cdot C_4H_4O_4$: 459.96

(2*R*)-2-{2-[(1*R*)-1-(4-Chlorophenyl)-1-phenylethoxy]ethyl}-1-methylpyrrolidine monofumarate
[14976-57-9]

Clemastine Fumarate, when dried, contains not less than 98.5% of $C_{21}H_{26}ClNO \cdot C_4H_4O_4$.

Description Clemastine Fumarate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 5 mg of Clemastine Fumarate add 5 mL of sulfuric acid, and shake to dissolve: a yellow color develops. Slowly drop this solution into 10 mL of water: the yellow color immediately disappears.

(2) To 0.01 g of Clemastine Fumarate add 1 mL of fuming nitric acid, and evaporate on a water bath to dryness. Then add 2 mL of diluted hydrochloric acid (1 in 2) and 0.2 g of zinc powder, heat for 10 minutes on a water bath, cool, and filter. Add 20 mL of water to the filtrate. The solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) To 5 mL of a solution of Clemastine Fumarate (1 in 50,000), add 5 mL of 4-dimethylaminobenzaldehyde TS, and warm for 10 minutes: a red-purple color develops.

(4) Perform the test with Clemastine Fumarate as directed under Flame Coloration Test <1.04> (2): a green color appears.

(5) Dissolve 0.04 g of Clemastine Fumarate and 0.01 g of fumaric acid for thin-layer chromatography in 2 mL each of a mixture of ethanol (95) and water (4:1) by gentle warming, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl ether, formic acid and water (90:7:3) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot with larger *R_f* value from the sample solution has the same *R_f* value as the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: +16 – +18° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Melting point <2.60> 176 – 180°C (with decomposition).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Clemastine Fumarate in 10 mL of methanol by warming: the

solution is clear and colorless.

(2) Heavy metals <1.07>—Perform the test with 1.0 g of Clemastine Fumarate according to Method 2. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Take 1.0 g of Clemastine Fumarate, prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(4) Related Substances—Dissolve 0.10 g of Clemastine Fumarate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 250 mL, and use this solution as the standard solution (1). Pipet 5 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (90:10:1) to a distance of about 10 cm, and air-dry the plate. After spraying evenly Dragendorff's TS on the plate, immediately spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and not more than 2 spots from the sample solution are more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

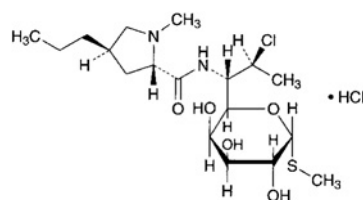
Assay Weigh accurately about 0.4 g of Clemastine Fumarate, previously dried, dissolved in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.00 mg of $C_{21}H_{26}ClNO \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.

Clindamycin Hydrochloride

クリンダマイシン塩酸塩



$C_{18}H_{33}ClN_2O_5S \cdot HCl$: 461.44

Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-threo- α -D-galacto-octopyranoside monohydrochloride [21462-39-5]

Clindamycin Hydrochloride is the hydrochloride of a derivative of lincomycin.

It contains not less than 759 μ g (potency) and not more than 902 μ g (potency) per mg. The potency of

Clindamycin Hydrochloride is expressed as mass (potency) of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Description Clindamycin Hydrochloride occurs as white to grayish white, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (95).

Identification Dissolve 0.1 g of Clindamycin Hydrochloride in 5 mL of water, add 2 mL of sodium hydroxide TS, and mix: a white turbidity is produced. To this solution add 0.3 mL of sodium pentacyanonitrosylferrate (III) TS, mix, allow to stand at 60 to 65°C for 10 minutes, and add 2 mL of dilute hydrochloric acid: a blue-green color develops.

Optical rotation <2.49> $[\alpha]_D^{25}$: +135 – +150° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Water <2.48> Not more than 6.0% (0.3 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Clindamycin Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 250 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 15°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 2 µg (potency) and 1 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Clindamycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 7.0 to make exactly 250 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 2 µg (potency) and 1 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Clindamycin Hydrochloride Capsules

クリンダマイシン塩酸塩カプセル

Clindamycin Hydrochloride Capsules contain not less than 90.0% and not more than 110.0% of the labeled amount of clindamycin ($C_{18}H_{33}ClN_2O_5S$: 424.98).

Method of preparation Prepare as directed under Capsules, with Clindamycin Hydrochloride.

Identification To an amount of the contents of Clindamycin Hydrochloride Capsules, equivalent to 10 mg (potency) of Clindamycin Hydrochloride, add 2 mL of methanol, shake well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of Clindamycin Hydrochloride Reference Standard in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, toluene and ammonia solution (28) (140:60:3) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of 500 mL of a solution of L-tartaric acid (1 in 5) and 50 mL of bismuth subnitrate TS on the plate: the R_f values of the principal spot with the sample solution and the spot with the standard solution are not different each other.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Clindamycin Hydrochloride Capsules add a suitable amount of the mobile phase, shake for 30 minutes, and add the mobile phase to make exactly V mL so that each mL contains 0.75 mg (potency) of Clindamycin Hydrochloride according to the labeled amount. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 75 mg (potency) of Clindamycin Hydrochloride Reference Standard, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of clindamycin.

$$\begin{aligned} &\text{Amount [mg (potency)] of clindamycin } (C_{18}H_{33}ClN_2O_5S) \\ &= W_S \times (A_T/A_S) \times (V/100) \end{aligned}$$

W_S : Amount [mg (potency)] of Clindamycin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.5 with 8 mol/L potassium hydroxide TS. To 550 mL of this solution add 450 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with

20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 1.0%.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Clindamycin Hydrochloride Capsules at 50 revolutions per minute according to the Paddle method, using the sinker, using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 15 minutes after starting the test for a 75-mg capsule or 30 minutes after starting the test for a 150-mg capsule, and filter through a membrane filter with a pore size not exceeding 0.45 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' so that each mL contains about 83 μg (potency) of clindamycin hydrochloride, and use this solution as the sample solution. Separately, weigh accurately about 17 mg (potency) of Clindamycin Hydrochloride Reference Standard, dissolve in water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, and determine the peak areas, A_T and A_S , of clindamycin. The dissolution rate of a 75-mg capsule in 15 minutes and that of a 150-mg capsule in 30 minutes are not less than 80%, respectively.

Dissolution rate (%) with respect to the labeled amount of clindamycin hydrochloride

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 450$$

W_S : Amount [mg (potency)] of Clindamycin Hydrochloride Reference Standard

C : Labeled amount [mg (potency)] of clindamycin ($\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.5 with 8 mol/L potassium hydroxide TS. To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of clindamycin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clindamycin is not more than 2.0%.

Assay Perform the test according to the Cylinder–plate method as directed under Microbial Assay for Antibiotics

<4.02> according to the following conditions.

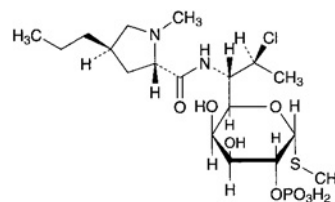
(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Clindamycin Hydrochloride.

(ii) Sample solutions—Weigh accurately the mass of not less than 20 Clindamycin Hydrochloride Capsules, cut the capsules and take out the contents, mix well, and powder, if necessary. If necessary, wash the empty capsules with a small amount of diethyl ether, allow to stand at room temperature to dry the capsules, weigh their mass accurately, and calculate the mass of the contents. Weigh accurately an amount of the contents, equivalent to about 25 mg (potency) according to the labeled amount, add a suitable amount of 0.1 mol/L phosphate buffer solution, pH 7.0, shake vigorously, then add 0.1 mol/L phosphate buffer solution to make a solution so that each mL contains about 100 μg (potency), and filter, if necessary. To exactly an amount of this solution add 0.1 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 2 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Clindamycin Phosphate

クリンダマイシンリン酸エステル



$\text{C}_{18}\text{H}_{34}\text{ClN}_2\text{O}_8\text{PS}$: 504.96

Methyl 7-chloro-6,7,8-trideoxy-6-[(2*S*,4*R*)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-L-threo- α -D-galacto-octopyranoside 2-dihydrogenphosphate [24729-96-2]

Clindamycin Phosphate is a derivative of clindamycin.

It contains not less than 800 μg (potency) and not more than 846 μg (potency) per mg, calculated on the anhydrous basis. The potency of Clindamycin Phosphate is expressed as mass (potency) of clindamycin ($\text{C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S}$: 424.98).

Description Clindamycin Phosphate occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Clindamycin Phosphate, previously dried at 100°C for 2 hours, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clindamycin Phosphate Reference Standard previously dried at 100°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +115 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Clindamycin Phosphate in 10 mL of water. The pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Clindamycin Phosphate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clindamycin Phosphate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clindamycin Phosphate according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.1 g of Clindamycin Phosphate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of clindamycin, having the relative retention time of about 1.8 with respect to clindamycin phosphate, obtained from the sample solution is not more than 1/2 of the peak area of clindamycin phosphate from the standard solution, and the total area of the peaks other than clindamycin phosphate from the sample solution is not more than 4 times the peak area of clindamycin phosphate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of clindamycin phosphate beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of clindamycin phosphate obtained from 20 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Clindamycin Phosphate and Clindamycin Phosphate Reference Standard, equivalent to about 20 mg (potency), add exactly 25 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of clindamycin phosphate to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of clindamycin (C}_{18}\text{H}_{33}\text{ClN}_2\text{O}_5\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Clindamycin Phosphate

Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in the mobile phase (3 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10.54 g of potassium dihydrogen phosphate in 775 mL of water, adjust the pH to 2.5 with phosphoric acid, and add 225 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of clindamycin phosphate is about 8 minutes.

System suitability—

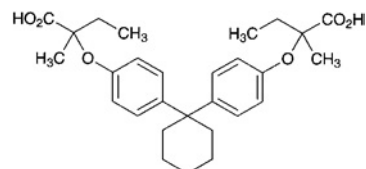
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, clindamycin phosphate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clindamycin phosphate to that of the internal standard is not more than 2.5%.

Containers and storage Containers—Tight containers.

Clinofibrate

クリノフィブラート



$\text{C}_{28}\text{H}_{36}\text{O}_6$: 468.58

2,2'-(4,4'-Cyclohexylidenediphenoxy)-2,2'-dimethyldibutanoic acid [30299-08-2]

Clinofibrate, when dried, contains not less than 98.5% of $\text{C}_{28}\text{H}_{36}\text{O}_6$.

Description Clinofibrate occurs as a white to yellowish white powder.

It is odorless and has no taste.

It is freely soluble in methanol, in ethanol (99.5), in acetone and in diethyl ether, and practically insoluble in water.

A solution of Clinofibrate in methanol (1 in 20) shows no optical rotation.

Melting point: about 146°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Clinofibrate in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clinofibrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Clinofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clinofibrate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Clinofibrate in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, cyclohexane and acetic acid (100) (12:5:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Isomer ratio To 50 mg of Clinofibrate add 0.4 mL of thionyl chloride, stopper tightly, heat on a water bath of 60°C for 5 minutes with occasional shaking, and evaporate the excess thionyl chloride at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 2 mL of toluene previously dried with synthetic zeolite for drying, add 2 mL of a solution of D-(+)- α -methylbenzylamine in toluene previously dried with synthetic zeolite for drying (3 in 100), mix gently, allow to stand for 10 minutes, and evaporate the toluene at a temperature not exceeding 60°C under reduced pressure. Dissolve the residue in 5 mL of chloroform, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area, A_a , A_b and A_c , of three peaks appear in order near the retention time of 40 minutes: a value, $\{A_b/(A_a + A_b + A_c)\} \times 100$, is between 40 and 70.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of hexane and 2-propanol (500:3).

Flow rate: Adjust the flow rate so that the retention time of the peak appearing first is about 35 minutes.

Selection of column: Proceed with 5 μ L of the sample solu-

tion under the above operating conditions. Use a column giving a complete separation of the three peaks.

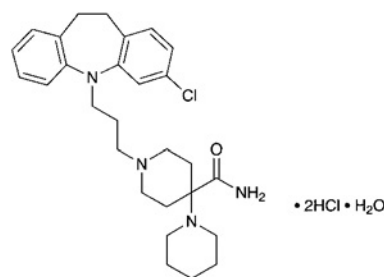
Assay Weigh accurately about 0.45 g of Clinofibrate, previously dried, dissolve in 40 mL of ethanol (99.5), add 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 23.43 mg of $C_{28}H_{36}O_6$

Containers and storage Containers—Tight containers.

Clozapramine Hydrochloride Hydrate

クロカプラミン塩酸塩水和物



$C_{28}H_{37}ClN_4O \cdot 2HCl \cdot H_2O$: 572.01

1'-[3-(3-Chloro-10,11-dihydro-5H-dibenz[b,f]azepin-5-yl)propyl]-1,4'-bipiperidine-4'-carboxamide dihydrochloride monohydrate [60789-62-0]

Clozapramine Hydrochloride Hydrate, when dried, contains not less than 98.0% of clozapramine hydrochloride ($C_{28}H_{37}ClN_4O \cdot 2HCl$: 553.99).

Description Clozapramine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), in chloroform and in isopropylamine, and practically insoluble in acetic anhydride and in diethyl ether.

It is gradually colored by light.

Melting point: about 260°C (with decomposition, after drying).

Identification (1) To 5 mL of a solution of Clozapramine Hydrochloride Hydrate (1 in 2500) add 1 mL of nitric acid: a blue color develops at first, and rapidly changes to deep blue, and then changes to green to yellow-green.

(2) Determine the absorption spectrum of a solution of Clozapramine Hydrochloride Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>., and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clozapramine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the

Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Dissolve 0.1 g of Clozapramine Hydrochloride Hydrate in 10 mL of water by warming, and after cooling, add 2 mL of ammonia TS, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Sulfate <1.14>—Dissolve 0.5 g of Clozapramine Hydrochloride Hydrate in 40 mL of water by warming, after cooling, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clozapramine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Clozapramine Hydrochloride Hydrate in 10 mL of a mixture of chloroform and isopropylamine (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and isopropylamine (99:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethyl acetate, methanol and ammonia solution (28) (100:70:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 2.0 – 3.5% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

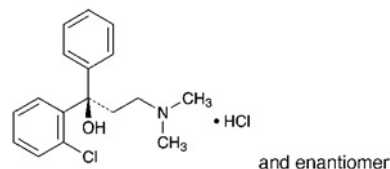
Assay Weigh accurately about 0.5 g of Clozapramine Hydrochloride Hydrate, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.70 mg of $C_{28}H_{37}ClN_4O_2 \cdot 2HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clofedanol Hydrochloride

クロフェダノール塩酸塩



$C_{17}H_{20}ClNO \cdot HCl$: 326.26
(1*S*)-1-(2-Chlorophenyl)-3-dimethylamino-1-phenylpropan-1-ol monohydrochloride [511-13-7]

Clofedanol Hydrochloride, when dried, contains not less than 98.5% of $C_{17}H_{20}ClNO \cdot HCl$.

Description Clofedanol Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in water, and practically insoluble in diethyl ether.

A solution of Clofedanol Hydrochloride in methanol (1 in 20) does not show optical rotation.

Melting point: about 190°C (after drying, with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Clofedanol Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofedanol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Clofedanol Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Clofedanol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.05 g of Clofedanol Hydrochloride in 25 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 3 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than clofedanol from the sample solution is not larger than the peak area of clofedanol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column about 4 mm in inside di-

ameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.34 g of potassium methanesulfonate in diluted phosphoric acid (1 in 1000) to make 1000 mL, and to 650 mL of this solution add 350 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of clofedanol is about 9 minutes.

Selection of column: Dissolve 0.01 g each of Clofedanol Hydrochloride and ethyl parahydroxybenzoate in methanol to make 100 mL. Proceed with 3 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofedanol and ethyl parahydroxybenzoate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of clofedanol obtained from 3 μL of the standard solution composes between 20% and 50% of the full scale.

Time span of measurement: About three times as long as the retention time of clofedanol beginning after the solvent peak.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, silica gel, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

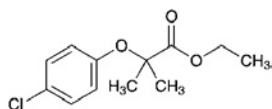
Assay Weigh accurately about 0.5 g of Clofedanol Hydrochloride, previously dried, dissolve in 15 mL of acetic acid (100), add 35 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.63 mg of $\text{C}_{17}\text{H}_{20}\text{ClNO}_2\text{HCl}$

Containers and storage Containers—Tight containers.

Clofibrate

クロフィブラート



$\text{C}_{12}\text{H}_{15}\text{ClO}_3$: 242.70

Ethyl 2-(4-chlorophenoxy)-2-methylpropanoate
[637-07-0]

Clofibrate, calculated on the anhydrous basis, contains not less than 98.0% of $\text{C}_{12}\text{H}_{15}\text{ClO}_3$.

Description Clofibrate occurs as a colorless or light yellow, clear, oily liquid. It has a characteristic odor and taste, which is bitter at first, and subsequently sweet.

It is miscible with methanol, with ethanol (95), with ethanol (99.5), with diethyl ether and with hexane, and practically insoluble in water.

It is gradually decomposed by light.

Identification (1) Determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Clofibrate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Clofibrate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Clofibrate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clofibrate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Clofibrate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.500 – 1.505

Specific gravity <2.56> d_{20}^{20} : 1.137 – 1.144

Purity (1) Acidity—Dissolve 2.0 g of Clofibrate in 100 mL of neutralized ethanol, and add 1 drop of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clofibrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—To 5.0 g of Clofibrate add 20 mL of nitric acid and 5 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, if necessary, add further 5 mL of nitric acid, heat until white fumes are evolved, and repeat this procedure until the solution is colorless to light yellow. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat again until white fumes are evolved. Cool, add water to make 25 mL, use 5 mL of this solution as the test solution, and perform the test.

Color standard: Prepare a solution according to the above procedure without using Clofibrate as the blank. Transfer 5 mL of the solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and then proceed as directed in the test solution (not more than 20 ppm).

(4) *p*-Chlorophenol—To 1.0 g of Clofibrate add exactly 1 mL of the internal standard solution, then add the mobile phase to make 5 mL, and use this solution as the sample solution. Separately, dissolve 0.010 g of 4-chlorophenol in a mixture of hexane and 2-propanol (9:1) to make exactly 100 mL. Pipet 10 mL of this solution, and add a mixture of hexane and 2-propanol (9:1) to make exactly 50 mL. Pipet 6 mL of this solution, add exactly 4 mL of the internal standard solution, then add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of 4-chlorophenol to that of the internal standard: Q_T is not greater than Q_S .

Internal standard solution—A solution of 4-ethoxyphenol in

the mobile phase (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with cyanopropyl-silanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, 2-propanol and acetic acid (100) (1970:30:1).

Flow rate: Adjust the flow rate so that the retention time of clofibrate is about 2 minutes.

Selection of column: Dissolve 10.0 g of clofibrate, 6 mg of 4-chlorophenol and 6 mg of 4-ethoxyphenol in 1000 mL of hexane. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of clofibrate, 4-chlorophenol and 4-ethoxyphenol in this order, with the resolution between the peaks of clofibrate and 4-chlorophenol is not less than 5, and with the resolution between the peaks of 4-chlorophenol and 4-ethoxyphenol is not less than 2.0.

Water <2.48> Not more than 0.2% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clofibrate, add exactly 50 mL of 0.1 mol/L potassium hydroxide-ethanol VS, and heat in a water bath under a reflux condenser with a carbon dioxide absorbing tube (soda-lime) for 2 hours with frequent shaking. Cool, and titrate <2.50> immediately the excess potassium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 24.27 mg of C₁₂H₁₅ClO₃

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clofibrate Capsules

クロフィブラートカプセル

Clofibrate Capsules contain not less than 93% and not more than 107% of the labeled amount of clofibrate (C₁₂H₁₅ClO₃: 242.70).

Method of preparation Prepare as directed under Capsules, with Clofibrate.

Identification Cut and open Clofibrate Capsules, and use the contents as the sample. Determine the absorption spectrum of a solution of the sample in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm, and it exhibits a maximum between 224 nm and 228 nm after diluting this solution 10 times with ethanol (99.5)

Purity *p*-Chlorophenol—Cut and open not less than 20 Clofibrate Capsules, and proceed with 1.0 g of the well-mixed contents as directed in the Purity (4) under Clofibrate.

Assay Weigh accurately not less than 20 Clofibrate Capsules, cut and open the capsules, rinse the inside of the capsules with a small amount of diethyl ether after taking out the contents, evaporate the diethyl ether by allowing the capsules to stand at room temperature, and weigh the capsules accurately. Weigh accurately an amount of the contents, equivalent to about 0.1 g of clofibrate (C₁₂H₁₅ClO₃), dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Clofibrate Reference Standard, proceed in the same manner as directed for the sample solution, and use the solution so obtained as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of clofibrate to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of clofibrate (C}_{12}\text{H}_{15}\text{ClO}_3) \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Clofibrate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of ibuprofen in the mobile phase (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:2).

Flow rate: Adjust the flow rate so that the retention time of clofibrate is about 10 minutes.

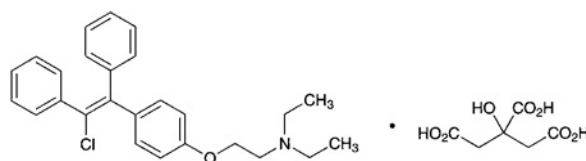
Selection of column: Dissolve 0.05 g of clofibrate and 0.3 g of ibuprofen in 50 mL of acetonitrile. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ibuprofen and clofibrate in this order with the resolution between these peaks being not less than 6.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clomifene Citrate

クロミフェンクエン酸塩



C₂₆H₂₈ClNO·C₆H₈O₇: 598.08

2-[4-(2-Chloro-1,2-diphenylvinyl)phenoxy]-*N,N*-

diethylethylamine monocitrate [50-41-9]

Clomifene Citrate, when dried, contains not less than 98.0% of $C_{26}H_{28}ClNO \cdot C_6H_8O_7$.

Description Clomifene Citrate occurs as a white to pale yellowish white powder. It is odorless.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes in color by light.

Melting point: about 115°C

Identification (1) To 2 mL of a solution of Clomifene Citrate in methanol (1 in 200) add 2 mL of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clomifene Citrate in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Clomifene Citrate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Clomifene Citrate in methanol (1 in 200) responds to the Qualitative Tests <1.09> (1) and (2) for citrate salt.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Clomifene Citrate in 30 mL of methanol is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clomifene Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio To 0.10 g of Clomifene Citrate add 10 mL of water and 1 mL of sodium hydroxide TS, and extract with three 15-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 20 mL of water, add 10 g of anhydrous sodium sulfate to the combined diethyl ether extracts, shake for 1 minute, filter, and evaporate the diethyl ether of the filtrate. Dissolve the residue in 10 mL of chloroform, and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b , having retention times of about 20 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: $A_b/(A_a + A_b)$ is between 0.3 and 0.5.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, having methylsilicone polymer coated at the ratio of 1% on siliceous earth for gas chromatography (125 to 150 μ m in particle diameter).

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the first peak of clomifene citrate is about 20 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the sample solution under the above operating conditions, the resolution between the two peaks is not less than 1.3.

System repeatability: When the test is repeated 5 times with 2 μ L of the sample solution under the above operating conditions, the relative standard deviation of $A_b/(A_a + A_b)$ is not more than 5.0%.

Assay Weigh accurately about 1 g of Clomifene Citrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.81 mg of $C_{26}H_{28}ClNO \cdot C_6H_8O_7$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clomifene Citrate Tablets

クロミフェンクエン酸塩錠

Clomifene Citrate Tablets contain not less than 93% and not more than 107% of the labeled amount of the clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$; 598.08).

Method of preparation Prepare as directed under Tablets, with Clomifene Citrate.

Identification (1) Weigh a portion of powdered Clomifene Citrate Tablets, equivalent to 1 g of Clomifene Citrate according to the labeled amount, shake vigorously with 100 mL of chloroform, and filter. Concentrate the filtrate on a water bath, allow to stand at room temperature, collect the crystals formed by filtration, and wash with a small quantity of chloroform. Proceed with the crystals as directed in the Identification (1) and (3) under Clomifene Citrate.

(2) Determine the absorption spectrum of a solution of the crystals obtained in (1) in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 233 nm and 237 nm, and between 290 nm and 294 nm.

Assay Weigh accurately, and powder not less than 20 Clomifene Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$), add 50 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Centrifuge a portion of this solution, pipet 4 mL of the supernatant liquid, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Clomifene Citrate Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, and dilute with methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of clomifene citrate ($C_{26}H_{28}ClNO \cdot C_6H_8O_7$)

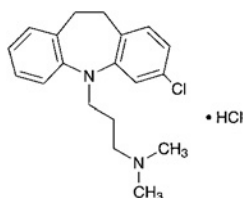
$$= W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Clomifene Citrate Reference Standard

Containers and storage Containers—Tight containers.

Clomipramine Hydrochloride

クロミプラミン塩酸塩



$C_{19}H_{23}ClN_2 \cdot HCl$: 351.31

3-(3-Chloro-10,11-dihydro-5*H*-dibenz[*b,f*]azepin-5-yl)-*N,N*-dimethylpropylamine monohydrochloride [17321-77-6]

Clomipramine Hydrochloride, when dried, contains not less than 98.5% of $C_{19}H_{23}ClN_2 \cdot HCl$.

Description Clomipramine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in chloroform, soluble in ethanol (95), sparingly soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in ethyl acetate and in diethyl ether.

Identification (1) Dissolve 3 mg of Clomipramine Hydrochloride in 1 mL of nitric acid: a deep blue color develops.

(2) Determine the absorption spectrum of a solution of Clomipramine Hydrochloride in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Take 1 g of Clomipramine Hydrochloride in a separator, dissolve in 10 mL of water, add 5 mL of sodium hydroxide TS, and extract with two 30-mL portions of diethyl ether [the water layer is used for Identification (4)]. Combine the diethyl ether extracts, add 20 mL of water, and shake. Take diethyl ether layer, dry with a small portion of anhydrous sodium sulfate, and filter. Evaporate the combined extracts by warming on a water bath, and proceed the test with the residue as directed under Flame Coloration Test <1.04> (2): a green color appears.

(4) The solution neutralized by adding dilute nitric acid to the water layer obtained in (3) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Clomipramine Hydrochloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point <2.60> 192 – 196°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Clomipramine Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clomipramine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clomipramine Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Clomipramine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Separately, weigh 20 mg of Imipramine Hydrochloride, dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution (1). Then pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and ammonia solution (28) (15:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the plate: the spot from the sample solution, corresponding to that from the standard solution (1), is not more intense than the spot from the standard solution (1). Each of the spots other than the principal spot and the above spot from the sample solution is not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clomipramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

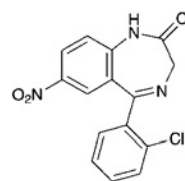
Each mL of 0.1 mol/L perchloric acid VS
= 35.13 mg of $C_{19}H_{23}ClN_2 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clonazepam

クロナゼパム



$C_{15}H_{10}ClN_3O_3$: 315.71

5-(2-Chlorophenyl)-7-nitro-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one [1622-61-3]

Clonazepam, when dried, contains not less than 99.0% of $C_{15}H_{10}ClN_3O_3$.

Description Clonazepam occurs as white to light yellow, crystals or crystalline powder.

It is sparingly soluble in acetic anhydride and in acetone, slightly soluble in methanol and in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Melting point: about 240°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Clonazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Clonazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Clonazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Chloride <1.03>—To 1.0 g of Clonazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. Discard the first 20 mL portion of the filtrate, take the subsequent 20 mL portion of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Clonazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Clonazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, then pipet 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and acetone (10:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clonazepam, previously dried, dissolve in 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

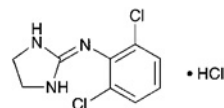
Each mL of 0.1 mol/L perchloric acid VS
= 31.57 mg of $C_{15}H_{10}ClN_3O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Clonidine Hydrochloride

クロニジン塩酸塩



$C_9H_9Cl_2N_3 \cdot HCl$: 266.55

2-(2,6-Dichlorophenylimino)imidazolidine
monohydrochloride [4205-91-8]

Clonidine Hydrochloride, when dried, contains not less than 99.0% of $C_9H_9Cl_2N_3 \cdot HCl$.

Description Clonidine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in water and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

Identification (1) To 5 mL of a solution of Clonidine Hydrochloride (1 in 1000) add 6 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the absorption spectrum of a solution of Clonidine Hydrochloride in 0.01 mol/L hydrochloric acid TS (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clonidine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Clonidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Clonidine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Clonidine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.5 g of Clonidine Hydrochloride according to Method 3, and perform the test (not more than 4 ppm).

(4) Related substances—Dissolve 0.20 g of Clonidine Hydrochloride in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL and 2 mL

of this solution, to each add methanol to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (10:8:2:1) to a distance of about 12 cm, air-dry the plate, and then dry at 100°C for 1 hour. Spray evenly sodium hypochlorite TS on the plate, air-dry the plate for 15 minutes, and then spray evenly potassium iodide starch TS on the plate: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (2), and the numbers of spots other than the principal spot and the spot of the starting point, which are more intense than the spot from the standard solution (1), are not more than 3.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

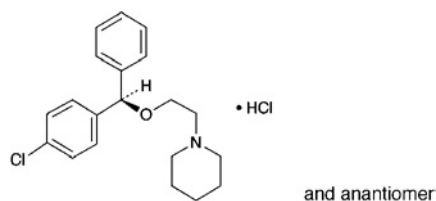
Assay Weigh accurately about 0.4 g of Cloperastine Hydrochloride, previously dried, and dissolve in 30 mL of acetic acid (100) by warming. After cooling, add 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.66 mg of $C_9H_9Cl_2N_3.HCl$

Containers and storage Containers—Tight containers.

Cloperastine Hydrochloride

クロペラスチン塩酸塩



$C_{20}H_{24}ClNO.HCl$: 366.32
1-{2-[(*RS*)-(4-Chlorophenyl)phenylmethoxy]ethyl}piperidine monohydrochloride [14984-68-0]

Cloperastine Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{24}ClNO.HCl$.

Description Cloperastine Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (95) and in acetic acid (100), and soluble in acetic anhydride.

A solution of Cloperastine Hydrochloride (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths.

Separately, determine the absorption spectrum of a solution of Cloperastine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cloperastine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Shake 10 mL of a solution of Cloperastine Hydrochloride (1 in 100) with 2 mL of ammonia TS and 20 mL of diethyl ether, separate the water layer, wash the water layer with 20 mL of diethyl ether, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 148 – 152°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cloperastine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Cloperastine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: The areas of two peaks corresponding to the relative retention times about 0.8 and 3.0 to the retention time of cloperastine obtained from the sample solution are not larger than the peak area from the standard solution, respectively, and the area of the peak corresponding to the relative retention time about 2.0 to cloperastine is not larger than 5/3 of the peak area from the standard solution, and the areas of the peaks other than cloperastine and other than the peaks mentioned above are all not larger than 3/5 of the peak area from the standard solution. The total area of these peaks is not larger than 2 times of the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, 0.1 mol/L monobasic potassium phosphate TS and perchloric acid (500:250:1).

Flow rate: Adjust the flow rate so that the retention time of cloperastine is about 7 minutes.

Selection of column: Dissolve 0.03 g of Cloperastine

Hydrochloride and 0.04 g of benzophenone in 100 mL of the mobile phase. To 2.0 mL of this solution add the mobile phase to make 50 mL. Perform the test with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cloperastine and benzophenone in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cloperastine obtained from 20 μ L of the standard solution is about 30% of the full scale.

Time span of measurement: About 4 times as long as the retention time of cloperastine, beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

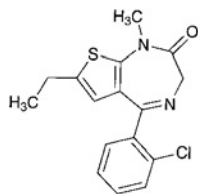
Assay Weigh accurately about 0.5 g of Cloperastine Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 36.63 \text{ mg of } C_{20}H_{24}ClNO.HCl \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Clotiazepam

クロチアゼパム



$C_{16}H_{15}ClN_2OS$: 318.82

5-(2-Chlorophenyl)-7-ethyl-1-methyl-1,3-dihydro-2H-thieno[2,3-e][1,4]-diazepin-2-one [33671-46-4]

Clotiazepam, when dried, contains not less than 98.5% of $C_{16}H_{15}ClN_2OS$.

Description Clotiazepam occurs as white to light yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetone, in acetic acid (100) and in ethyl acetate, soluble in diethyl ether, and practically insoluble in water.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Clotiazepam in 3 mL of sulfuric acid: the solution shows a light yellow fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Clotiazepam in 0.1 mol/L hydrochloric acid TS (1 in

100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Prepare the test solution with 0.01 g of Clotiazepam as directed under Oxygen Flask Combustion Method <1.06>, using 10 mL of diluted hydrogen peroxide (30) (1 in 5) as the absorbing liquid. Apply a small amount of water to the upper part of the Apparatus A, pull out C carefully, wash C, B and the inner side of A with 15 mL of methanol, and use the obtained solution as the test solution. Add 0.5 mL of dilute nitric acid to 15 mL of the test solution: this solution responds to the Qualitative Tests <1.09> (2) for chloride. The remaining test solution responds to the Qualitative Tests <1.09> (1) for sulfate.

Melting point <2.60> 106 – 109°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Clotiazepam in 10 mL of ethanol (95): the solution is clear and is not more colored than the following control solution.

Control solution: To 5 mL of Matching Fluid C add 0.01 mol/L hydrochloric acid TS to make 10 mL.

(2) Chloride <1.03>—To 1.0 g of Clotiazepam add 50 mL of water, shake for 30 minutes, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Clotiazepam according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clotiazepam, according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Clotiazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 20 mL, pipet 2 mL of this solution, add acetone to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition <2.44> not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Clotiazepam, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid (potentiometric titration). Perform a blank determination in, and make any necessary correction.

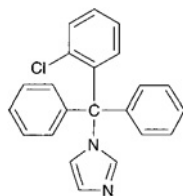
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 31.88 \text{ mg of } C_{16}H_{15}ClN_2OS \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Clotrimazole

クロトリマゾール



$C_{22}H_{17}ClN_2$: 344.84
1-[(2-Chlorophenyl)(diphenyl)methyl]-1H-imidazole
[23593-75-1]

Clotrimazole, when dried, contains not less than 98.0% of $C_{22}H_{17}ClN_2$.

Description Clotrimazole occurs as a white, crystalline powder. It is odorless and tasteless.

It is freely soluble in dichloromethane and in acetic acid (100), soluble in *N,N*-dimethylformamide, in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 0.1 g of Clotrimazole add 10 mL of 5 mol/L hydrochloric acid TS, dissolve by heating, and cool. To this solution add 3 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Clotrimazole in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Clotrimazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Clotrimazole as directed under Flame Coloration Test (2) <1.04>: a green color appears.

Melting point <2.60> 142 – 145°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Clotrimazole in 10 mL of dichloromethane: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Clotrimazole in 40 mL of *N,N*-dimethylformamide, add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.60 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of *N,N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Clotrimazole in 10 mL of methanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.05 mL of 0.005 mol/L sulfuric acid VS, 10 mL of

methanol, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Clotrimazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Clotrimazole according to Method 3, and perform the test (not more than 2 ppm).

(6) Imidazole—Dissolve 0.10 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 25 mg of imidazole for thin-layer chromatography in dichloromethane to make exactly 50 mL. Pipet 5 mL of this solution, add dichloromethane to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and chloroform (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly sodium hydrochloride TS on the plate, and air-dry the plate for 15 minutes, then spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

(7) (2-Chlorophenyl)-diphenylmethanol—Dissolve 0.20 g of Clotrimazole in exactly 10 mL of dichloromethane, and use this solution as the sample solution. Separately, dissolve 0.010 g of (2-chlorophenyl)-diphenylmethanol for thin-layer chromatography in dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Clotrimazole, previously dried, and dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

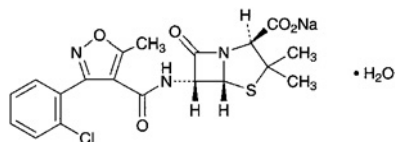
Each mL of 0.1 mol/L perchloric acid VS
= 34.48 mg of $C_{22}H_{17}ClN_2$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Cloxacillin Sodium Hydrate

クロキサシリンナトリウム水和物



$C_{19}H_{17}ClN_3NaO_5S \cdot H_2O$: 475.88

Monosodium (2*S*,5*R*,6*R*)-6-[[3-(2-chlorophenyl)-5-methylisoxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [7081-44-9]

Cloxacillin Sodium Hydrate contains not less than 900 μ g (potency) and not more than 960 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Cloxacillin Sodium Hydrate is expressed as mass (potency) of cloxacillin ($C_{19}H_{18}ClN_3O_5S$: 435.88).

Description Cloxacillin Sodium Hydrate occurs as white to light yellowish white, crystals or crystalline powder.

It is freely soluble in water, in *N,N*-dimethylformamide and in methanol, and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Cloxacillin Sodium Hydrate in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cloxacillin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelength.

(2) Determine the infrared absorption spectrum of Cloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Cloxacillin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Cloxacillin Sodium Hydrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +163 – +171° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Cloxacillin Sodium Hydrate in 10 mL of water is clear and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cloxacillin Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Cloxacillin Sodium Hydrate according to Method 5, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Cloxacillin

Sodium Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than cloxacillin obtained from the sample solution is not more than the peak area of cloxacillin obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.953 g of diammonium hydrogen phosphate in 700 mL of water, and add 250 mL of acetonitrile. Adjust the pH to 4.0 with phosphoric acid, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of cloxacillin is about 24 minutes.

Time span of measurement: About 3 times as long as the retention time of cloxacillin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of cloxacillin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that obtained from the standard solution.

System performance: Weigh accurately about 50 mg of Cloxacillin Sodium Reference Standard, dissolve in a suitable amount of the mobile phase, add 5 mL of a solution of guaifenesin in the mobile phase (1 in 200), then add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. When the procedure is run with 10 μ L of the solution for system suitability test under the above operating conditions, guaifenesin and cloxacillin are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of cloxacillin to that of guaifenesin is not more than 1.0%.

Water <2.48> 3.0 – 4.5% (0.2 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Cloxacillin Sodium Reference Standard equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L phos-

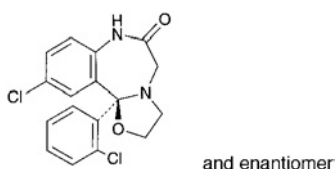
phate buffer solution, pH 7.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Cloxacillin Sodium Hydrate equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Cloxazolam

クロキサゾラム



$\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}_2$: 349.21
(11*bRS*)-10-Chloro-11*b*-(2-chlorophenyl)-2,3,7,11*b*-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one [24166-13-0]

Cloxazolam, when dried, contains not less than 99.0% of $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}_2$.

Description Cloxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), sparingly soluble in dichloromethane, slightly soluble in ethanol (99.5) and in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 200°C (with decomposition).

Identification (1) Dissolve 0.01 g of Cloxazolam in 10 mL of ethanol (99.5) by heating, and add 1 drop of hydrochloric acid: the solution shows a light yellow color and a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 0.01 g of Cloxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Place 2 g of Cloxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of sodium hydroxide TS, and boil under a reflux condenser for 4 hours. After cooling, neutralize with dilute hydrochloric acid, and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 5 mL of methanol by heating

on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry the crystals in vacuum at 60°C for 1 hour: it melts <2.60> between 87°C and 91°C.

(4) Determine the absorption spectrum of a solution of Cloxazolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Cloxazolam as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color appears.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (244 nm): 390 – 410 (after drying, 1 mg, ethanol (99.5), 100 mL).

Purity (1) Chloride <1.03>—To 1.0 g of Cloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Place 1.0 g of Cloxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue heating until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate solution, and heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Cloxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of toluene and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

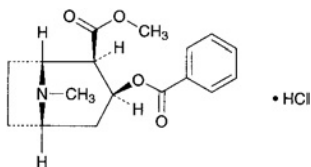
Assay Weigh accurately about 0.5 g of Cloxazolam, previously dried, and dissolve in 50 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 34.92 mg of $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cocaine Hydrochloride

コカイン塩酸塩



$C_{17}H_{21}NO_4 \cdot HCl$: 339.81
(1*R*,2*R*,3*S*,5*S*)-2-Methoxycarbonyl-8-methyl-8-azabicyclo[3.2.1]oct-3-yl benzoate monohydrochloride
[53-21-4]

Cocaine Hydrochloride, when dried, contains not less than 98.0% of $C_{17}H_{21}NO_4 \cdot HCl$.

Description Cocaine Hydrochloride occurs as colorless crystals or a white crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Cocaine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cocaine Hydrochloride, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Cocaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-70 - -73^\circ$ (after drying, 0.5 g, water, 20 mL, 100 mm).

Purity (1) Acidity—Dissolve 0.5 g of Cocaine Hydrochloride in 10 mL of water, add 1 drop of methyl red TS, and neutralize with 0.01 mol/L sodium hydroxide VS: the consumed volume is not more than 1.0 mL.

(2) Cinnamyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 5 mL of water, and add 0.3 mL of diluted sulfuric acid (1 in 20) and 0.10 mL of 0.02 mol/L potassium permanganate VS: the red color does not disappear within 30 minutes.

(3) Isoatropyl cocaine—Dissolve 0.10 g of Cocaine Hydrochloride in 30 mL of water in a beaker. Transfer 5 mL of this solution to a test tube, add 1 drop of ammonia TS,

and mix. After the precipitate is coagulated, add 10 mL of water, and transfer the mixture to the former beaker, to which 30 mL of water has been added previously. Wash the test tube with 10 mL of water, combine the washings with the mixture in the beaker, add 3 drops of ammonia TS to the combined mixture, and mix gently: a crystalline precipitate is produced. Allow to stand for 1 hour: the supernatant liquid is clear.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Cocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.98 mg of $C_{17}H_{21}NO_4 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Coconut Oil

Oleum Cocois

ヤシ油

Coconut oil is the fixed oil obtained from the seeds of *Cocos nucifera* Linné (*Palmae*).

Description Coconut Oil is a white to light yellow mass or a colorless or light yellow, clear oil. It has a slight, characteristic odor and a mild taste.

It is freely soluble in diethyl ether and in petroleum ether. It is practically insoluble in water.

At a temperature below 15°C, it congeals to a hard and brittle solid.

Melting point: 20 – 28°C

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 246 – 264

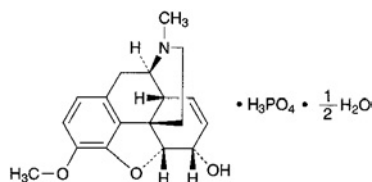
Unsaponifiable matter <1.13> Not more than 1.0%.

Iodine value <1.13> 7 – 11

Containers and storage Containers—Tight containers.

Codeine Phosphate Hydrate

コデインリン酸塩水和物



$C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$: 406.37
(5*R*,6*S*)-4,5-Epoxy-3-methoxy-17-methyl-7,8-didehydromorphinan-6-ol monophosphate hemihydrate
[41444-62-6]

Codeine Phosphate Hydrate contains not less than 98.0% of codeine phosphate ($C_{18}H_{21}NO_3 \cdot H_3PO_4$; 397.36), calculated on the anhydrous basis.

Description Codeine Phosphate Hydrate occurs as white to yellowish white crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Codeine Phosphate Hydrate (1 in 10) is between 3.0 and 5.0.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Codeine Phosphate Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Codeine Phosphate Hydrate, previously dried at 105°C for 4 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Codeine Phosphate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> (1) for phosphate.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-98 - -102^\circ$ (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

Purity (1) Chloride <1.03>—Perform the test with 0.5 g of Codeine Phosphate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(2) **Sulfate** <1.14>—Perform the test with 0.20 g of Codeine Phosphate Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) **Related substances**—Dissolve 0.20 g of Codeine Phosphate Hydrate in 10 mL of a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and ethanol (99.5) (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography

<2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 1.5 – 3.0% (0.5 g, direct titration).

Assay Dissolve about 0.5 g of Codeine Phosphate Hydrate, accurately weighed, in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.74 mg of $C_{18}H_{21}NO_3 \cdot H_3PO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

1% Codeine Phosphate Powder

コデインリン酸塩散 1%

10% Codeine Phosphate Powder contains not less than 0.90% and not more than 1.10% of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$; 406.37).

Method of preparation

Codeine Phosphate Hydrate	10 g
Lactose Hydrate	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1% Codeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Assay Weigh accurately about 5 g of 1% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of codeine to that of the internal standard.

Amount (mg) of codeine phosphate Hydrate
($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$)

$$= W_s \times (Q_T/Q_S) \times 1.0227$$

W_s : Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

10% Codeine Phosphate Powder

コデインリン酸塩散 10%

10% Codeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of codeine phosphate Hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$; 406.37).

Method of preparation

Codeine Phosphate Hydrate	100 g
Lactose Hydrate	a sufficient quantity
<hr/>	
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% Codeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Assay Weigh accurately about 2.5 g of 10% Codeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previ-

ously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of codeine to that of the internal standard:

$$\begin{aligned} &\text{Amount (mg) of codeine phosphate Hydrate} \\ &(C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O) \\ &= W_s \times (Q_T/Q_S) \times 5 \times 1.0227 \end{aligned}$$

W_s : Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Codeine Phosphate Tablets

コデインリン酸塩錠

Codeine Phosphate Tablets contain not less than 93% and not more than 107% of the labeled amount of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$; 406.37)

Method of preparation Prepare as directed under Tablets, with Codeine Phosphate Hydrate.

Identification To a quantity of powdered Codeine Phosphate Tablets, equivalent to 0.1 g of Codeine Phosphate Hydrate according to the labeled amount, add 20 mL of water, shake, and filter. To 2 mL of the filtrate add water to make

100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Assay Weigh accurately and powder not less than 20 Codeine Phosphate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of codeine phosphate hydrate ($C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O$), add 30 mL of water, shake, add 20 mL of diluted dilute sulfuric acid (1 in 20), treat the mixture with ultrasonic waves for 10 minutes, and add water to make exactly 100 mL. Filter the solution, then pipet 5 mL of the filtrate, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of codeine phosphate for assay (previously determine the water <2.48> in the same manner as Codeine Phosphate Hydrate), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of codeine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of codeine phosphate hydrate} \\ & (C_{18}H_{21}NO_3 \cdot H_3PO_4 \cdot \frac{1}{2}H_2O) \\ & = W_S \times (Q_T/Q_S) \times 2 \times 1.0227 \end{aligned}$$

W_S : Amount (mg) of codeine phosphate for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of codeine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, codeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of codeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Cod Liver Oil

肝油

Cod Liver Oil is the fatty oils obtained from fresh livers and pyloric appendages of *Gadus macrocephalus* Tilesius or *Theragra chalcogramma* Pallas (*Gadidae*).

Cod Liver Oil contains not less than 2000 Vitamin A Units and not more than 5000 Vitamin A Units per g.

Description Cod Liver Oil is a yellow to orange oily liquid. It has a characteristic, slightly fishy odor and a mild taste.

It is miscible with chloroform.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air or by light.

Identification Dissolve 0.1 g of Cod Liver Oil in 10 mL of chloroform, and to 1 mL of this solution add 3 mL of antimony (III) chloride TS: a blue color develops immediately, but the color fades rapidly.

Specific gravity <1.13> d_{20}^{20} : 0.918 – 0.928

Acid value <1.13> Not more than 1.7.

Saponification value <1.13> 180 – 192

Unsaponifiable matter <1.13> Not more than 3.0%.

Iodine value <1.13> 130 – 170

Purity Rancidity—No unpleasant odor of rancid oil is perceptible on warming Cod Liver Oil.

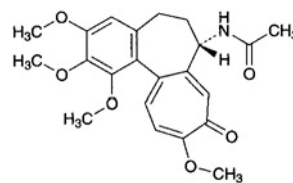
Assay Proceed with about 0.5 g of Cod Liver Oil, accurately weighed, as directed in Method 2 under the Vitamin A Determination <2.55>, and perform the test.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere.

Colchicine

コルヒチン



$C_{22}H_{25}NO_6$: 399.44

N-[(7*S*)-(1,2,3,10-Tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl)]acetamide [64-86-8]

Colchicine contains not less than 97.0% and not more than 102.0% of $C_{22}H_{25}NO_6$, calculated on the anhydrous basis and corrected by the amount of ethyl acetate.

Description Colchicine occurs as a yellowish white powder.

It is very soluble in methanol, freely soluble in *N,N*-dimethylformamide, in ethanol (95) and in acetic anhydride, and sparingly soluble in water.

It is colored by light.

Identification (1) Determine the absorption spectrum of a solution of Colchicine in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) To 1 g of potassium bromide for infrared absorption spectrum add 0.5 mL of a solution of Colchicine in methanol (1 in 50), grind thoroughly, and dry in vacuum at 80°C for 1 hour. Determine the infrared absorption spectrum of this powder as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -235 – -250° (0.1 g calculated on the anhydrous basis and corrected by the amount of ethyl acetate, ethanol (95), 10 mL, 100 mm).

Purity (1) Colchicine—Dissolve 0.10 g of Colchicine in 10 mL of water, and to 5 mL of this solution add 2 drops of iron (III) chloride TS: no definite green color develops.

(2) Chloroform and ethyl acetate—Weigh accurately about 0.6 g of Colchicine, dissolve in exactly 2 mL of the internal standard solution, add *N,N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh 0.30 g of chloroform using a 100-mL volumetric flask containing about 20 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add *N,N*-dimethylformamide to make exactly 200 mL, and use this solution as the standard solution (1). Separately, weigh accurately about 1.8 g of ethyl acetate using a 100-mL volumetric flask containing about 20 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution and *N,N*-dimethylformamide to make 10 mL, and use this solution as the standard solution (2). Perform the test with 2 μ L each of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following conditions: the peak area of chloroform is not more than that from the standard solution (1). Determine the ratios of the peak area of ethyl acetate to that of the internal standard, Q_T and Q_S , of the sample solution and standard solution (2), and calculate the amount of ethyl acetate by the following formula: the amount of ethyl acetate is not more than 6.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of ethyl acetate (C}_4\text{H}_8\text{O}_2) \\ = (W_S/W_T) \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount (g) of ethyl acetate

W_T : Amount (g) of the sample

Internal standard solution—A solution of 1-propanol in *N,N*-dimethylformamide (3 in 200)

Operating conditions—

Detector: A hydrogen flame-ionization detector

Column: A fused silica column 0.53 mm in inside diameter and 30 m in length, coated inside surface with polyethylene glycol 20 M for gas chromatography 1.0 μ m in thickness.

Column temperature: 60°C for 7 minutes, then up to 100°C at a rate of 40°C per minute if necessary, and hold at 100°C for 10 minutes.

Injection port temperature: A constant temperature of about 130°C

Detector temperature: A constant temperature of about 200°C

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 3 minutes.

Split ratio: 1:20

System suitability—

Test for required detectability: Pipet 2 mL of the standard solution (2), and add *N,N*-dimethylformamide to make exactly 25 mL. Pipet 1 mL of this solution, and add *N,N*-dimethylformamide to make exactly 50 mL. Confirm that the peak area of ethyl acetate obtained from 2 μ L of this solution is equivalent to 0.11 to 0.21% of that obtained from 2 μ L of the standard solution (2).

System performance: To 1 mL of chloroform add *N,N*-dimethylformamide to make 10 mL. To 1 mL of this solution add 2 mL of ethyl acetate and *N,N*-dimethylformamide to make 100 mL. To 2 mL of this solution add 2 mL of the internal standard solution and *N,N*-dimethylformamide to make 10 mL. When the procedure is run with 2 μ L of this solution under the above operating conditions, ethyl acetate, chloroform and the internal standard are eluted in this order with the resolution between the peaks of chloroform and the internal standard being not less than 2.0.

System repeatability: When the test is repeated 3 times with 2 μ L of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl acetate to that of the internal standard is not more than 3.0%.

(3) Related substances Dissolve 60 mg of Colchicine in 100 mL of diluted methanol (1 in 2). Pipet 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method. Calculate the total amount of the peaks other than colchicine by the area percentage method: not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 450 mL of 0.05 mol/L potassium dihydrogen phosphate TS add methanol to make 1000 mL. Adjust the pH to 5.5 with diluted phosphoric acid (7 in 200).

Flow rate: Adjust the flow rate so that the retention time of colchicine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of colchicine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, and add diluted methanol (1 in 2) to make exactly 50 mL. Confirm that the peak area of colchicine obtained

from 20 μL of this solution is equivalent to 1.4 to 2.6% of that obtained from 20 μL of the sample solution.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of colchicine are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of colchicine is not more than 2.0%.

Water <2.48> Not more than 2.0% (0.5 g, volumetric titration, back titration).

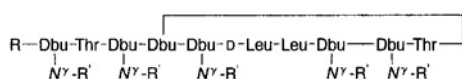
Assay Weigh accurately about 0.4 g of Colchicine, dissolve in 25 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 19.97 mg of $\text{C}_{22}\text{H}_{25}\text{NO}_6$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Colistin Sodium Methanesulfonate

コリスチンメタンスルホン酸ナトリウム



Colistin A Sodium Methanesulfonate : R = 6-Methyloctanoic acid
Dbu = L- α, γ -Diaminobutyric acid
R' = SO_3Na

Colistin B Sodium Methanesulfonate : R = 6-Methylheptanoic acid
Dbu = L- α, γ -Diaminobutyric acid
R' = SO_3Na

[8068-28-8, Colistin Sodium Methanesulfonate]

Colistin Sodium Methanesulfonate is the sodium salt of colistin derivatives, and is a mixture of colistin A sodium methanesulfonate and colistin B sodium methanesulfonate. It, when dried, contains not less than 11,500 Units per mg. The unit of Colistin Sodium Methanesulfonate is expressed as mass of colistin A (R = 6-methyloctanoic acid, R' = H; $\text{C}_{53}\text{H}_{100}\text{N}_{16}\text{O}_{13}$: 1169.46).

Description Colistin Sodium Methanesulfonate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) Dissolve 20 mg of Colistin Sodium Methanesulfonate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, and add 5 drops of copper (II) sulfate TS while shaking: a blue-purple color develops.

(2) Dissolve 40 mg of Colistin Sodium Methanesulfonate in 1 mL of 1 mol/L hydrochloric acid TS, and add 0.5 mL of dilute iodine TS: the color of iodine disappears.

(3) Determine the infrared absorption spectrum of

Colistin Sodium Methanesulfonate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Colistin Sodium Methanesulfonate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Colistin Sodium Methanesulfonate responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 0.1 g of Colistin Sodium Methanesulfonate in 10 mL of water, and allow to stand for 30 minutes: the pH of the solution is between 6.5 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.16 g of Colistin Sodium Methanesulfonate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Colistin Sodium Methanesulfonate according to Method 4, and perform the test (not more than 2 ppm).

(4) Free colistin—Dissolve 80 mg of Colistin Sodium Methanesulfonate in 3 mL of water, add 0.05 mL of a solution of silicotungstic acid 26-water (1 in 10), and immediately compare the solution with the reference suspension described under Test Methods for Plastic Containers <7.02>: the turbidity is not greater than that of the reference suspension (not more than 0.25%).

Loss on drying <2.41> Not more than 3.0% (0.1 g, reduced pressure, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—To 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 20.0 g of agar add 1000 mL of water, then add a suitable amount of sodium hydroxide TS so that the pH of the medium is being 6.5 to 6.6 after sterilization, sterile, and use this as the seeded agar medium and the agar medium for base layer.

(iii) Standard solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate Reference Standard, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing 100,000 Units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 10°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

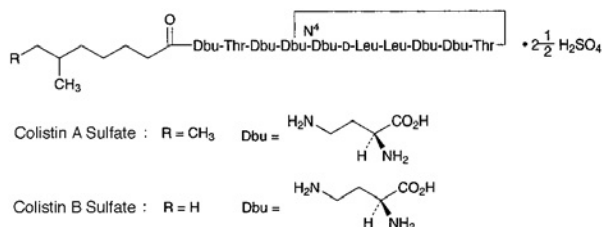
(iv) Sample solutions—Weigh accurately an amount of Colistin Sodium Methanesulfonate, previously dried, dissolve in phosphate buffer solution, pH 6.0 to make a solution containing about 100,000 Units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 Units and 2500 Units, and use these solutions as the high con-

centration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Colistin Sulfate

コリスチン硫酸塩



Colistin A Sulfate $C_{53}H_{100}N_{16}O_{13} \cdot 2\frac{1}{2}H_2SO_4$: 1414.66

Colistin B Sulfate $C_{52}H_{98}N_{16}O_{13} \cdot 2\frac{1}{2}H_2SO_4$: 1400.63

[1264-72-8]

Colistin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa* var. *colistinus*.

It, when dried, contains not less than 16,000 units per mg. The potency of Colistin Sulfate is expressed as unit calculated from the amount of colistin A ($C_{53}H_{100}N_{16}O_{13}$; 1169.46). One unit of Colistin Sulfate is equivalent to 0.04 μ g of colistin A ($C_{53}H_{100}N_{16}O_{13}$).

Description Colistin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 20 mg of Colistin Sulfate in 2 mL of water, add 0.5 mL of sodium hydroxide TS, then add 5 drops of copper (II) sulfate TS while shaking: a purple color develops.

(2) Dissolve 50 mg of Colistin Sulfate in 10 mL of diluted hydrochloric acid (1 in 2). Transfer 1 mL of this solution in a tube for hydrolysis, seal, and heat at 135°C for 5 hours. After cooling, open the tube, and evaporate the content to dryness until the odor of hydrochloric acid is no more perceptible. Dissolve the residue in 0.5 mL of water, and use this solution as the sample solution. Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine in 10 mL of water, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, acetic acid (100), water, pyridine and ethanol (99.5) (60:15:10:6:5) to a distance of about 10 cm, and dry the plate at 105°C for 10 minutes. Spray evenly ninhydrin TS on the plate, and heat at 110°C for 5 minutes: three principal spots are obtained from the sample solution, the *R_f* values of two spots of them are the same with those of the corresponding

spots obtained from the standard solution (1) and the standard solution (2), and the *R_f* value of the rest principal spot is about 0.1. No spot is observed at the position corresponding to the spots obtained from the standard solution (3) and the standard solution (4).

(3) A solution of Colistin Sulfate (1 in 20) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-63 - -73^\circ$ (1.25 g, after drying, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Colistin Sulfate in 10 mL of water is between 4.0 and 6.0.

Purity (1) Sulfuric acid—Weigh accurately about 0.25 g of previously dried Colistin Sulfate, dissolve in a suitable amount of water, adjust the pH to 11 with ammonia solution (28), and add water to make 100 mL. To this solution add exactly 10 mL of 0.1 mol/L barium chloride VS and 50 mL of ethanol (99.5), and titrate with <2.50> 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue-purple color of the solution disappears (indicator: 0.5 mg of phthalein purple): the amount of sulfuric acid (SO_4) is 16.0 to 18.0%.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of SO_4

(2) Related substances—Dissolve 50 mg of Colistin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of pyridine, 1-butanol, water and acetic acid (100) (6:5:4:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly ninhydrin-butanol TS on the plate, and heat at 100°C for about 20 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Escherichia coli* NIHJ

(ii) Culture medium—Dissolve 10.0 g of peptone, 30.0 g of sodium chloride, 3.0 g of meat extract and 15.0 g of agar in 1000 mL of water, adjust the pH with sodium hydroxide TS so that the solution will be 6.5 to 6.6 after sterilization, and use as the agar media for seed layer and for base layer.

(iii) Standard solutions—Weigh accurately an amount of Colistin Sulfate Reference Standard, previously dried, equivalent to about 1,000,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 10°C, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration stan-

standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Colistin Sulfate, previously dried, equivalent to about 1,000,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 10 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10,000 units and 2500 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Corn Oil

Oleum Maydis

トウモロコシ油

Corn Oil is the fixed oil obtained from the embryo of *Zea mays* Linné (*Gramineae*).

Description Corn Oil is a clear, light yellow oil. It is odorless or has a slight odor, and a mild taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95), and practically insoluble in water.

At -7°C , it congeals to an unguentary mass.

Specific gravity d_{25}^{25} : 0.915 – 0.921

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 187 – 195

Unsaponifiable matter <1.13> Not more than 1.5%.

Iodine value <1.13> 103 – 130

Containers and storage Containers—Tight containers.

Corn Starch

Amylum Maydis

トウモロコシデンプン

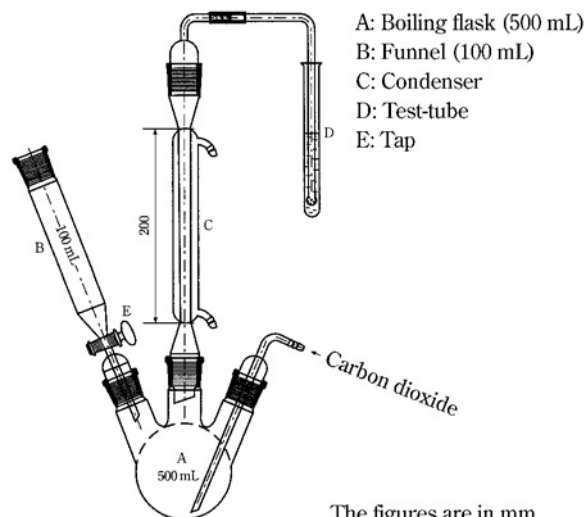
This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Corn Starch consists of starch granules derived from the ripen seeds of *Zea mays* Linné (*Gramineae*).

♦**Description** Corn Starch occurs as white to pale yellowish white masses or powder.

It is practically insoluble in water and in ethanol (99.5).♦

Identification (1) Under a microscope, Corn Starch, preserved in a mixture of water and glycerin (1:1), appears as irregularly polygonal simple grains about 2 – 23 μm in diameter, or irregularly orbicular or spherical simple grains about 25 – 35 μm in diameter; hilum appears as distinct cave or 2 – 5 radial clefts; concentric striation absent; a black cross, its intersection point on hilum, is observed when grains



The figures are in mm.

are put between two nicol prisms fixed at right angle to each other.

(2) To 1 g of Corn Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed and the color disappears by heating.

pH <2.54> Put 5.0 g of Corn Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 4.0 and 7.0.

Purity (1) Iron—To 1.5 g of Corn Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (1 in 5) and 0.1 mL of mercapto acetic acid, and mix. Alkalinize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Corn Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the following figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place

10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Corn Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = (V/W) \times 1000 \times 3.203$$

W: Amount (g) of the sample

V: Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

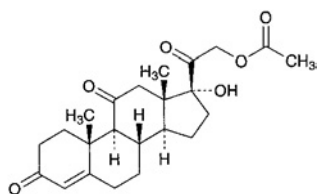
Loss on drying <2.41> Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

♦**Containers and storage** Containers—Well-closed containers. ♦

Cortisone Acetate

コルチゾン酢酸エステル



$C_{23}H_{30}O_6$: 402.48

17,21-Dihydroxypregn-4-ene-3,11,20-trione 21-acetate
[50-04-4]

Cortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of $C_{23}H_{30}O_6$.

Description Cortisone Acetate occurs as white crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

Melting point: about 240°C (with decomposition).

Identification (1) To 2 mg of Cortisone Acetate add 2 mL of sulfuric acid, and allow to stand for a while: a yellowish green color is produced, and it gradually changes to yellow-orange. Examine the solution under ultraviolet light: the solution shows a light green fluorescence. Add carefully 10 mL of water to this solution: the color of the solution is dis-

charged, and the solution remains clear.

(2) Determine the absorption spectrum of a solution of Cortisone Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cortisone Acetate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Cortisone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cortisone Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Cortisone Acetate and Cortisone Acetate Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +207 – +216° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 25 mg of Cortisone Acetate in 10 mL of a mixture of acetonitrile, water and acetic acid (100) (70:30:1), and use this solution as the sample solution. Pipet 1 mL of this solution add the mixture of acetonitrile, water and acetic acid (100) (70:30:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than cortisone acetate is not larger than 1/2 times the peak area of cortisone acetate obtained with the standard solution, and the total area of the peak other than cortisone acetate is not larger than 1.5 times the peak area of cortisone acetate with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and acetonitrile (7:3).

Mobile phase B: A mixture of acetonitrile and water (7:3).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 5	90	10
5 – 25	90 → 10	10 → 90
25 – 30	10	90

Flow rate: About 1 mL per minute.

Time span of measurement: About 3 times as long as the retention time of cortisone acetate beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile, water and acetic acid (100) (70:30:1) to make exactly 10 mL. Confirm that the peak area of cortisone acetate obtained with 15 μ L of this solution is equivalent to 8 to 12% of that with 15 μ L of the standard solution.

System performance: When the procedure is run with 15 μ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of cortisone acetate are not less than 10,000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 3 times with 15 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of cortisone acetate is not more than 5.0%.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 10 mg each of Cortisone Acetate and Cortisone Acetate Reference Standard, previously dried and accurately weighed, in 50 mL of methanol, add exactly 5 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of cortisone acetate to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } C_{23}H_{30}O_6 \\ & = W_s \times (Q_T/Q_S) \end{aligned}$$

W_s : Amount (mg) of Cortisone Acetate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of cortisone acetate is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, cortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of cortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Creosote

クレオソート

Creosote is a mixture of phenols obtained from wood tar.

Description Creosote is a colorless or pale yellow, clear liquid. It has a characteristic odor, and a burning taste.

It is miscible with ethanol (95) and with diethyl ether.

It is slightly soluble in water.

Its saturated solution is neutral.

It is highly refractive.

It gradually changes in color by light or by air.

Identification To 10 mL of a saturated solution of Creosote add 1 drop of iron (III) chloride TS: a purple color develops, and the solution becomes rapidly turbid, and changes through blue and muddy green to brown.

Specific gravity <2.56> d_{20}^{20} : not less than 1.076.

Purity (1) Bases and hydrocarbons—Shake 1.0 mL of Creosote with 9 mL of sodium hydroxide TS: the solution is clear and does not darken. On further addition of 50 mL of water, the solution is practically clear.

(2) Phenol or coal-tar creosote—Shake Creosote with an equal volume of collodion: no coagulum is produced.

(3) Other impurities—To 1.0 mL of Creosote add 2 mL of petroleum benzin and 2 mL of barium hydroxide TS, shake, and allow to stand: no blue or muddy brown color develops in the upper layer of the mixture, and no red color develops in the lower layer.

Distilling range <2.57> 200 – 220°C, not less than 85 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cresol

クレゾール

C_7H_8O : 108.14

Cresol is a mixture of isomeric cresols.

Description Cresol is a clear, colorless or yellow to yellow-brown liquid. It has a phenol-like odor.

It is miscible with ethanol (95) and with diethyl ether.

It is sparingly soluble in water.

It dissolves in sodium hydroxide TS.

A saturated solution of Cresol is neutral to bromocresol purple TS.

It is a highly refractive liquid.

It becomes dark brown by light or on aging.

Identification To 5 mL of a saturated solution of Cresol add 1 to 2 drops of dilute iron (III) chloride TS: a blue-purple color develops.

Specific gravity <2.56> d_{20}^{20} : 1.032 – 1.041

Purity (1) Hydrocarbons—Dissolve 1.0 mL of Cresol in 60 mL of water: the solution shows no more turbidity than that produced in the following control solution.

Control solution: To 54 mL of water add 6.0 mL of 0.005 mol/L sulfuric acid and 1.0 mL of barium chloride TS, and after thorough shaking, allow to stand for 5 minutes.

(2) Sulfur compounds—Transfer 20 mL of Cresol in a 100-mL conical flask, place a piece of moistened lead (II) acetate paper on the mouth of the flask, and warm for 5 minutes on a water bath: the lead (II) acetate paper may develop a yellow color, but neither a brown nor a dark tint.

Distilling range <2.57> 196 – 206°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Cresol Solution

クレゾール水

Cresol Solution contains not less than 1.25 vol% and not more than 1.60 vol% of cresol.

Method of preparation

Saponated Cresol Solution	30 mL
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Prepare by mixing the above ingredients.

Description Cresol Solution is a clear or slightly turbid, yellow solution. It has the odor of cresol.

Identification Shake 0.5 mL of the oily layer obtained in the Assay with 30 mL of water, filter, and perform the following tests using this filtrate as the sample solution:

(1) To 5 mL of the sample solution add 1 to 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) To 5 mL of the sample solution add 1 to 2 drops of bromine TS: a light yellow, flocculent precipitate is produced.

Assay Transfer 200 mL of Cresol Solution, exactly measured, to a 500-mL distilling flask. Add 40 g of sodium chloride and 3 mL of dilute sulfuric acid, and connect the distilling apparatus with the distilling flask, and distil into a cassia flask which contains 30 g of powdered sodium chloride and 3 mL of kerosene, exactly measured, until the distillate measures 90 mL. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Shake often the cassia flask in warm water to dissolve the sodium chloride, and allow to stand for 15 minutes. After cooling to 15°C, add a saturated solution of sodium chloride, and allow to stand for more than 3 hours with occasional shaking. Allow to stand for 1 to 2 minutes with gentle shaking to combine the separated oil drops with the oil layer. The difference between the number of mL of the oil layer measured and 3 mL represents the amount (mL) of cresol.

Containers and storage Containers—Tight containers.

Saponated Cresol Solution

クレゾール石ケン液

Saponated Cresol Solution contains not less than 42 vol% and not more than 52 vol% of cresol.

Method of preparation

Cresol	500 mL
Fixed Oil	300 mL
Potassium Hydroxide	a suitable quantity
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Dissolve Potassium Hydroxide, in required quantity for saponification, in a sufficient quantity of Water or Purified Water, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol, if necessary, heat in a water bath by thorough stirring, and continue the saponification. After complete saponification, add Cresol, stir thoroughly until the mixture becomes clear, and add sufficient Water or Purified Water to make 1000 mL. A corresponding amount of Sodium Hydroxide may be used in place of Potassium Hydroxide.

Description Saponated Cresol Solution is a yellow-brown to red-brown, viscous liquid. It has the odor of cresol.

It is miscible with water, with ethanol (95) and with glycerin.

It is alkaline.

Identification Proceed as directed in the Identification under Cresol, using the distillate in the Purity (3).

Purity (1) Alkalinity—Mix well 0.50 mL of Saponated Cresol Solution with 10 mL of neutralized ethanol, add 2 to 3 drops of phenolphthalein TS and 0.10 mL of 1 mol/L hydrochloric acid VS: no red color develops.

(2) Unsaponified matter—To 1.0 mL of Saponated Cresol Solution add 5 mL of water, and shake: the solution is clear.

(3) Cresol fraction—Transfer 180 mL of Saponated Cresol Solution to a 2000-mL distilling flask, add 300 mL of water and 100 mL of dilute sulfuric acid, and distil with steam until the distillate becomes clear. Draw off the water from the condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Cool the condenser again, and continue distillation for 5 minutes. Dissolve 20 g of sodium chloride per 100 mL of the distillate, allow to stand, and collect the separated clear oil layer. After adding about 15 g of powdered calcium chloride for drying in small portions with frequent shaking, allow to stand for 4 hours. Filter, and distil exactly 50 mL of the filtrate: the distillate is not less than 43 mL between 196°C and 206°C.

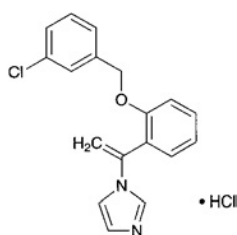
Assay Transfer 5 mL of Saponated Cresol Solution, exactly measured, to a 500-mL distilling flask, holding the pipet vertically for 15 minutes to draw off the solution into the flask. Add 200 mL of water, 40 g of sodium chloride and 3 mL of dilute sulfuric acid, connect the distilling apparatus with the distilling flask, and distil into a cassia flask which contains 30 g of powdered sodium chloride and exactly 3 mL of kerosene, until the distillate reaches 90 mL. Draw off the water from the

condenser, and continue the distillation until water vapor begins to come out of the tip of the condenser. Allow the cassia flask to stand in warm water for 15 minutes to dissolve the sodium chloride with frequent shaking. Cool to 15°C, add a saturated solution of sodium chloride, and allow to stand for more than 3 hours with occasional shaking. Allow to stand for 1 to 2 minutes with gentle shaking, and combine the separated oil drops with the oil layer. The volume (mL) subtracted 3 (mL) from the oil layer measured represents the amount (mL) of cresol.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Croconazole Hydrochloride

クロコナゾール塩酸塩



$C_{18}H_{15}ClN_2O \cdot HCl$: 347.24
1-[1-[2-(3-Chlorobenzoyloxy)phenyl]vinyl]-1*H*-imidazole
monohydrochloride [77174-66-4]

Croconazole Hydrochloride, when dried, contains not less than 98.5% of $C_{18}H_{15}ClN_2O \cdot HCl$.

Description Croconazole Hydrochloride occurs as white to pale yellowish white crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Croconazole Hydrochloride in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Croconazole Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.05 g of Croconazole Hydrochloride in 10 mL of water, add 2 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake. Wash the separated aqueous layer with two 10-mL portions of diethyl ether, and acidify the solution with 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 148 – 153°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Croconazole Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of

Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Croconazole Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, methanol and ammonia solution (28) (30:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Croconazole Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS [indicator: 1 to 2 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)] until the color of the solution changes from blue-green through green to yellow-green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.72 mg of $C_{18}H_{15}ClN_2O \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Croscarmellose Sodium

クロスカルメロースナトリウム

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (♦ ◆).

Croscarmellose Sodium is the sodium salt of a cross-linked poly carboxymethylether of cellulose.

♦**Description** Croscarmellose Sodium occurs as a white to yellowish white powder.

It is practically insoluble in ethanol (99.5) and in diethyl ether.

It swells with water and becomes a suspension.

It is hygroscopic. ◆

Identification (1) To 1 g of Croscarmellose Sodium add 100 mL of a solution of methylene blue (1 in 250,000), stir well, and allow to stand: blue cotton-like precipitates appear.

(2) To 1 g of Croscarmellose Sodium add 50 mL of water, and stir well to make a suspension. To 1 mL of this suspension add 1 mL of water and 5 drops of freshly prepared solution of 1-naphthol in methanol (1 in 25), and gently add 2 mL of sulfuric acid along a wall of the vessel: a red-purple color appears at the zone of contact.

(3) The suspension obtained in (2) responds to the

Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> To 1.0 g of Croscarmellose Sodium add 100 mL of water, and stir for 5 minutes: the pH of the supernatant liquid is between 5.0 and 7.0.

Purity ♦(1) Heavy metals <1.07>—Proceed with 2.0 g of Croscarmellose Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm). ♦

♦(2) Sodium chloride and sodium glycolate—The total amount of sodium chloride and sodium glycolate is not more than 0.5%.

(i) Sodium chloride: Weigh accurately about 5 g of Croscarmellose Sodium, add 50 mL of water and 5 mL of hydrogen peroxide (30), and heat on a water bath for 20 minutes with occasional stirring. After cooling, add 100 mL of water and 10 mL of nitric acid, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Perform a blank determination in the same manner and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 5.844 mg of NaCl

(ii) Sodium glycolate: Weigh accurately about 0.5 g of Croscarmellose Sodium, add 2 mL of acetic acid (100) and 5 mL of water, and stir for 15 minutes. Add gradually 50 mL of acetone with stirring, then add 1 g of sodium chloride, stir for 3 minutes, and filter through a filter paper moistened with acetone. Wash the residue thoroughly with 30 mL of acetone, combine the filtrate and washings, add acetone to make exactly 100 mL, and use this solution as the sample stock solution. Separately, dissolve 0.100 g of glycolic acid in water to make 200 mL. Pipet 0.5 mL, 1 mL, 2 mL, 3 mL and 4 mL of this solution, add water to make them exactly 5 mL, then add 5 mL of acetic acid (100) and acetone to make exactly 100 mL, and designate them standard stock solution (1), standard stock solution (2), standard stock solution (3), standard stock solution (4) and standard stock solution (5), respectively. Pipet 2 mL each of the sample stock solution and the standard stock solutions (1), (2), (3), (4) and (5), and heat them in a water bath for 20 minutes to evaporate acetone. After cooling, add exactly 5 mL of 2,7-dihydroxynaphthalene TS, mix, then add 15 mL of 2,7-dihydroxynaphthalene TS, mix, cover the mouth of the vessels with aluminum foil, and heat in a water bath for 20 minutes. After cooling, add sulfuric acid to make exactly 25 mL, mix, and designate them sample solution, standard solution (1), standard solution (2), standard solution (3), standard solution (4) and standard solution (5), respectively. Separately, to 10 mL of a mixture of water and acetic acid (100) (1:1) add acetone to make exactly 100 mL, and proceed with exactly 2 mL of this solution in the same manner for preparation of the sample solution, and use the solution so obtained as the blank solution. Determine the absorbances, A_T , A_{S1} , A_{S2} , A_{S3} , A_{S4} and A_{S5} , of the sample solution and the standard solutions (1), (2), (3), (4) and (5), respectively, at 540 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the blank solution as the control. Determine the amount (g) of glycolic acid, X, in 100 mL of the sample solution from the calibration curve obtained with the standard solutions, and calculate the amount of sodium glycolate by the following formula.

$$\begin{aligned} \text{Amount (\%)} & \text{ of sodium glycolate} \\ & = (X/W) \times 100 \times 1.2890 \end{aligned}$$

W : Amount (g) of sample ♦

♦(3) Water-soluble substance—Weigh accurately about 10 g of Croscarmellose Sodium, disperse in 800 mL of water by stirring for 1 minute every 10 minutes during 30 minutes, and allow to stand for at most 1 hour to precipitate. Filter by suction or centrifuge the clear upper portion, and weigh accurately the mass of about 150 mL of the filtrate or supernatant liquid. Heat to concentrate this liquid avoiding to dryness, then dry at 105°C for 4 hours, and weigh the mass of the residue accurately. Calculate the amount of the water-soluble substance by the following formula: not less than 1.0 % and not more than 10.0%.

$$\begin{aligned} \text{Amount (\%)} & \text{ of water-soluble substance} \\ & = 100 W_3 (800 + W_1) / W_1 W_2 \end{aligned}$$

W_1 : Amount (g) of sample

W_2 : Amount (g) of the filtrate or supernatant liquid of about 150 mL

W_3 : Amount (g) of the residue ♦

Precipitation test Put 75 mL of water in a 100-mL glass-stoppered graduated cylinder, and add portion by portion with 1.5 g of Croscarmellose Sodium divided into three portions while shaking vigorously at each time. Then, add water to make 100 mL, shake until to get a homogenous dispersion, and allow to stand for 4 hours: the volume of the settled layer is not less than 10.0 mL and not more than 30.0 mL.

Degree of substitution Weigh accurately about 1 g of Croscarmellose Sodium, put in a 500-mL glass-stoppered conical flask, add 300 mL of sodium chloride TS, then add 25.0 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 5 minutes with occasional shaking. Add 5 drops of *m*-cresol purple TS, then add exactly 15 mL of 0.1 mol/L hydrochloric acid VS using a buret, stopper the flask, and shake. If the color of the solution is purple, add exactly 1-mL portions of 0.1 mol/L hydrochloric acid VS using the buret, with shaking each time, until the color of the solution changes to yellow, then titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to purple. Perform a blank determination in the same manner. Calculate the degrees of substitution of acid-carboxymethyl group and sodium-carboxymethyl group, A and S : $A + S$ is not less than 0.60 and not more than 0.85.

$$\begin{aligned} A & = 1150M / (7102 - 412M - 80C) \\ S & = (162 + 58A)C / (7102 - 80C) \end{aligned}$$

M : Amount (mmol) of sodium hydroxide needed to neutralize 1 g of sample, calculated on the dried basis

C : The value (%) obtained in Residue on ignition

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.44> 14.0 – 28.0% (after drying, 1 g).

Containers and storage Containers—Tight containers.

Cyanamide

シアナミド



CH₂N₂: 42.04
Aminonitrile [420-04-2]

Cyanamide contains not less than 97.0% and not more than 101.0% of CH₂N₂, calculated on the anhydrous basis.

Description Cyanamide occurs as white crystals or crystalline powder.

It is very soluble in water, in methanol, in ethanol (99.5) and in acetone.

The pH of a solution of Cyanamide (1 in 100) is between 5.0 and 6.5.

It is hygroscopic.

Melting point: about 46°C

Identification (1) To 1 mL of a solution of Cyanamide (1 in 100) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) Drop one or two drops of a solution of Cyanamide in acetone (1 in 100) onto a potassium bromide disk prepared as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and air-dry the disk. Determine the infrared absorption spectrum of the disk as directed in the film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cyanamide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Cyanamide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Water <2.48> Not more than 1.0% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Cyanamide, and dissolve in water to make exactly 250 mL. Pipet 15 mL of this solution, add 2 to 3 drops of dilute nitric acid, 10 mL of ammonia TS and exactly 50 mL of 0.1 mol/L silver nitrate VS, and allow to stand for 15 minutes with occasional shaking. Add water to make exactly 100 mL, filter, discard the first 20 mL of the filtrate, and pipet the subsequent 50 mL. After neutralizing this solution with dilute nitric acid, add 3 mL of dilute nitric acid, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 2.102 mg of CH₂N₂

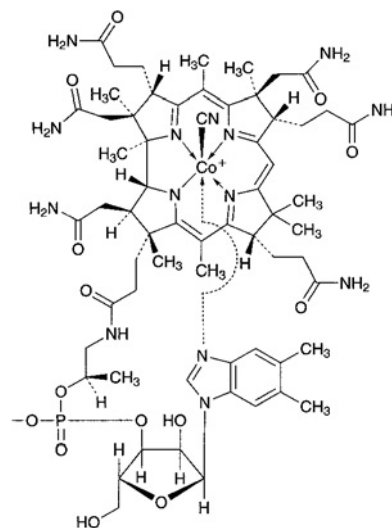
Containers and storage Containers—Tight containers.

Storage—In a cold place.

Cyanocobalamin

Vitamin B₁₂

シアノコバラミン



C₆₃H₈₈CoN₁₄O₁₄P: 1355.37

Co α -[α -(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]-Co β -cyanocobamide [68-19-9]

Cyanocobalamin contains not less than 96.0% and not more than 102.0% of C₆₃H₈₈CoN₁₄O₁₄P, calculated on the dried basis.

Description Cyanocobalamin occurs as dark red crystals or powder.

It is sparingly soluble in water, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Cyanocobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Cyanocobalamin with 0.05 g of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Transfer 5 mg of Cyanocobalamin to a 50-mL distilling flask, dissolve in 5 mL of water, and add 2.5 mL of hypophosphorous acid. Connect the flask with a short con-

denser, and dips its tip into a test tube containing 1 mL of a solution of sodium hydroxide (1 in 50). Heat gently for 10 minutes, then distil 1 mL into a test tube. To the test tube add 4 drops of a saturated solution of ammonium iron (II) sulfate hexahydrate, shake gently, then add about 0.03 g of sodium fluoride, and heat the contents to boil. Immediately add dropwise diluted sulfuric acid (1 in 7) until a clear solution results, then add 3 to 5 drops more of diluted sulfuric acid (1 in 7): a blue to blue-green color develops.

pH <2.54> Dissolve 0.10 g of Cyanocobalamin in 20 mL of water: the pH of this solution is between 4.2 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Cyanocobalamin in 10 mL of water: the solution is clear and red in color.

(2) Pseudocyanocobalamin—Dissolve 1.0 mg of Cyanocobalamin in 20 mL of water, transfer the solution to a separator, add 5 mL of a mixture of *m*-cresol and carbon tetrachloride (1:1), and shake vigorously for 1 minute. Allow to separate, draw off the lower layer into another separator, add 5 mL of diluted sulfuric acid (1 in 7), shake vigorously, and allow to separate completely. If necessary, centrifuge the mixture: the supernatant liquid is colorless or not more colored than the following control solution.

Control solution: Dilute 0.6 mL of 0.02 mol/L potassium permanganate VS with water to make 1000 mL.

Loss on drying <2.41> Not more than 12% (50 mg, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 4 hours).

Assay Weigh accurately about 20 mg each of Cyanocobalamin and Cyanocobalamin Reference Standard (previously determine the loss on drying <2.41> in the same manner as Cyanocobalamin), dissolve in water to make exactly 1000 mL, respectively, and use these solutions as the sample solution and the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution, respectively, at 361 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{63}H_{88}CoN_{14}O_{14}P \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Cyanocobalamin Reference Standard, calculated on the dried basis

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Cyanocobalamin Injection

Vitamin B₁₂ Injection

シアノコバラミン注射液

Cyanocobalamin Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 115.0% of the labeled amount of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$: 1355.37)

Method of preparation Prepare as directed under Injections, with Cyanocobalamin.

Description Cyanocobalamin Injection is a clear, light red to red liquid

It is gradually affected by light.

pH: 4.0 – 5.5

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 277 nm and 279 nm, between 360 nm, and 362 nm and between 548 nm and 552 nm. Determine the absorbances, A_1 and A_2 , of this solution at the wavelengths of maximum absorption between 360 nm and 362 nm, and between 548 nm and 552 nm, respectively: the ratio A_2/A_1 is not less than 0.29 and not more than 0.32.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Cyanocobalamin Injection, equivalent to about 2 mg of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$), add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin Reference Standard (previously determine the loss on drying <2.41> in the same manner as Cyanocobalamin), add water to make exactly 1000 mL, and use this solution as the standard solution. With these solutions, proceed as directed in the Assay under Cyanocobalamin.

$$\begin{aligned} \text{Amount (mg) of cyanocobalamin } (C_{63}H_{88}CoN_{14}O_{14}P) \\ = W_S \times (A_T/A_S) \times (1/10) \end{aligned}$$

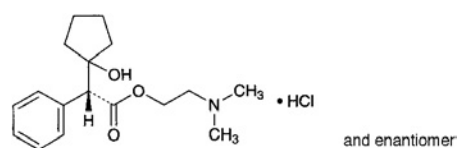
W_S : Amount (mg) of Cyanocobalamin Reference Standard, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Cyclopentolate Hydrochloride

シクロペントラート塩酸塩



$C_{17}H_{25}NO_3 \cdot HCl$: 327.85

2-(Dimethylamino)ethyl (2*RS*)-2-(1-hydroxycyclopentyl)phenylacetate monohydrochloride [5870-29-1]

Cyclopentolate Hydrochloride, when dried, contains not less than 98.5% of $C_{17}H_{25}NO_3 \cdot HCl$.

Description Cyclopentolate Hydrochloride occurs as a white, crystalline powder. It is odorless, or has a characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), in acetic acid (100) and in chloroform, sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 1 mL of a solution of Cyclopentolate Hydrochloride (1 in 100) add 1 mL of Reinecke salt TS: a

light red precipitate is formed.

(2) Dissolve 0.2 g of Cyclopentolate Hydrochloride in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute. After cooling, add 2 drops of nitric acid: a phenylacetic acid-like odor is perceptible.

(3) Determine the infrared absorption spectrum of Cyclopentolate Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Cyclopentolate Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.20 g of Cyclopentolate Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 5.5.

Melting point <2.60> 135 – 138°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cyclopentolate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cyclopentolate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Cyclopentolate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, *n*-butyl acetate, water and ammonia solution (28) (100:60:23:17) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in ethanol (99.5) (1 in 10) on the plate, and heat at 120°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.05% (1 g).

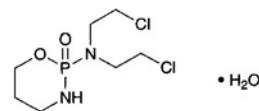
Assay Weigh accurately about 0.5 g of Cyclopentolate Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to yellow-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.79 mg of C₁₇H₂₅NO₃·HCl

Containers and storage Containers—Tight containers.

Cyclophosphamide Hydrate

シクロホスファミド水和物



C₇H₁₅Cl₂N₂O₂P·H₂O: 279.10

N,N-Bis(2-chloroethyl)-3,4,5,6-tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide monohydrate
[6055-19-2]

Cyclophosphamide Hydrate contains not less than 97.0% of C₇H₁₅Cl₂N₂O₂P·H₂O.

Description Cyclophosphamide Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in acetic acid (100), freely soluble in ethanol (95), in acetic anhydride and in chloroform, and soluble in water and in diethyl ether.

Melting point: 45 – 53°C

Identification (1) Dissolve 0.1 g of Cyclophosphamide Hydrate in 10 mL of water, and add 5 mL of silver nitrate TS: no precipitate is produced. Then boil this solution: a white precipitate is produced. Collect the precipitate, and add dilute nitric acid to a portion of this precipitate: it does not dissolve. Add excess ammonia TS to another portion of the precipitate: it dissolves.

(2) Add 1 mL of diluted sulfuric acid (1 in 25) to 0.02 g of Cyclophosphamide Hydrate, and heat until white fumes are evolved. After cooling, add 5 mL of water, and shake. Neutralize with ammonia TS, then acidify with dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (2) for phosphate.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Cyclophosphamide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.40 g of Cyclophosphamide Hydrate at a temperature not exceeding 20°C. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Cyclophosphamide Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Water <2.48> 5.5 – 7.0% (0.5 g, direct titration).

Assay Weigh accurately about 0.3 g of Cyclophosphamide Hydrate, add 15 mL of hydrogen chloride-ethanol TS, and heat in a water bath under a reflux condenser for 3.5 hours while protecting from moisture. Distil the ethanol under reduced pressure. Dissolve the residue in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from blue through green to yellow. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS

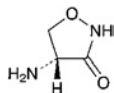
= 13.96 mg of $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Cycloserine

サイクロセリン



$C_3H_6N_2O_2$: 102.09

(4*R*)-4-Aminoisoxazolidin-3-one [68-41-7]

Cycloserine contains not less than 950 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the dried basis. The potency of Cycloserine is expressed as mass (potency) of cycloserine ($C_3H_6N_2O_2$).

Description Cycloserine occurs as white to light yellowish white, crystals or crystalline powder.

It is soluble in water, and sparingly soluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Cycloserine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Cycloserine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +108 – +114° (2.5 g calculated on the dried basis, 2 mol/L sodium hydroxide TS, 50 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Cycloserine in 20 mL of water: the pH of the solution is between 5.0 and 7.4.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Cycloserine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Condensation products—Dissolve 20 mg of Cycloserine in sodium hydroxide TS to make exactly 50 mL, and determine the absorbance of this solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.8.

Loss on drying <2.41> Not more than 1.5% (0.5 g, reduced pressure, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.0 to 6.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Cycloserine Reference Standard, previously dried at 60°C for 3 hours under reduced pressure of not exceeding 0.67 kPa,

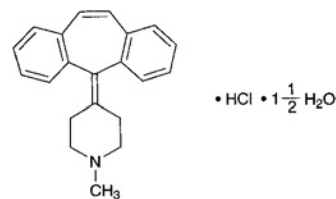
equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, and add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 100 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(4) Sample solutions—Weigh accurately an amount of Cycloserine equivalent to about 40 mg (potency), dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 100 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Cyproheptadine Hydrochloride Hydrate

シプロヘプタジン塩酸塩水和物



$C_{21}H_{21}N \cdot HCl \cdot 1\frac{1}{2}H_2O$: 350.88

4-(5*H*-Dibenzo[*a,d*]cyclohepten-5-ylidene)-1-methylpiperidine monohydrochloride sesquihydrate [41354-29-4]

Cyproheptadine Hydrochloride Hydrate, when dried, contains not less than 98.5% of cyproheptadine hydrochloride ($C_{21}H_{21}N \cdot HCl$: 323.86).

Description Cyproheptadine Hydrochloride Hydrate occurs as a white to pale yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in methanol and in acetic acid (100), soluble in chloroform, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.1 g of Cyproheptadine Hydrochloride Hydrate in 10 mL of methanol, apply 1 drop of this solution on filter paper, air-dry, and examine under ultraviolet light (main wavelength: 254 nm): the solution shows a pale blue fluorescence.

(2) Weigh 0.1 g of Cyproheptadine Hydrochloride Hydrate, transfer to a separator, dissolve in 5 mL of chloroform, add 4 mL of water and 1 mL of sodium carbonate TS, and shake. Transfer the chloroform layer to another separator, and wash with 4 mL of water by shaking well. Filter the chloroform layer through absorbent cotton moistened previously with chloroform, and evaporate the

filtrate to dryness. Dissolve the residue in 8 mL of dilute ethanol by warming at 65°C. Rub the inner wall of the container with a glass rod while cooling until crystallization begins, and allow to stand for 30 minutes. Collect the crystals, and dry at 80°C for 2 hours: the crystals melt <2.60> between 111°C and 115°C.

(3) Determine the absorption spectrum of a solution of Cyproheptadine Hydrochloride Hydrate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A saturated solution of Cyproheptadine Hydrochloride Hydrate responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Acidity—Dissolve 2.0 g of Cyproheptadine Hydrochloride Hydrate in 25 mL of methanol, and add 1 drop of methyl red TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Cyproheptadine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> 7.0 – 9.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

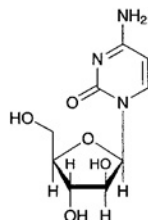
Assay Weigh accurately about 0.5 g of Cyproheptadine Hydrochloride Hydrate, previously dried, and dissolve in 20 mL of acetic acid (100) by warming at 50°C. After cooling, add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.39 mg of C₂₁H₂₁N.HCl

Containers and storage Containers—Well-closed containers.

Cytarabine

シタラビン



C₉H₁₃N₃O₅: 243.22
1-β-D-Arabinofuranosylcytosine
[147-94-4]

Cytarabine, when dried, contains not less than 98.5% and not more than 101.0% of C₉H₁₃N₃O₅.

Description Cytarabine occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in acetic acid (100), and

very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 214°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Cytarabine in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cytarabine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +154 – +160° (after drying, 0.1 g, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of Cytarabine in 20 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Cytarabine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 1.0 g of Cytarabine. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Cytarabine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Cytarabine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add water to make exactly 25 mL and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with 1-butanol saturated with water to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the number of them which are more intense than the spot from the standard solution (2) is not more than two. Spray evenly acidic potassium permanganate TS on the plate: any spot other than the principal spot does not appear.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

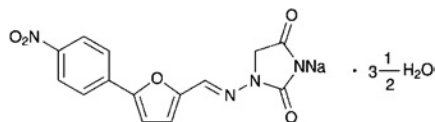
Assay Weigh accurately about 0.2 g of Cytarabine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.16 mg of C₉H₁₃N₃O₅

Containers and storage Containers—Tight containers.

Dantrolene Sodium Hydrate

ダントロレンナトリウム水和物



$C_{14}H_9N_4NaO_5 \cdot 3\frac{1}{2}H_2O$: 399.29

Monosodium 3-[5-(4-nitrophenyl)furan-2-ylmethylene]amino-2,5-dioxo-1,3-imidazolidinate hemiheptahydrate [14663-23-1, anhydride]

Dantrolene Sodium Hydrate contains not less than 98.0% of dantrolene sodium ($C_{14}H_9N_4NaO_5$: 336.23), calculated on the anhydrous basis.

Description Dantrolene Sodium Hydrate occurs as a yellowish orange to deep orange, crystalline powder.

It is soluble in propylene glycol, sparingly soluble in methanol, slightly soluble in ethanol (95), very slightly soluble in water and in acetic acid (100), and practically insoluble in acetone, in tetrahydrofuran and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Dantrolene Sodium Hydrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dantrolene Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Dantrolene Sodium Hydrate add 20 mL of water and 2 drops of acetic acid (100), shake well, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Alkalinity—To 0.7 g of Dantrolene Sodium Hydrate add 10 mL of water, shake well, and centrifuge or filter through a membrane filter. To 5 mL of the supernatant or the filtrate add 45 mL of water, 3 drops of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS: a red color is not produced.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Dantrolene Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related Substances—Dissolve 50 mg of Dantrolene Sodium Hydrate in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100), add ethanol (99.5) to make 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total

area of all peaks other than the peak of dantrolene from the sample solution is not larger than the peak area of dantrolene from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 300 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of hexane, acetic acid (100) and ethanol (99.5) (90:10:9).

Flow rate: Adjust the flow rate so that the retention time of dantrolene is about 8 minutes.

Selection of column: Dissolve 5 mg of Dantrolene Sodium Hydrate and 0.1 g of theophylline in 20 mL of tetrahydrofuran and 2 mL of acetic acid (100), and add ethanol (99.5) to make 100 mL. To 10 mL of this solution add ethanol (99.5) to make 100 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of theophylline and dantrolene in this order with the resolution between these peaks being not less than 6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dantrolene from 10 μ L of the standard solution is 10% to 40% of the full scale.

Time span of measurement: About twice as long as the retention time of dantrolene beginning after the solvent peak.

Water <2.48> 14.5 – 17.0% (0.2 g, direct titration).

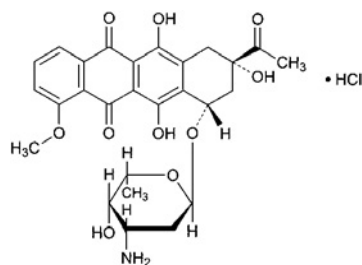
Assay Weigh accurately about 0.7 g of Dantrolene Sodium Hydrate, dissolve in 180 mL of a mixture of propylene glycol and acetone (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.624 mg of $C_{14}H_9N_4NaO_5$

Containers and storage Containers—Tight containers.

Daunorubicin Hydrochloride

ダウノルビシン塩酸塩



$C_{27}H_{29}NO_{10} \cdot HCl$: 563.98

(2S,4S)-2-Acetyl-4-(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyloxy)-2,5,12-trihydroxy-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride [23541-50-6]

Daunorubicin Hydrochloride is the hydrochloride of

an anthracycline substance having antitumor activity produced by the growth of *Streptomyces peucetius*.

It contains not less than 940 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the dried basis. The potency of Daunorubicin Hydrochloride is expressed as mass (potency) of daunorubicin hydrochloride ($\text{C}_{27}\text{H}_{29}\text{NO}_{10}\cdot\text{HCl}$).

Description Daunorubicin Hydrochloride occurs as a red powder.

It is soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Daunorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Daunorubicin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) A solution of Daunorubicin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +250 – +275° (15 mg calculated on the dried basis, methanol, 10 mL, 100 mm).

pH <2.54> Dissolve 0.15 g of Daunorubicin Hydrochloride in 30 mL of water: the pH of the solution is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Daunorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Daunorubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Daunorubicin Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water and acetic acid (100) (15:5:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the naked eye: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 7.5% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Daunorubicin Hydrochloride and Daunorubicin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve each in a suitable amount of the mobile phase, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of

the peak area of daunorubicin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{27}\text{H}_{29}\text{NO}_{10}\cdot\text{HCl} \\ = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Daunorubicin Hydrochloride Reference Standard

Internal standard solution—A solution of 2-naphthalenesulfonic acid in the mobile phase (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of a mixture of water and acetonitrile (31:19) to 2.2 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of daunorubicin is about 9 minutes.

System suitability—

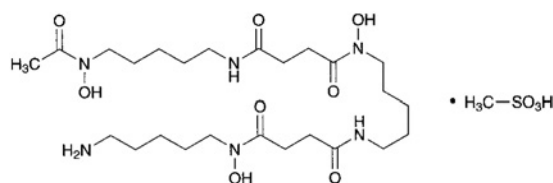
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and daunorubicin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of daunorubicin to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Deferoxamine Mesilate

デフェロキサミンメシル酸塩



$\text{C}_{25}\text{H}_{48}\text{N}_6\text{O}_8\cdot\text{CH}_4\text{O}_3\text{S}$: 656.79

N-[5-(Acetylhydroxycarbonyl)pentyl]-*N'*-(5-{3-[(5-aminopentyl)hydroxycarbonyl]propanoylamino}pentyl)-*N'*-hydroxysuccinamide monomethanesulfonate [138-14-7]

Deferoxamine Mesilate contains not less than 98.0% and not more than 102.0% of $\text{C}_{25}\text{H}_{48}\text{N}_6\text{O}_8\cdot\text{CH}_4\text{O}_3\text{S}$, calculated on the anhydrous basis.

Description Deferoxamine Mesilate occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5), in 2-propanol and in diethyl ether.

Melting point: about 147°C (with decomposition).

Identification (1) To 5 mL of a solution of Deferoxamine Mesilate (1 in 500) add 1 drop of iron (III) chloride TS: a deep red color develops.

(2) To 0.05 g of Deferoxamine Mesilate add 0.2 g of sodium hydroxide, melt by heating over a small flame, and heat further for 2 to 3 seconds. To the residue add 0.5 mL of water, acidify with dilute hydrochloric acid, and warm: the gas evolved changes moistened potassium iodate-starch paper to blue.

(3) Determine the infrared absorption spectrum of Deferoxamine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Deferoxamine Mesilate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Deferoxamine Mesilate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—Perform the test with 1.0 g of Deferoxamine Mesilate. Prepare the control solution with 0.90 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.032%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of Deferoxamine Mesilate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.040%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Deferoxamine Mesilate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Deferoxamine Mesilate according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Deferoxamine Mesilate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the peak of deferoxamine is not larger than the peak area of deferoxamine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.32 g of diammonium hydrogen phosphate, 0.37 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate and 1.08 g of sodium 1-heptanesulfonate in 950 mL of water, and adjust the pH of this solution

to 2.8 with phosphoric acid. To 800 mL of this solution add 100 mL of 2-propanol.

Flow rate: Adjust the flow rate so that the retention time of deferoxamine is about 15 minutes.

Time span of measurement: About two times as long as the retention time of deferoxamine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of deferoxamine obtained from 20 μ L of this solution is equivalent to 1.5 to 2.5% of that of deferoxamine obtained from 20 μ L of the standard solution.

System performance: Dissolve 16 mg of Deferoxamine Mesilate and 4 mg of methyl parahydroxybenzoate in 50 mL of the mobile phase. When the procedure is run with 20 μ L of this solution under the above operating conditions, deferoxamine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of deferoxamine is not more than 3.0%.

Water <2.48> Not more than 2.0% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Deferoxamine Mesilate and Deferoxamine Mesilate Reference Standard (previously determine the water <2.48> in the same manner as Deferoxamine Mesilate), dissolve each in 20 mL of water, add 10 mL of 0.05 mol/L sulfuric acid TS, and add water to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of 0.05 mol/L sulfuric acid TS and exactly 0.2 mL of iron (III) chloride TS, then add water to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 0.05 mol/L sulfuric acid TS to 0.2 mL of iron (III) chloride TS to make exactly 50 mL as the blank, and determine the absorbances, A_T and A_S , of each solution from the sample solution and the standard solution at 430 nm.

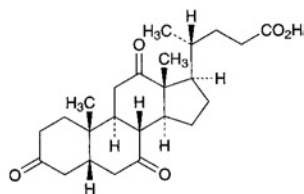
$$\begin{aligned} \text{Amount (mg) of } C_{25}H_{48}N_6O_8 \cdot CH_4O_3S \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Deferoxamine Mesilate Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Dehydrocholic Acid

デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5 β -cholan-24-oic acid [81-23-2]

Dehydrocholic Acid, when dried, contains not less than 98.5% of $C_{24}H_{34}O_5$.

Description Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and a blue-green fluorescence.

(2) To 0.02 g of Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation <2.49> $[\alpha]_D^{20}$: +29 - +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 233 - 242°C

Purity (1) Odor—To 2.0 g of Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—To 0.10 g of Dehydrocholic Acid, previously powdered in a mortar, add 30 mL of ethanol (95), and dissolve by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride <1.03>—To 2.0 g of Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate <1.14>—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Pre-

pare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes. Cool, filter, and wash with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

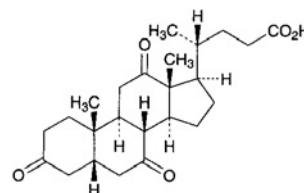
Assay Weigh accurately about 0.5 g of Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, titrate <2.50> with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.

Purified Dehydrocholic Acid

精製デヒドロコール酸



$C_{24}H_{34}O_5$: 402.52
3,7,12-Trioxo-5 β -cholan-24-oic acid [81-23-2]

Purified Dehydrocholic Acid, when dried, contains not less than 99.0% of $C_{24}H_{34}O_5$.

Description Purified Dehydrocholic Acid occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is sparingly soluble in 1,4-dioxane, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Purified Dehydrocholic Acid in 1 mL of sulfuric acid and 1 drop of formaldehyde solution, and allow to stand for 5 minutes. Add 5 mL of water to the solution: the solution shows a yellow color and blue-green fluorescence.

(2) To 0.02 g of Purified Dehydrocholic Acid add 1 mL of ethanol (95), shake, add 5 drops of 1,3-dinitrobenzene TS and 0.5 mL of a solution of sodium hydroxide (1 in 8), and allow to stand: a purple to red-purple color develops, and gradually changes to brown.

Optical rotation <2.49> $[\alpha]_D^{20}$: +29 – +32° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 237 – 242°C

Purity (1) Odor—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, and boil for 2 minutes: the solution is odorless.

(2) Clarity and color of solution—Dissolve 0.10 g of Purified Dehydrocholic Acid, previously powdered in a mortar, in 30 mL of ethanol (95) by shaking for 10 minutes: the solution is clear and colorless.

(3) Chloride <1.03>—To 2.0 g of Purified Dehydrocholic Acid add 100 mL of water, shake for 5 minutes and filter, and use this filtrate as the sample solution. To 25 mL of the sample solution add 6 mL of dilute nitric acid, heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(4) Sulfate <1.14>—Add 1 mL of dilute hydrochloric acid to 25 mL of the sample solution obtained in (3), heat in a water bath for 6 minutes, filter after cooling, and collect the clear filtrate. Wash the residue with 10 mL of water, combine the washings and the filtrate, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Purified Dehydrocholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, and boil for 2 minutes, cool, filter, and wash the filter with water until 100 mL of the filtrate is obtained. To 10 mL of the filtrate add 1 mL of dilute sulfuric acid: no turbidity is produced.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Purified Dehydrocholic Acid, previously dried, add 40 mL of neutralized ethanol and 20 mL of water, and dissolve by warming. Add 2 drops of phenolphthalein TS, then titrate <2.50> with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Well-closed containers.

Dehydrocholic Acid Injection

Dehydrocholate Sodium Injection

デヒドロコール酸注射液

Dehydrocholic Acid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of dehydrocholic acid ($C_{24}H_{34}O_5$; 402.52).

Method of preparation Dissolve Purified Dehydrocholic Acid in a solution of Sodium Hydroxide, and prepare as directed under Injections.

Description Dehydrocholic Acid Injection is a clear, colorless to light yellow liquid, and has a bitter taste.

pH: 9 – 11

Identification Transfer a volume of Dehydrocholic Acid Injection, equivalent to 0.1 g of Purified Dehydrocholic Acid according to the labeled amount, to a separator, and add 10 mL of water and 1 mL of dilute hydrochloric acid: a white precipitate is produced. Extract the mixture with three 15-mL portions of chloroform, combine all the chloroform extracts, evaporate the chloroform on a water bath, and dry the residue at 105°C for 1 hour: the residue so obtained melts <2.60> between 235°C and 242°C.

Purity Heavy metals <1.07>—Evaporate a volume of Dehydrocholic Acid Injection, equivalent to 1.0 g of Purified Dehydrocholic Acid according to the labeled amount, on a water bath to dryness. Proceed with the residue according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Bacterial endotoxins <4.01> Less than 0.30 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay Transfer an exactly measured volume of Dehydrocholic Acid Injection, equivalent to about 0.5 g of dehydrocholic acid ($C_{24}H_{34}O_5$), to a 100-mL separator, and add, if necessary, water to make 25 mL. Add 2 mL of hydrochloric acid, and extract with 25-mL, 20-mL and 15-mL portions of chloroform successively. Combine the chloroform extracts, wash with cold water until the washings become negative to acid, and evaporate the chloroform on a water bath. Dissolve the residue in 40 mL of neutralized ethanol and 20 mL of water by warming. Add 2 drops of phenolphthalein TS to this solution, titrate <2.50> with 0.1 mol/L sodium hydroxide VS, adding 100 mL of freshly boiled and cooled water as the end point is approached, and continue the titration.

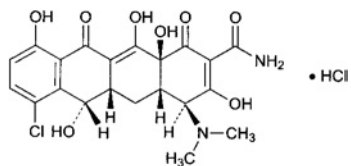
Each mL of 0.1 mol/L sodium hydroxide VS
= 40.25 mg of $C_{24}H_{34}O_5$

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Demethylchlortetracycline Hydrochloride

デメチルクロルテトラサイクリン塩酸塩



$C_{21}H_{21}ClN_2O_8 \cdot HCl$: 501.31
(4*S*,4*aS*,5*aS*,6*S*,12*aS*)-7-Chloro-4-dimethylamino-3,6,10,12,12*a*-pentahydroxy-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracycane-2-carboxamide monohydrochloride [64-73-3]

Demethylchlortetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity produced by the growth of the mutant of *Streptomyces aureofaciens*.

It contains not less than 900 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Demethylchlortetracycline Hydrochloride is expressed as mass (potency) of demethylchlortetracycline hydrochloride ($C_{21}H_{21}ClN_2O_8 \cdot HCl$).

Description Demethylchlortetracycline Hydrochloride occurs as a yellow crystalline powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Dissolve 40 mg of Demethylchlortetracycline Hydrochloride in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Demethylchlortetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Demethylchlortetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Demethylchlortetracycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Demethylchlortetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-248 - -263^\circ$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Demethylchlortetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 2.0 and 3.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of

Demethylchlortetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Demethylchlortetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Demethylchlortetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from the chromatograms of these solutions by the automatic integration method: the peak area other than demethylchlortetracycline obtained from the sample solution is not more than 6/5 times that of demethylchlortetracycline from the standard solution, and the sum of the areas of the peaks other than demethylchlortetracycline from the sample solution is not more than 2 times the peak area of demethylchlortetracycline from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of demethylchlortetracycline beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 10 mL of the standard solution, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Confirm that the peak area of demethylchlortetracycline obtained from 20 μL of this solution is equivalent to 7 to 13% of that from 20 μL of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Demethylchlortetracycline Hydrochloride and Demethylchlortetracycline Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve each in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of demethylchlortetracycline.

Amount [μg (potency)] of $C_{21}H_{21}ClN_2O_8 \cdot HCl$

$$= W_S \times (A_T/A_S) \times 1000$$

W_S : Amount [mg (potency)] of Demethylchlortetracycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.1 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate, 1.5 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, and adjust the pH to 8.5 with sodium hydroxide TS. To this solution add 75.0 g of *t*-butanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of demethylchlortetracycline is about 8 minutes.

System suitability—

System performance: Heat 10 mL of the standard solution on a water bath for 60 minutes. When the procedure is run with 20 μ L of this solution so obtained under the above operating conditions, 4-epidemethylchlortetracycline and demethylchlortetracycline are eluted in this order with the resolution between these peaks being not less than 3. The relative retention time of 4-epidemethylchlortetracycline with respect to demethylchlortetracycline is about 0.7.

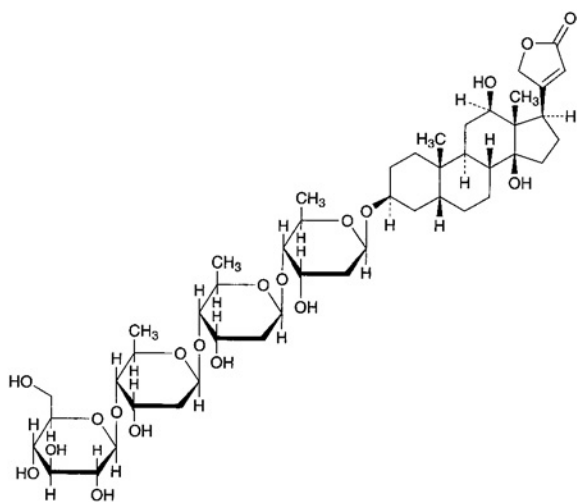
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of demethylchlortetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Deslanoside

デスラノシド



$C_{47}H_{74}O_{19}$: 943.08

3 β -[β -D-Glucopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-

β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy-
 β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-
hexopyranosyloxy]-12 β ,14-dihydroxy-5 β ,14 β -card-
20(22)-enolide [17598-65-1]

Deslanoside, when dried, contains not less than 90.0% and not more than 102.0% of $C_{47}H_{74}O_{19}$.

Description Deslanoside occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is freely soluble in anhydrous pyridine, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Transfer 1 mg of Deslanoside to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 1000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of two liquids a brown ring is produced, and the color of the upper layer near to the contact zone changes gradually to blue through purple, and the entire acetic acid layer shows a blue-green color through a deep blue color.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Deslanoside in 10 mL of ethanol (95) and 3 mL of water by warming, cool, and dilute to 100 mL with water: the solution is clear and colorless.

(2) Related substances—Dissolve 10 mg of Deslanoside in exactly 5 mL of methanol, and use this solution as the sample solution. Dissolve 1.0 mg of Deslanoside Reference Standard in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not larger than and not more intense than the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: +6.5 – +8.5° (after drying, 0.5 g, anhydrous pyridine, 25 mL, 100 mm).

Loss on drying <2.41> Not more than 8.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Dissolve about 12 mg each of Deslanoside and Deslanoside Reference Standard, previously dried and accurately weighed, in 20 mL each of methanol, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of these solutions, transfer to light-resistant, 25-mL volumetric flasks, shake well with 5 mL each of 2,4,6-trinitrophenol TS and 0.5 mL each of a solution of sodium hydroxide (1 in 10), add diluted methanol (1 in 4) to make 25 mL, and allow to stand at a temperature between 18°C and 22°C for 25 minutes. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution, respectively, at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared

with 5 mL of diluted methanol (1 in 5) in the same manner as the blank.

$$\begin{aligned} \text{Amount (mg) of } C_{47}H_{74}O_{19} \\ = W_s \times (A_T/A_S) \end{aligned}$$

W_s : Amount (mg) of Deslanoside Reference Standard

Containers and storage Containers—Tight containers.

Deslanoside Injection

デスラノシド注射液

Deslanoside Injection is an aqueous solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of deslanoside ($C_{47}H_{74}O_{19}$; 943.08).

Method of preparation Dissolve Deslanoside in 10 vol% ethanol and prepare as directed under Injections. It may contain Glycerin. It may be prepared with a suitable amount of Ethanol and Water for Injection.

Description Deslanoside Injection is a clear and colorless liquid.

pH: 5.0 – 7.0

Identification (1) Place a volume of Deslanoside Injection, equivalent to 2 mg of Deslanoside according to the labeled amount, in a separator, add sodium chloride in the ratio of 0.2 g to each mL of this solution, and extract with three 10-mL portions of chloroform. Combine the chloroform extracts, mix uniformly, pipet 15 mL of this solution, and evaporate the chloroform under reduced pressure. Proceed with the residue as directed in the Identification under Deslanoside.

(2) Place a volume of Deslanoside Injection, equivalent to 1 mg of Deslanoside according to the labeled amount, in a separator, add sodium chloride in the ratio of 0.2 g to each mL of this solution, and extract with three 5-mL portions of chloroform. Combine the chloroform extracts, evaporate the chloroform under reduced pressure, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of Deslanoside Reference Standard in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat the plate at 110°C for 10 minutes: the spots from the sample solution and the standard solution show a black color and have the same R_f value.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Deslanoside Injection, equivalent to about 3 mg of deslanoside ($C_{47}H_{74}O_{19}$). Add 5 mL of methanol and water to make 25 mL. Use this solution as the sample solution, and proceed as directed in the Assay under Deslanoside.

$$\text{Amount (mg) of deslanoside } (C_{47}H_{74}O_{19})$$

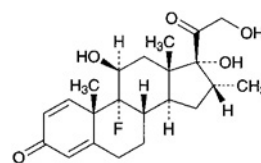
$$= W_s \times (A_T/A_S) \times (1/4)$$

W_s : Amount (mg) of Deslanoside Reference Standard

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Dexamethasone

デキサメタゾン



$C_{22}H_{29}FO_5$; 392.46

9-Fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione [50-02-2]

Dexamethasone, when dried, contains not less than 97.0% and not more than 102.0% of $C_{22}H_{29}FO_5$.

Description Dexamethasone occurs as white to pale yellow crystals or crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in acetonitrile, and practically insoluble in water.

Melting point: about 245°C (with decomposition).

Identification (1) Proceed with 10 mg of Dexamethasone as directed under Oxygen Flask Combustion Method, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the solution obtained responds to the Qualitative Tests <1.09> for fluoride

(2) Dissolve 1 mg of Dexamethasone in 10 mL of ethanol (95). Mix 2 mL of the solution with 10 mL of phenylhydrazine hydrochloride TS, heat in a water bath at 60°C for 20 minutes, and cool the solution. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using as the blank the solution prepared with 2 mL of ethanol (95) in the same manner as the former solution, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dexamethasone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dexamethasone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Dexamethasone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Dexamethasone and Dexamethasone Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +86 – +94° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of

Dexamethasone according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.18 g of Dexamethasone in 100 mL of acetonitrile. To 33 mL of this solution add a solution, prepared by dissolving 1.32 g of ammonium formate in water to make 1000 mL and adjusted to pH 3.6 with formic acid, to make 100 mL, and use this solution as the sample solution. To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than dexamethasone is not larger than the peak area of dexamethasone obtained from the standard solution, and the total area of the peaks other than the peak of dexamethasone from the sample solution is not larger than 2 times the peak area of dexamethasone from the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.32 g of ammonium formate in 1000 mL of water, and adjust the pH to 3.6 with formic acid. To 670 mL of this solution add 330 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 13 minutes.

Time span of measurement: About 4 times as long as the retention time of dexamethasone beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of dexamethasone obtained with 10 μ L of this solution is equivalent to 8 to 12% of that with 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of dexamethasone are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dexamethasone is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (0.2 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 10 mg each of Dexamethasone and Dexamethasone Reference Standard, previously dried and accurately weighed, in 70 mL each of diluted methanol (1 in 2), add exactly 5 mL each of the internal standard solution, then add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and standard solution.

Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dexamethasone to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{22}H_{29}FO_5 = W_S \times Q_T / Q_S$$

W_S : Amount (mg) of Dexamethasone Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of dexamethasone is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, dexamethasone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dexamethasone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dextran 40

デキストラン 40

Dextran 40 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 40,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 40.

Description Dextran 40 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 40 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH <2.54> Dissolve 1.0 g of Dextran 40 in 10 mL of water:

the pH of this solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dextran 40 in 10 mL of water by warming: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 2.0 g of Dextran 40. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid (not more than 0.018%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextran 40 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.5 g of Dextran 40 according to Method 1, and perform the test (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 40, previously dried, and perform the test as directed under Nitrogen Determination <1.08>, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.01) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.450 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL each of these solutions, add 5 mL of alkaline copper TS, exactly measured, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodine (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Viscosity <2.53> (1) Dextran 40—Weigh accurately 0.2 to 0.5 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.16 and 0.19.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually 80 to 90 mL) at 25 ± 1°C with stirring. Dissolve the precipitate at 35°C in a water bath with occasional shaking, and allow to stand for more than 15 hours at 25 ± 1°C. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not more than 0.27.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 40, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 115 to 135 mL) at 25 ± 1°C with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath.

Dry the residue, and determine the intrinsic viscosity of the dried substance as directed in (1): the value is not less than 0.09.

Antigenicity Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit no signs mentioned above.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Pyrogen <4.04> Dissolve 10.0 g of Dextran 40 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirement.

Assay Weigh accurately about 3 g of Dextran 40, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under Optical Rotation Determination <2.49> in a 100-mL cell at 20 ± 1°C.

$$\text{Amount (mg) of dextran 40} = \alpha_D \times 253.8$$

Containers and storage Containers—Tight containers.

Dextran 40 Injection

デキストラン 40 注射液

Dextran 40 Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of dextran 40.

Method of preparation

Dextran 40	10 g
Isotonic Sodium Chloride Solution	a sufficient quantity
To make 100 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Dextran 40 Injection is a clear and colorless liquid. It is slightly viscous.

Identification (1) Dilute 1 mL of Dextran 40 Injection with water to 200 mL, and to 1 mL of the diluted solution add 2 mL of anthrone TS: a blue-green color develops and

turns gradually dark blue-green. Add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution does not change in color.

(2) Dextran 40 Injection responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

pH <2.54> 4.5 – 7.0

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Viscosity <2.53> Measure exactly 2 to 5 mL of Dextran 40 Injection, add isotonic sodium chloride solution to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with isotonic sodium chloride solution as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.16 and 0.19. Calculate the concentration of the sample solution (g/100 mL) as directed in the Assay.

Assay To exactly 30 mL of Dextran 40 Injection add water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D with the sample solution as directed under Optical Rotation Determination <2.49> in a 100-mL cell at $20 \pm 1^\circ\text{C}$.

$$\begin{aligned} &\text{Amount (mg) of dextran 40 in 100 mL of} \\ &\text{Dextran 40 Injection} \\ &= \alpha_D \times 846.0 \end{aligned}$$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Storage—Avoid exposure to undue fluctuations in temperature.

Dextran 70

デキストラン 70

Dextran 70 is a product obtained by partial decomposition of polysaccharide, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* van Tieghem (*Lactobacillaceae*), and the average molecular mass is about 70,000.

When dried, it contains not less than 98.0% and not more than 102.0% of dextran 70.

Description Dextran 70 occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves gradually in water.

It is hygroscopic.

Identification To 1 mL of a solution of Dextran 70 (1 in 3000) add 2 mL of anthrone TS: a blue-green color develops and turns gradually dark blue-green. Then to this solution add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100): the solution does not change in color.

pH <2.54> Dissolve 3.0 g of Dextran 70 in 50 mL of water: the pH of this solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dextran 70 in 10 mL of water with warming: the solution is

clear and colorless.

(2) Chloride <1.03>—With 2.0 g of Dextran 70, perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextran 70 according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.5 g of Dextran 70 according to Method 1, and perform the test (not more than 1.3 ppm).

(5) Nitrogen—Weigh accurately about 2 g of Dextran 70, previously dried, perform the test as directed under Nitrogen Determination <1.08>, where 10 mL of sulfuric acid is used for decomposition, and 45 mL of a solution of sodium hydroxide (2 in 5) is added: the amount of nitrogen (N: 14.007) is not more than 0.010%.

(6) Reducing substances—Weigh exactly 3.00 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 0.300 g of glucose, previously dried, dissolve in water to make exactly 500 mL, and use this solution as the control solution. Pipet 5 mL each of the sample solution and the control solution, and add water to make exactly 50 mL, respectively. Pipet 5 mL of these diluted solutions, add exactly 5 mL of alkaline copper TS, and heat for 15 minutes in a water bath. After cooling, add 1 mL of a solution of potassium iodide (1 in 40) and 1.5 mL of dilute sulfuric acid, and titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

The titrant consumed for the sample solution is not less than that for the control solution.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 6 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Viscosity <2.53> (1) Dextran 70—Weigh accurately 0.2 to 0.5 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and with water as directed in Method 1 at 25°C: the intrinsic viscosity is between 0.21 and 0.26.

(2) High-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 7% to 10% of the precipitate (usually, 75 to 85 mL) at $25 \pm 1^\circ\text{C}$ with stirring. Dissolve the precipitate in a water bath at 35°C with occasional shaking, and allow to stand for more than 15 hours at $25 \pm 1^\circ\text{C}$. Remove the supernatant liquid by decantation, and heat the precipitate of the lower layer on a water bath to dryness. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not more than 0.35.

(3) Low-molecular fraction—Weigh accurately about 6 g of Dextran 70, previously dried, dissolve in water to make exactly 100 mL, and transfer to a flask. Add slowly enough methanol to get 90% to 93% of the precipitate (usually 110 to 130 mL) at $25 \pm 1^\circ\text{C}$ with stirring, centrifuge at 25°C, and evaporate the supernatant liquid to dryness on a water bath. Dry the residue, and determine the intrinsic viscosity of the dried residue as directed in (1): the value is not less than 0.10.

Antigenicity Dissolve 6.0 g of Dextran 70 in isotonic sodi-

um chloride solution to make 100 mL, sterilize, and use this solution as the sample solution. Inject 1.0 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Separately, inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously to each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit not signs.

All the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Pyrogen <4.04> Dissolve 6.0 g of Dextran 70 in isotonic sodium chloride solution to make 100 mL, and perform the test: this solution meets the requirement.

Assay Weigh accurately about 3 g of Dextran 70, previously dried, dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Determine the optical rotation α_D as directed under Optical Rotation Determination <2.49> in a 100-mL cell at $20 \pm 1^\circ\text{C}$.

$$\text{Amount (mg) of dextran 70} \times \alpha_D = 253.8$$

Containers and storage Containers—Tight containers.

Dextran Sulfate Sodium Sulfur 5

デキストラン硫酸エステルナトリウム イオウ 5

Dextran Sulfate Sodium Sulfur 5 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* Van Tieghem (*Lactobacillaceae*).

Description Dextran Sulfate Sodium Sulfur 5 occurs as a white to light yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification (1) To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

(2) To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 5 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

(3) A solution of Dextran Sulfate Sodium Sulfur 5 (1 in 100) responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +135.0 – +155.0° (calculated

on the dried basis, 1.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 5 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

Purity (1) Clarity of solution—Dissolve 2.5 g of Dextran Sulfate Sodium Sulfur 5 in 50 mL of water: the solution is clear. And, determine the absorbance of the solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.090.

(2) Chloride <1.03>—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfur 5. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.106%).

(3) Sulfate <1.14>—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 5 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid add 6 mL of water, and proceed in the same manner (not more than 0.240%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 5 according to Method 3, and perform the test (not more than 2 ppm).

Sulfur content Weigh accurately about 1.0 g of Dextran Sulfate Sodium Sulfur 5, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 3.0 and 6.0%.

$$\begin{aligned} \text{Each mL of 0.02 mol/L barium chloride VS} \\ = 0.6413 \text{ mg of S} \end{aligned}$$

Loss on drying <2.41> Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Viscosity <2.53> Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 5, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at $25 \pm 0.02^\circ\text{C}$ as directed: the intrinsic viscosity is between 0.030 and 0.040.

Containers and storage Containers—Tight containers.

Dextran Sulfate Sodium Sulfur 18

デキストラン硫酸エステルナトリウム イオウ 18

Dextran Sulfate Sodium Sulfur 18 is a sodium salt of sulfate ester obtained by sulfation of partial decomposition products of dextran, which is produced by fermentation of sucrose with *Leuconostoc mesenteroides* Van Tieghem (*Lactobacillaceae*).

Description Dextran Sulfate Sodium Sulfur 18 occurs as a white to light yellowish white powder. It is odorless, and has a saline taste.

It is freely soluble in water and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification (1) To 10 mL of a solution of toluidine blue (1 in 100,000) add 0.05 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (3 in 50) dropwise: a color of the solution changes from blue to red-purple.

(2) To 1 mL of a solution of Dextran Sulfate Sodium Sulfur 18 (1 in 1500) add 2 mL of anthrone TS: a blue-green color develops, which turns dark blue-green gradually. Then, add 1 mL of diluted sulfuric acid (1 in 2) or 1 mL of acetic acid (100) to this solution: the solution remains dark blue-green.

(3) A solution of Dextran Sulfate Sodium Sulfur 18 (1 in 100) responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +90.0 – +110.0° (calculated on the dried basis, 1.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Dextran Sulfate Sodium Sulfur 18 in 20 mL of water: the pH of this solution is between 5.5 and 7.5.

Purity (1) Chloride <1.03>—Perform the test with 0.10 g of Dextran Sulfate Sodium Sulfur 18. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.106%).

(2) Sulfate <1.14>—Dissolve 0.10 g of Dextran Sulfate Sodium Sulfur 18 in 6 mL of water, add 0.6 mL of barium chloride TS, and heat in a water bath for 4 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL, allow to stand for 10 minutes, and observe: the turbidity of the solution is not more intense than that of the control solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid add 6 mL of water, and proceed in the same manner (not more than 0.480%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dextran Sulfate Sodium Sulfur 18 according to Method 3, and perform the test (not more than 2 ppm).

Sulfur content Weigh accurately about 0.5 g of Dextran Sulfate Sodium Sulfur 18, dissolve in 5 mL of water, add 1.5 mL of hydrochloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. To exactly 10 mL of the

sample solution add exactly 20 mL of 0.02 mol/L barium chloride VS, add 5 mL of methanol, and heat in a water bath for 30 minutes. After cooling, neutralize with sodium hydroxide TS, and add 70 mL of water, 10 mL of a solution of zinc disodium ethylenediamine tetraacetate tetrahydrate (1 in 20), 3 mL of ammonium chloride TS and 7 mL of strong ammonium water, and titrate <2.50> with 0.02 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red to light blue (indicator: 5 drops of eriochrome black T TS). Perform a blank determination. Amount of sulfur (S: 32.07), calculated on the dried basis, is between 15.0 and 20.0%.

Each mL of 0.02 mol/L barium chloride VS
= 0.6413 mg of S

Loss on drying <2.41> Not more than 10.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Viscosity <2.53> Weigh accurately about 1.5 g of Dextran Sulfate Sodium Sulfur 18, calculated on the dried basis, dissolve in a solution of sodium chloride (29 in 500) to make exactly 100 mL, and use this solution as the sample solution. Perform the test with the sample solution and a solution of sodium chloride (29 in 500) at $25 \pm 0.02^\circ\text{C}$ as directed: the intrinsic viscosity is between 0.020 and 0.032.

Containers and storage Containers—Tight containers.

Dextrin

デキストリン

Description Dextrin occurs as a white or light yellow, amorphous powder or granules. It has a slight, characteristic odor and a sweet taste. It does not irritate the tongue. Dextrin is freely soluble in boiling water, soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification To 0.1 g of Dextrin add 100 mL of water, shake, and filter if necessary. To 5 mL of the filtrate add 1 drop of iodine TS: a light red-brown or light red-purple color develops.

Purity (1) Clarity and color of solution—Take 2.0 g of Dextrin in a Nessler tube, add 40 mL of water, dissolve by heating, cool, and add water to make 50 mL: the solution is colorless or light yellow. It is clear, and even if turbid, the turbidity is not more than that of the following control solution.

Control solution: To 1.0 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 46 mL of water and 2 mL of barium chloride TS, allow to stand for 10 minutes, and shake before use.

(2) Acidity—To 1.0 g of Dextrin add 5 mL of water, dissolve by heating, cool, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—To 2.0 g of Dextrin add 80 mL of water, dissolve by heating, cool, add water to make 100 mL, and filter. Take 40 mL of the filtrate, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more

than 0.013%).

(4) Sulfate <1.14>—To 45 mL of the filtrate obtained in (3) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(5) Oxalate—To 1.0 g of Dextrin add 20 mL of water, dissolve by heating, cool, add 1 mL of acetic acid (31), and filter. To 5 mL of the filtrate add 5 drops of calcium chloride TS: no turbidity is produced immediately.

(6) Calcium—To a 5-mL portion of the filtrate obtained in (5) add 5 drops of ammonium oxalate TS: no turbidity is immediately produced.

(7) Heavy metals <1.07>—Proceed with 0.5 g of Dextrin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

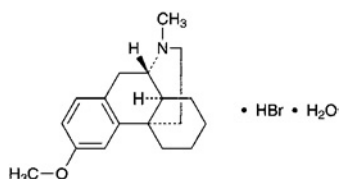
Loss on drying <2.41> Not more than 10% (0.5 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.5 g).

Containers and storage Containers—Well-closed containers.

Dextromethorphan Hydrobromide Hydrate

デキストロメトルファン臭化水素酸塩水和物



$C_{18}H_{25}NO \cdot HBr \cdot H_2O$: 370.32
(9*S*, 13*S*, 14*S*)-3-Methoxy-17-methylmorphinan
monohydrobromide monohydrate [6700-34-1]

Dextromethorphan Hydrobromide Hydrate contains not less than 98.0% of dextromethorphan hydrobromide ($C_{18}H_{25}NO \cdot HBr$: 352.31), calculated on the anhydrous basis.

Description Dextromethorphan Hydrobromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetic acid (100), and sparingly soluble in water.

Melting point: about 126°C (Insert the capillary tube into the bath preheated to 116°C, and continue the heating so that the temperature rises at a rate of about 3°C per minute.)

Identification (1) Determine the absorption spectrum of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dextromethorphan Hydrobromide Hydrate as directed in the potassium bromide disk method under Infrared Spec-

trophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 50 mL of a solution of Dextromethorphan Hydrobromide Hydrate (1 in 100) add 2 drops of phenolphthalein TS and sodium hydroxide TS until a red color develops. Add 50 mL of chloroform, shake, and add 5 mL of dilute nitric acid to 40 mL of the water layer. This solution responds to the Qualitative Tests <1.09> for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: +26 – +30° (0.34 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Dextromethorphan Hydrobromide Hydrate in 100 mL of water: the pH of this solution is between 5.2 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Dextromethorphan Hydrobromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) *N,N*-dimethylaniline—To 0.50 g of Dextromethorphan Hydrobromide Hydrate add 20 mL of water, and dissolve by heating on a water bath. After cooling, add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL: the solution has no more color than the following control solution.

Control solution: Dissolve 0.10 g of *N,N*-dimethylaniline in 400 mL of water by warming on a water bath, cool, and add water to make 500 mL. Pipet 5 mL of this solution, and add water to make 200 mL. To 1.0 mL of this solution add 2 mL of dilute acetic acid, 1 mL of sodium nitrite TS and water to make 25 mL.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dextromethorphan Hydrobromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Phenolic compounds—Dissolve 5 mg of Dextromethorphan Hydrobromide Hydrate in 1 drop of dilute hydrochloric acid and 1 mL of water, add 2 drops of iron (III) chloride TS and 2 drops of potassium hexacyanoferrate (III) TS, shake, and allow to stand for 15 minutes: no blue-green color develops.

(5) Related substances—Dissolve 0.25 g of Dextromethorphan Hydrobromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene, ethyl acetate, methanol, dichloromethane and 13.5 mol/L ammonia TS (55:20:13:10:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly bismuth potassium iodide TS on the plate, and then spray evenly hydrogen peroxide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 4.0 – 5.5% (0.2 g, back titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Dextromethorphan Hydrobromide Hydrate, dissolve in 10 mL of acetic acid (100) and add 40 mL of acetic anhydride. Titrate <2.50> with

0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.23 mg of $C_{18}H_{25}NO \cdot HBr$

Containers and storage Containers—Well-closed containers.

Diastase

ジアスターゼ

Diastase is an enzyme drug mainly prepared from malt.

It has amylolytic activity.

It contains not less than 440 starch saccharifying activity units per g.

It is usually diluted with suitable diluents.

Description Diastase occurs as a light yellow to light brown powder.

It is hygroscopic.

Purity Rancidity—Diastase has no unpleasant or rancid odor, and has no unpleasant or rancid taste.

Loss on drying <2.41> Not more than 4.0% (1 g, 105°C, 5 hours).

Assay (i) Substrate solution—Use potato starch TS for amylolytic activity test.

(ii) Sample solution—Weigh accurately about 0.1 g of Diastase, and dissolve in water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in (i) Measurement of starch saccharifying activity of (1) Assay for starch digestive activity under Digestion Test <4.03>.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Diastase and Sodium Bicarbonate Powder

ジアスターゼ・重曹散

Method of preparation

Diastase	200 g
Sodium Bicarbonate	300 g
Precipitated Calcium Carbonate	400 g
Magnesium Oxide	100 g
To make 1000 g	

Prepare before use as directed under Powders, with the above ingredients.

Description Diastase and Sodium Bicarbonate Powder occurs as a light yellow powder. It has a characteristic, salty taste.

Containers and storage Containers—Well-closed containers.

Compound Diastase and Sodium Bicarbonate Powder

複方ジアスターゼ・重曹散

Method of preparation

Diastase	200 g
Sodium Bicarbonate	600 g
Magnesium Oxide	150 g
Powdered Gentian	50 g
To make 1000 g	

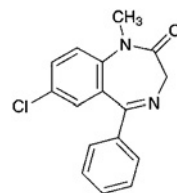
Prepare before use as directed under Powders, with the above ingredients.

Description Compound Diastase and Sodium Bicarbonate Powder occurs as a slightly brownish, light yellow powder. It has a characteristic odor and a bitter taste.

Containers and storage Containers—Well-closed containers.

Diazepam

ジアゼパム



$C_{16}H_{13}ClN_2O$: 284.74

7-Chloro-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one [439-14-5]

Diazepam, when dried, contains not less than 98.0% of $C_{16}H_{13}ClN_2O$.

Description Diazepam occurs as a white to light yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, soluble in acetic anhydride and in ethanol (95), sparingly soluble in diethyl ether, slightly soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 0.01 g of Diazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(2) Dissolve 2 mg of Diazepam in 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Diazepam as directed under Flame Coloration Test <1.04> (2): a blue to blue-green color appears.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (285 nm): 425 – 445 [after drying, 2 mg, a solution of sulfuric acid in ethanol (99.5) (3 in 1000), 200 mL].

Melting point <2.60> 130 – 134°C

Purity (1) Clarity of solution—Dissolve 0.10 g of Diazepam in 20 mL of ethanol (95); the solution is clear.

(2) Chloride <1.03>—To 1.0 g of Diazepam add 50 mL of water, allow to stand for 1 hour, with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Diazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 1.0 g of Diazepam in 10 mL of acetone, and use this solution as the sample solution. Pipette 1 mL of the sample solution, and add acetone to make exactly 100 mL. Pipette 1 mL of this solution, add acetone to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Diazepam, previously dried, dissolve in 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.48 mg of $\text{C}_{16}\text{H}_{13}\text{ClN}_2\text{O}$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dibasic Sodium Phosphate Hydrate

リン酸水素ナトリウム水和物

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$: 358.14

Dibasic Sodium Phosphate Hydrate, when dried, contains not less than 98.0% of disodium hydrogen phosphate (Na_2HPO_4 : 141.96)

Description Dibasic Sodium Phosphate Hydrate occurs as colorless or white crystals. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It effloresces in warm, dry air.

Identification A solution of Dibasic Sodium Phosphate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> (1) and (2) for sodium salt, and the Qualitative Tests <1.09> for phosphate.

pH <2.54> Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 50 mL of water: the pH of this solution is between 9.0 and 9.4.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Dibasic Sodium Phosphate Hydrate in 7 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Dibasic Sodium Phosphate Hydrate in 2 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Carbonate—To 2.0 g of Dibasic Sodium Phosphate Hydrate add 5 mL of water, boil, and add 2 mL of hydrochloric acid after cooling: the solution does not effervesce.

(5) Heavy metals <1.07>—Dissolve 2.0 g of Dibasic Sodium Phosphate Hydrate in 4 mL of acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution by adding 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dibasic Sodium Phosphate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> 57.0 – 61.0% (10 g, at 40°C for 3 hours at first and then at 105°C for 5 hours).

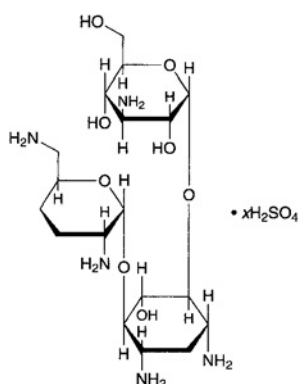
Assay Dissolve about 3 g of Dibasic Sodium Phosphate Hydrate, previously dried and accurately weighed, in 50 mL of water. Titrate <2.50> it with 0.5 mol/L sulfuric acid VS at 15°C until the green color of the solution changes to dark-greenish red-purple (indicator: 3 to 4 drops of methyl orange-xylencyanol FF TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 142.0 mg of Na_2HPO_4

Containers and storage Containers—Tight containers.

Dibekacin Sulfate

ジベカシン硫酸塩



$C_{18}H_{37}N_5O_8 \cdot xH_2SO_4$

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,3,4,6-tetrahydroxy- α -D-erythro-hexopyranosyl-(1 \rightarrow 4)]-2-deoxy-D-streptamine sulfate [58580-55-5]

Dibekacin Sulfate is the sulfate of a derivative of bekanamycin.

It contains not less than 640 μ g (potency) and not more than 740 μ g (potency) per mg, calculated on the dried basis. The potency of Dibekacin Sulfate is expressed as mass (potency) of dibekacin ($C_{18}H_{37}N_5O_8$: 451.52).

Description Dibekacin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 20 mg each of Dibekacin Sulfate and Dibekacin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28) and methanol (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same R_f value.

(2) To 5 mL of a solution of Dibekacin Sulfate (1 in 50) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +96 – +106° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Dibekacin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dibekacin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Dibekacin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 5.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Dibekacin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

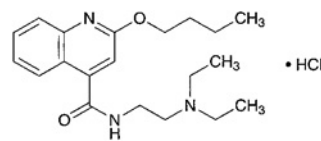
(iv) Sample solutions—Weigh accurately an amount of Dibekacin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Dibucaine Hydrochloride

Cinchocaine Hydrochloride

ジブカイン塩酸塩



$C_{20}H_{29}N_3O_2 \cdot HCl$: 379.92

2-Butyloxy-*N*-(2-diethylaminoethyl)-4-quinolinecarboxamide monohydrochloride [61-12-1]

Dibucaine Hydrochloride, when dried, contains not less than 98.0% of $C_{20}H_{29}N_3O_2 \cdot HCl$.

Description Dibucaine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, in ethanol (95) and in acetic acid (100), freely soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Dibucaine Hydrochloride in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dibucaine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dibucaine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 95 – 100 °C Charge Dibucaine Hydrochloride into a capillary tube for melting point determination, and dry in vacuum over phosphorus (V) oxide at 80 °C for 5 hours. Seal immediately the open end of the tube, and determine the melting point.

pH <2.54> Dissolve 1.0 g of Dibucaine Hydrochloride in 50 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dibucaine Hydrochloride in 20 mL of water: the solution is clear and colorless. Determine the absorbance of this solution at 430 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: it is not more than 0.03.

(2) Sulfate <1.14>—Perform the test with 0.30 g of Dibucaine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.056%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dibucaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.20 g of Dibucaine Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 20 mL, then pipet 2 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 80 °C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

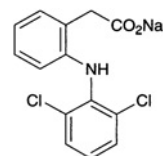
Assay Weigh accurately about 0.3 g of Dibucaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.00 mg of C₂₀H₂₉N₃O₂·HCl

Containers and storage Containers—Tight containers.

Diclofenac Sodium

ジクロフェナクナトリウム



C₁₄H₁₀Cl₂NNaO₂: 318.13

Monosodium 2-(2,6-dichlorophenylamino)phenylacetate
[15307-79-6]

Diclofenac Sodium, when dried, contains not less than 98.5% of C₁₄H₁₀Cl₂NNaO₂.

Description Diclofenac Sodium occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), sparingly soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To 1 mL of a solution of Diclofenac Sodium in methanol (1 in 250) add 1 mL of nitric acid: a dark red color develops.

(2) Perform the test with 5 mg of Diclofenac Sodium as directed under Flame Coloration Test <1.04> (2): a light green color appears.

(3) Determine the infrared absorption spectrum of Diclofenac Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Diclofenac Sodium (1 in 100) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Diclofenac Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Diclofenac Sodium according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Diclofenac Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of these solutions as directed under Liquid Chromatography <2.01>. Determine each peak area of these solutions by the automatic integration method: the area of each peak other than the peak of diclofenac from the sample solution is not larger than the peak area of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and diluted acetic acid (100) (3 in 2500) (4:3).

Flow rate: Adjust the flow rate so that the retention time of diclofenac is about 20 minutes.

Time span of measurement: About twice as long as the retention time of diclofenac beginning after the solvent peak.

System suitability—

System performance: Dissolve 35 mg of ethyl parahydroxybenzoate and 0.05 g of propyl parahydroxybenzoate in 100 mL of the mobile phase. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, ethyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of diclofenac is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

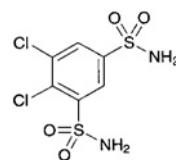
Assay Weigh accurately about 0.5 g of Diclofenac Sodium, previously dried, dissolve with 40 mL of water in a separator, add 2 mL of dilute hydrochloric acid, and extract the precipitate formed with 50 mL of chloroform. Extract again with two 20-mL portions of chloroform, and filter the extract each time through a pledget of absorbent cotton moistened with chloroform. Wash the tip of the separator and the absorbent cotton with 15 mL of chloroform, combine the washing with the extracts, add 10 mL of a solution of 1 mol/L hydrochloric acid TS in ethanol (99.5) (1 in 100), and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS from the first equivalent point to the second equivalent point (potentiometric titration).

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 31.81 mg of C₁₄H₁₀Cl₂NNaO₂

Containers and storage Containers—Tight containers.

Diclofenamide**Dichlorphenamide**

ジクロフェナミド



C₆H₆Cl₂N₂O₄S₂: 305.16

4,5-Dichlorobenzene-1,3-disulfonamide [120-97-8]

Diclofenamide, when dried, contains not less than 98.0% of C₆H₆Cl₂N₂O₄S₂.

Description Diclofenamide occurs as a white, crystalline powder.

It is very soluble in *N,N*-dimethylformamide, soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.01 g of Diclofenamide in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of the solution add 0.1 mL of hydrochloric acid. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Diclofenamide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Diclofenamide, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Diclofenamide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 237 – 240°C

Purity (1) Chloride <1.03> —Dissolve 0.10 g of Diclofenamide in 10 mL of *N,N*-dimethylformamide, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of *N,N*-dimethylformamide, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.160%).

(2) Selenium—To 0.10 g of Diclofenamide add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yellow clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Ab-

sorption Spectrophotometry <2.23> according to the following conditions, and determine constant absorbances, A_T and A_S , obtained on a recorder after rapid increasing of the absorption: A_T is smaller than A_S (not more than 30 ppm).

Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: An selenium hollow cathode lamp

Wavelength: 196.0 nm

Temperature of sample atomizer: When an electric furnace is used, about 1000°C.

Carrier gas: Nitrogen or Argon

(3) Heavy metals <1.07>—Proceed with 2.0 g of Diclofenamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Diclofenamide in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of diclofenamide from the sample solution is not larger than the peak area of diclofenamide from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of diclofenamide.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of diclofenamide obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that of diclofenamide obtained from 10 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diclofenamide is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Diclofenamide and Diclofenamide Reference Standard, previously dried, and dissolve each in 30 mL of the mobile phase. To each add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of diclofenamide to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_6H_6Cl_2N_2O_4S_2 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Diclofenamide Reference Standard

Internal standard solution—A solution of butyl parahydroxy benzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of sodium phosphate TS and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of diclofenamide is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, diclofenamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diclofenamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Diclofenamide Tablets

Dichlorphenamide Tablets

ジクロフェナミド錠

Diclofenamide Tablets contain not less than 92% and not more than 108% of the labeled amount of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$; 305.16).

Method of preparation Prepare as directed under Tablets, with Diclofenamide.

Identification To a quantity of powdered Diclofenamide Tablets, equivalent to 0.2 g of Diclofenamide according to the labeled amount, add 20 mL of methanol, shake, and filter. Evaporate the filtrate on a water bath to dryness, and dissolve 0.01 g of the residue in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of this solution add 0.1 mL of hydrochloric acid TS, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 284 nm and 288 nm, and between 293 nm and 297 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Diclofenamide Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 60 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μ m. Discard the first 10 mL of the filtrate, and use the subsequent as the sample solution. Separately, weigh accurately about 55 mg of Diclofenamide Reference Stan-

ard, previously dried in vacuum at a pressure not exceeding 0.67 kPa at 100°C for 5 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Diclofenamide Tablets in 60 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$)

$$= W_S \times (A_T/A_S) \times (1/C) \times 90$$

W_S : Amount (mg) of Diclofenamide Reference Standard.

C : Labeled amount (mg) of diclofenamide

($C_6H_6Cl_2N_2O_4S_2$) in 1 tablet.

Assay Weigh accurately, and powder not less than 20 tablets of Diclofenamide Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$), add exactly 25 mL of the mobile phase, shake for 15 minutes, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 4 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Diclofenamide Reference Standard, previously dried at 100°C in vacuum at a pressure not exceeding 0.67 kPa for 5 hours, dissolve in 30 mL of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Diclofenamide.

Amount (mg) of diclofenamide ($C_6H_6Cl_2N_2O_4S_2$)

$$= W_S \times (Q_T/Q_S)$$

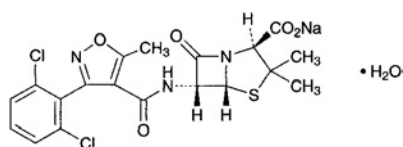
W_S : Amount (mg) of Diclofenamide Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in the mobile phase (3 in 5000).

Containers and storage Containers—Well-closed containers.

Dicloxacillin Sodium Hydrate

ジクロキサシリンナトリウム水和物



$C_{19}H_{16}Cl_2N_3NaO_5S \cdot H_2O$: 510.32

Monosodium (2*S*,5*R*,6*R*)-6-[[3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrate [13412-64-1]

Dicloxacillin Sodium Hydrate contains not less than 910 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Dicloxacillin Sodium Hydrate is expressed as

mass (potency) of dicloxacillin ($C_{19}H_{17}Cl_2N_3O_5S$: 470.33).

Description Dicloxacillin Sodium Hydrate occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Dicloxacillin Sodium Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Dicloxacillin Sodium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dicloxacillin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Dicloxacillin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dicloxacillin Sodium Hydrate responds to the Quantitative Tests <1.09> (1) for sodium salt.

Water <2.48> Not less than 3.0% and not more than 4.5% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.5 to 6.6 after sterilization.

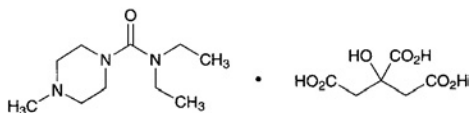
(iii) Standard solutions—Weigh accurately an amount of Dicloxacillin Sodium Reference Standard equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 24 hours. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10 μ g (potency) and 2.5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Dicloxacillin Sodium Hydrate equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 10 μ g (potency) and 2.5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Diethylcarbamazine Citrate

ジエチルカルバマジンクエン酸塩



$C_{10}H_{21}N_3O \cdot C_6H_8O_7$; 391.42
N,N-Diethyl-4-methylpiperazine-1-carboxamide
 monocitrate [1642-54-2]

Diethylcarbamazine Citrate, when dried, contains not less than 98.0% of $C_{10}H_{21}N_3O \cdot C_6H_8O_7$.

Description Diethylcarbamazine Citrate occurs as a white, crystalline powder. It is odorless, and has an acid and bitter taste.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in acetone, in chloroform and in diethyl ether.

A solution of Diethylcarbamazine Citrate (1 in 20) is acid. It is hygroscopic.

Identification (1) Dissolve 0.5 g of Diethylcarbamazine Citrate in 2 mL of water, add 10 mL of sodium hydroxide TS, and extract with four 5-mL portions of chloroform. Wash the combined chloroform extracts with 10 mL of water, and evaporate the chloroform on a water bath. Add 1 mL of iodoethane to the residue, and boil gently under a reflux condenser for 5 minutes. Evaporate the excess iodoethane with the aid of a current of air, and dissolve the residue in 4 mL of ethanol (95). Cool the ethanol solution in an ice bath, with continuous stirring, add diethyl ether until precipitates are formed, and stir until crystallization is evident. Allow to stand in the ice bath for 30 minutes, and collect the precipitate. Dissolve the precipitate in 4 mL of ethanol (95), repeat the recrystallization in the same manner, then dry at 105°C for 4 hours: the crystals so obtained melt <2.60> between 151°C and 155°C.

(2) Neutralize the remaining aqueous layer obtained in (1) with dilute sulfuric acid: the solution responds to the Qualitative Tests <1.09> (2) and (3) for citrate.

Melting point <2.60> 135.5 – 138.5°C

Purity Heavy metals <1.07>—Proceed with 2.0 g of Diethylcarbamazine Citrate according to Method 4, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 1.0% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.75 g of Diethylcarbamazine Citrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
 = 39.14 mg of $C_{10}H_{21}N_3O \cdot C_6H_8O_7$

Containers and storage Containers—Tight containers.

Diethylcarbamazine Citrate Tablets

ジエチルカルバマジンクエン酸塩錠

Diethylcarbamazine Citrate Tablets contain not less than 95% and not more than 105% of the labeled amount of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$; 391.42).

Method of preparation Prepare as directed under Tablets, with Diethylcarbamazine Citrate.

Identification (1) To a quantity of powdered Diethylcarbamazine Citrate Tablets, equivalent to 0.5 g of Diethylcarbamazine Citrate according to the labeled amount, add 10 mL of water, shake, and filter. Add 10 mL of sodium hydroxide TS to the filtrate, and proceed as directed in the Identification (1) under Diethylcarbamazine Citrate.

(2) To a quantity of powdered Diethylcarbamazine Citrate Tablets, equivalent to 0.8 g of diethylcarbamazine citrate according to the labeled amount, add 10 mL of water, shake, centrifuge, and filter the supernatant liquid. To 5 mL of the filtrate add 5 mL of sodium hydroxide TS, and extract with two 20-mL portions of chloroform. Separate the aqueous layer, and neutralize with dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> (2) and (3) for citrate.

Assay Weigh accurately and powder not less than 20 Diethylcarbamazine Citrate Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of diethylcarbamazine citrate ($C_{10}H_{21}N_3O \cdot C_6H_8O_7$), add 10 mL of water, shake well, add 5 mL of sodium hydroxide TS, then add exactly 20 mL of the internal standard solution, and shake vigorously for 10 minutes. Centrifuge, discard the aqueous layer, and use the chloroform layer as the sample solution. Separately, weigh accurately about 50 mg of Diethylcarbamazine Citrate Reference Standard, previously dried at 105°C for 4 hours, dissolve in 10 mL of water, add 5 mL of sodium hydroxide TS, proceed in the same manner as the preparation of the sample solution, and use the chloroform layer as the standard solution. Perform the test with 2 μ L of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of diethylcarbamazine to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of diethylcarbamazine citrate} \\ & (C_{10}H_{21}N_3O \cdot C_6H_8O_7) \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Diethylcarbamazine Citrate Reference Standard

Internal standard solution—A solution of *n*-octadecane in chloroform (1 in 1250).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass tube 3 mm in inside diameter and 1 m in length, packed with silanized siliceous earth for gas chro-

matography (180 to 250 μm in particle diameter) coated with 35% methylphenyldimethyl silicone polymer for gas chromatography in the ratio of 3%.

Column temperature: A constant temperature of about 145 $^{\circ}\text{C}$.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of diethylcarbamazine is about 4 minutes.

System suitability—

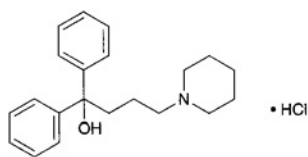
System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, diethylcarbamazine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of diethylcarbamazine to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Difenidol Hydrochloride

ジフェニドール塩酸塩



$\text{C}_{21}\text{H}_{27}\text{NO}\cdot\text{HCl}$: 345.91

1,1-Diphenyl-4-piperidin-1-ylbutan-1-ol
monohydrochloride [3254-89-5]

Difenidol Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_{21}\text{H}_{27}\text{NO}\cdot\text{HCl}$.

Description Difenidol Hydrochloride occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in water and in acetic acid (100), and practically insoluble in diethyl ether.

Melting point: about 217 $^{\circ}\text{C}$ (with decomposition).

Identification (1) Dissolve 0.01 g of Difenidol Hydrochloride in 1 mL of sulfuric acid: an orange-red color develops. To this solution add carefully 3 drops of water: the solution becomes yellowish brown, and colorless on the addition of 10 mL of water.

(2) To 5 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(3) To 10 mL of a solution of Difenidol Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 15-mL portions of chloroform. Combine the extracts, wash with three 10-mL portions of water, evaporate the chloroform on a water bath, and dry the residue in a desiccator (in vacuum, silica gel, 55 $^{\circ}\text{C}$) for 5 hours: the residue melts <2.60> between 103 $^{\circ}\text{C}$ and 106 $^{\circ}\text{C}$.

(4) A solution of Difenidol Hydrochloride (1 in 100)

responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Difenidol Hydrochloride in 100 mL of freshly boiled and cooled water: the pH of this solution is between 4.7 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Difenidol Hydrochloride in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Difenidol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Difenidol Hydrochloride according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.10 g of Difenidol Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 1,1-diphenyl-4-piperidino-1-butene hydrochloride for thin-layer chromatography in methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, methanol and acetic acid (100) (10:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

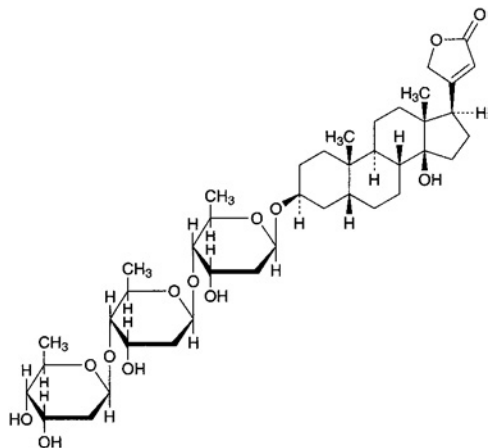
Assay Weigh accurately about 0.35 g of Difenidol Hydrochloride, previously dried, dissolve in 30 mL of acetic acid (100) by warming if necessary, cool, add 30 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 17.30 mg of $\text{C}_{21}\text{H}_{27}\text{NO}\cdot\text{HCl}$

Containers and storage Containers—Well-closed containers.

Digitoxin

ジギトキシシ

 $C_{41}H_{64}O_{13}$: 764.94

3 β -[2,6-Dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyloxy]-14-hydroxy-5 β ,14 β -card-20(22)-enolide [71-63-6]

Digitoxin, when dried, contains not less than 90.0% of $C_{41}H_{64}O_{13}$.

Description Digitoxin occurs as a white to light yellowish white, crystalline powder. It is odorless.

It is soluble in chloroform, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Transfer 1 mg of Digitoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the upper layer near the contact zone changes to green through purple. Finally the color of the entire acetic acid layer changes to green through deep blue.

(2) To 2 mg of Digitoxin add 25 mL of a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100), and dissolve by shaking. Take 2 mL of this solution, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and mix: a red-purple color develops slowly, and then fades.

(3) Dissolve 1 mg each of Digitoxin and Digitoxin Reference Standard in a mixture of chloroform and ethanol (95) (1:1) to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 110°C for 10 minutes: the spot from the sample solution shows the same *R_f* value as

the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: +16 – +18° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity Digitonin—Dissolve 10 mg of Digitoxin in 2 mL of ethanol (95) in a test tube, having the inner walls which are free from scratches, add 2 mL of a solution of cholesterol in ethanol (95) (1 in 200), mix gently, and allow to stand for 10 minutes: no turbidity is produced.

Loss on drying <2.41> Not more than 1.5% (0.5 g, in vacuum, 100°C, 2 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Dissolve about 20 mg each of Digitoxin and Digitoxin Reference Standard, previously dried and accurately weighed, in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution to each solution, add 12.5 mL of water, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of digitoxin to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{41}H_{64}O_{13} \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Digitoxin Reference Standard

Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 20 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol and water (3:1).

Flow rate: Adjust the flow rate so that the retention time of digitoxin is about 5 minutes.

Selection of column: Proceed with 50 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of digitoxin and the internal standard in this order with the resolution between these peaks being not less than 6.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Digitoxin Tablets

ジギトキシシ錠

Digitoxin Tablets contain not less than 90% and not more than 110% of the labeled amount of digitoxin ($C_{41}H_{64}O_{13}$: 764.94).

Method of preparation Prepare as directed under Tablets, with Digitoxin.

Identification (1) Place a portion of powdered Digitoxin Tablets, equivalent to 2 mg of digitoxin ($C_{41}H_{64}O_{13}$) according to the labeled amount, in a separator, shake with 30 mL of water, and shake vigorously with 30 mL of chloroform. Filter the chloroform extract with a funnel on which a small amount of anhydrous sodium sulfate is placed, and transfer to a round-bottomed flask connected by a universal joint. Evaporate the solution to dryness by warming under reduced pressure, and dissolve the residue in 10 mL of chloroform. Transfer 5 mL of this solution to a small test tube about 10 mm in inside diameter, and evaporate to dryness on a water bath with the aid of a current of air. Proceed with the residue as directed in the Identification (1) under Digitoxin.

(2) Evaporate 4 mL of the chloroform solution obtained in (1) to dryness, by warming under reduced pressure, add a freshly prepared solution of 1,3-dinitrobenzene in ethanol (95) (1 in 100) to the residue, and dissolve by shaking. Proceed with 2 mL of this solution as directed in the Identification (2) under Digitoxin.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Digitoxin Tablets to a 50-mL beaker, add 0.5 mL of water to disintegrate the tablet, add 5 mL of acetonitrile, and warm on a water bath for 5 minutes, covering the beaker with a watch glass. After cooling, transfer the solution to separator A, rinse the beaker with 30 mL of chloroform and then with 20 mL of water, transfer the rinsings to separator A, and extract by vigorous shaking. Transfer the chloroform extract to separator B containing 5 mL of a solution of sodium hydrogen carbonate (1 in 100), and shake to wash. Filter the chloroform layer through a pledget of absorbent cotton, previously moistened with chloroform. Extract the water layer in separator A with two 30-mL portions of chloroform, wash the chloroform extract with a solution of sodium hydrogen carbonate (1 in 100) in separator B, filter in the same manner, and combine the filtrate with the first one. Evaporate this filtrate to dryness under reduced pressure by warming, add diluted ethanol (95) (4 in 5) to make exactly V mL of a solution containing $5 \mu\text{g}$ of digitoxin ($C_{41}H_{64}O_{13}$) per mL. Shake vigorously for 20 minutes to dissolve, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Digitoxin Reference Standard, previously dried at 100°C for 2 hours, and dissolve in diluted ethanol (95) (4 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted ethanol (95) (4 in 5) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (4 in 5) into brown glass-stoppered test tubes T, S and B. Add exactly 10 mL each of 0.02 w/v% L-ascorbic acid-hydrochloric acid TS, shake well, and immediately add exactly 1 mL each of dilute hydrogen peroxide TS. Shake vigorously, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_T , F_S and F_B , of these solutions at 400 nm of the excitation wavelength and at about 570 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

$$\begin{aligned} & \text{Amount (mg) of digitoxin (C}_{41}\text{H}_{64}\text{O}_{13}\text{)} \\ & = W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (V/2000) \end{aligned}$$

W_S : Amount (mg) of Digitoxin Reference Standard

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Take 1 tablet of Digitoxin Tablets, and perform the test using 500 mL of diluted hydrochloric acid (3 in 500), degassed by a suitable method, as the test solution at 100 revolutions per minute as directed in the Basket method. At 30 minutes after starting the test, take $a + 15$ mL of the dissolved solution, and immediately add the same volume of fresh test solution, previously warmed at $37 \pm 0.5^\circ\text{C}$, to the vessel carefully. Filter $a + 15$ mL of the dissolved solution through a membrane filter (less than $0.8 \mu\text{m}$ in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Measure exactly a mL of the sample solution, equivalent to about $2 \mu\text{g}$ of digitoxin ($C_{41}H_{64}O_{13}$) according to the labeled amount, transfer to a glass-stoppered centrifuge tube T_{30} , and warm at $37 \pm 0.5^\circ\text{C}$ for 30 minutes. Further, at 60 minutes after starting the test, take $a + 15$ mL of the dissolved solution, proceed in the same manner, measure exactly a mL of the sample solution, and transfer to a glass-stoppered centrifuge tube T_{60} . Separately, weigh accurately 100 times the labeled amount of Digitoxin Reference Standard, previously dried under reduced pressure at 100°C for 2 hours, and dissolve in ethanol (95) to make exactly 100 mL. Measure exactly 1 mL of this solution, add the test solution to make exactly 500 mL, warm at $37 \pm 0.5^\circ\text{C}$ for 60 minutes, and filter through a membrane filter (less than $0.8 \mu\text{m}$ in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the standard solution. Measure exactly a mL each of the standard solution and the test solution, transfer to glass-stoppered centrifuge tubes T_S and T_B , respectively. Add exactly 7 mL of chloroform to each of the glass-stoppered centrifuge tubes T_{30} , T_{60} , T_S and T_B , shake vigorously for 10 minutes and centrifuge. Discard the aqueous layer, measure exactly 5 mL of the chloroform layer, transfer to brown test tubes T'_{30} , T'_{60} , T'_S and T'_B , evaporate the chloroform, add exactly 4 mL each of 0.05 w/v% L-ascorbic acid-hydrochloric acid TS, shake well, and allow to stand for 10 minutes. Then add exactly 0.5 mL each of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_{30} , F_{60} , F_S and F_B , of these solutions at about 395 nm of the excitation wavelength and at about 560 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively. Dissolution rates of Digitoxin Tablets after 30 minutes and 60 minutes should be not less than 60% and 85%, respectively.

No retest requirement is applied to Digitoxin Tablets.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ with respect to the labeled} \\ & \text{amount of digitoxin (C}_{41}\text{H}_{64}\text{O}_{13}\text{)} \text{ for 30 minutes} \\ & = W_S \times \{(F_{30} - F_B)/(F_S - F_B)\} \times (1/C) \end{aligned}$$

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ with respect to the labeled} \\ & \text{amount of digitoxin (C}_{41}\text{H}_{64}\text{O}_{13}\text{)} \text{ for 60 minutes} \\ & = W_S \times \left(\frac{F_{60} - F_B}{F_S - F_B} + \frac{F_{30} - F_B}{F_S - F_B} \times \frac{a + 15}{500} \right) \\ & \quad \times \frac{1}{C} \end{aligned}$$

W_S : Amount (mg) of Digitoxin Reference Standard.

C : The labeled amount (mg) of digitoxin ($C_{41}H_{64}O_{13}$) in 1 tablet.

$a + 15$: Measured volume (mL) of dissolved solution at the

specified time.

Assay Weigh accurately and powder not less than 20 Digoxin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of digitoxin ($C_{41}H_{64}O_{13}$), and shake with 12.5 mL of water for 10 minutes. Add exactly 10 mL of the internal standard solution, shake for 20 minutes, and add methanol to make 50 mL. Centrifuge this solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Digoxin Reference Standard, previously dried in vacuum at 100°C for 2 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of the solution, add exactly 10 mL of the internal standard solution, add 12.5 mL of water, then methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Digitoxin.

$$\begin{aligned} &\text{Amount (mg) of digitoxin (C}_{41}\text{H}_{64}\text{O}_{13}) \\ &= W_S \times (Q_T/Q_S) \times 0.025 \end{aligned}$$

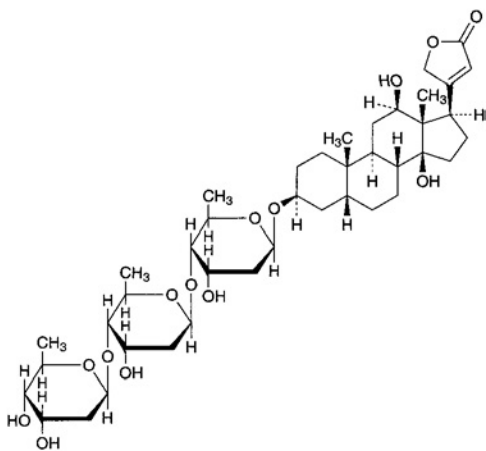
W_S : Amount (mg) of Digoxin Reference Standard

Internal standard solution—A solution of acenaphthene in methanol (3 in 1,000,000).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Digoxin

ジゴキシン



$C_{41}H_{64}O_{14}$: 780.94

3 β -[2,6-Dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribo-hexopyranosyloxy]-12 β ,14-dihydroxy-5 β ,14 β -card-20(22)-enolide [20830-75-5]

Digoxin, when dried, contains not less than 96.0% and not more than 106.0% of $C_{41}H_{64}O_{14}$.

Description Digoxin occurs as colorless or white crystals or a white crystalline powder.

It is freely soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in acetic acid (100), and practically insoluble in water.

Identification (1) Transfer 1 mg of Digoxin to a small test tube about 10 mm in inside diameter, dissolve in 1 mL of a

solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids a brown ring free from a reddish color is produced, and the color of the upper layer near the contact zone changes to green through purple. Finally the entire acetic acid layer shows a green color through a deep blue color.

(2) Determine the infrared absorption spectrum of Digoxin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +10.0 – +13.0° (after drying, 0.20 g, dehydratead pyridine, 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Digoxin in 15 mL of diluted ethanol (95) (4 in 5) by warming: the solution is clear and colorless.

(2) Related substances—Dissolve exactly 25.0 mg of Digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the sample solution. Separately, dissolve exactly 5.0 mg of Gitoxin Reference Standard, previously dried under reduced pressure at 105°C for 1 hour, in a mixture of acetonitrile and water (7:3) to make exactly 200 mL. Pipet 2 mL of this solution, add dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of gitoxin: A_T is not larger than A_S , and the total of the areas of the peaks other than digitoxin and gitoxin, obtained by the area percentage method, is not more than 3%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of digoxin beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of digoxin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from the solution for system suitability test.

System performance: Dissolve 25 mg of digoxin in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of a solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000), 10 mL of water and dilute ethanol to make 50 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, digoxin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of digoxin is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 25 mg each of Digoxin and Digoxin Reference Standard, previously dried, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of these solutions, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of digoxin to that of the internal standard.

$$\text{Amount (mg) of } C_{41}H_{64}O_{14} = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Digoxin Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Digoxin Injection

ジゴキシン注射液

Digoxin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 105.0% of the labeled amount of digoxin ($C_{41}H_{64}O_{14}$; 780.94).

Method of preparation Prepare as directed under Injections, with a solution of Digoxin in 5 to 50 vol% ethanol.

Description Digoxin Injection is a clear, colorless liquid.

Identification Dilute Digoxin Injection, if necessary, with methanol so that each mL contains about 0.25 mg of Digoxin according to the labeled amount, and use this solution as the sample solution. In case where ingredients are suspected to affect the test, remove them by means of a solid-phase extraction. Separately, dissolve 0.5 mg of Digoxin Reference Standard in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of octadecylsilanized silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium toluenesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the R_f values of the principal spots with the sample solution and the standard solution are not different each other.

Bacterial endotoxins <4.01> Less than 200 EU/mg.

Extractable volume <6.05> It meets the requirements.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay To an exact volume of Digoxin Injection, equivalent to about 2.5 mg of digoxin ($C_{41}H_{64}O_{14}$), add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Digoxin Reference Standard, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of digoxin to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of digoxin (} C_{41}H_{64}O_{14} \text{)} \\ = W_S \times (Q_T/Q_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of Digoxin Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Digoxin Tablets

ジゴキシン錠

Digoxin Tablets contain not less than 90.0% and not more than 105.0% of the labeled amount of digoxin ($\text{C}_{41}\text{H}_{64}\text{O}_{14}$; 780.94).

Method of preparation Prepare as directed under Tablets, with Digoxin.

Identification To an amount of pulverized Digoxin Tablets, equivalent to 0.5 mg of Digoxin according to the labeled amount, add 2 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 0.5 mg of Digoxin Reference Standard in 2 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of octadecylsilylated silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and water (7:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of a solution of trichloroacetic acid in ethanol (99.5) (1 in 4) and a freshly prepared solution of sodium toluenesulfonchloramide trihydrate (3 in 100) (4:1) on the plate, heat at 110°C for 10 minutes, and examine under ultraviolet light (main wavelength: 366 nm): the R_f values of the principal spots with the sample solution and the standard solution are not different each other.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Digoxin Tablets add 0.5 mL of water to disintegrate, then add exactly 0.5 mL of the internal standard solution, and add V mL of dilute ethanol so that each mL contains about 21 μg of digoxin ($\text{C}_{41}\text{H}_{64}\text{O}_{14}$). Exposure this solution to ultrasonic waves for 20 minutes, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin Reference Standard, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet

10 mL of this solution, and add ethanol (95) to make exactly 20 mL. Pipet 1 mL of this solution, add exactly 0.5 mL of the internal standard solution, then add 1.5 mL of water and ($V - 2$) mL of dilute ethanol, and use this as the standard solution. Proceed with the sample solution and standard solution as directed in the Assay.

$$\begin{aligned} & \text{Amount (mg) of digoxin (C}_{41}\text{H}_{64}\text{O}_{14}) \\ & = W_S \times (Q_T/Q_S) \times (1/200) \end{aligned}$$

W_S : Amount (mg) of Digoxin Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 40,000/ V).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Digoxin Tablets, using 500 mL of diluted hydrochloric acid (3 in 500) at 100 revolutions per minute according to the Basket method as the dissolution medium. Withdraw 30 mL or more of the dissolved solution 60 minutes after starting the test, and filter through a membrane filter (less than 0.8 μm in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Digoxin Reference Standard, previously dried in vacuum at 105°C for 1 hour, dissolve in a small portion of ethanol (95), and add a mixture of ethanol (95) and water (4:1) to make exactly 500 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 500 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and the dissolution medium, and transfer to brown glass-stoppered test tubes. Add exactly 10 mL of 0.012 g/dL L-ascorbic acid-hydrochloric acid TS to these tubes, and shake. Immediately add exactly 1 mL of dilute hydrogen peroxide TS, shake well, and allow to stand at a constant temperature between 25°C and 30°C for 45 minutes. Determine the fluorescence intensities, F_T , F_S , and F_B , of these solutions at 360 nm of the excitation wavelength and at 485 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively: the dissolution rate in 60 minutes is not less than 65%. No retest requirement is applied to Digoxin Tablets.

Dissolution rate (%) with respect to the labeled amount of digoxin ($\text{C}_{41}\text{H}_{64}\text{O}_{14}$)

$$= W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (1/C)$$

W_S : Amount (mg) of Digoxin Reference Standard

C : The labeled amount (mg) of digoxin ($\text{C}_{41}\text{H}_{64}\text{O}_{14}$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Digoxin Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 2.5 mg of digoxin ($\text{C}_{41}\text{H}_{64}\text{O}_{14}$), add 30 mL of dilute ethanol, exposure to ultrasonic waves for 20 minutes, and shake for 5 minutes. Add exactly 5 mL of the internal standard solution and dilute ethanol to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of Digoxin Reference Standard, previously dried under reduced pressure at 105°C for 1 hour, dissolve in 50 mL of warm ethanol (95), cool, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, 10 mL of water and dilute ethanol to make 50 mL, and use this solution as the standard solution. Perform

the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of digoxin to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of digoxin (C}_{41}\text{H}_{64}\text{O}_{14}) \\ = W_S \times (Q_T/Q_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of Digoxin Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of digoxin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, digoxin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

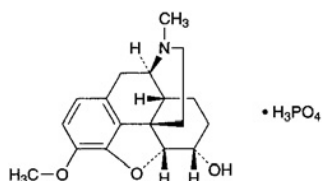
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of digoxin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dihydrocodeine Phosphate

ジヒドロコデインリン酸塩



$\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{H}_3\text{PO}_4$: 399.38

(5*R*,6*S*)-4,5-Epoxy-3-methoxy-17-methylmorphinan-6-ol monophosphate [24204-13-5]

Dihydrocodeine Phosphate contains not less than 98.0% of $\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{H}_3\text{PO}_4$, calculated on the dried basis.

Description Dihydrocodeine Phosphate occurs as a white to yellowish white, crystalline powder.

It is freely soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Dihydrocodeine Phosphate (1 in 10) is between 3.0 and 5.0.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Dihydrocodeine Phosphate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Dihydrocodeine Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dihydrocodeine Phosphate (1 in 20) responds to the Qualitative Tests <1.09> (1) for phosphate.

Purity (1) Chloride <1.03>—Perform the test with 0.5 g of Dihydrocodeine Phosphate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.021%).

(2) Sulfate <1.14>—Perform the test with 0.20 g of Dihydrocodeine Phosphate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid (not more than 0.240%).

(3) Related substances—Dissolve 0.20 g of Dihydrocodeine Phosphate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Dihydrocodeine Phosphate, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to greenish blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 39.94 \text{ mg of } \text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{H}_3\text{PO}_4 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

1% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散 1%

1% Dihydrocodeine Phosphate Powder contains not less than 0.90% and not more than 1.10% of dihydrocodeine phosphate ($C_{18}H_{23}NO_3 \cdot H_3PO_4$; 399.38).

Method of preparation

Dihydrocodeine Phosphate	10 g
Lactose Hydrate	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 1% Dihydrocodeine Phosphate Powder (1 in 100) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 281 nm and 285 nm.

Assay Weigh accurately about 5 g of 1% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay (previously determine the loss on drying <2.41> (105°C, 4 hours)), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dihydrocodeine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of dihydrocodeine phosphate} \\ & (C_{18}H_{23}NO_3 \cdot H_3PO_4) \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

Internal standard solution—A solution of ethylefurin hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

10% Dihydrocodeine Phosphate Powder

ジヒドロコデインリン酸塩散 10%

10% Dihydrocodeine Phosphate Powder contains not less than 9.3% and not more than 10.7% of dihydrocodeine phosphate ($C_{18}H_{23}NO_3 \cdot H_3PO_4$; 399.38).

Method of preparation

Dihydrocodeine Phosphate	100 g
Lactose Hydrate	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% Dihydrocodeine Phosphate Powder (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 281 nm and 285 nm.

Assay Weigh accurately about 2.5 g of 10% Dihydrocodeine Phosphate Powder, dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of dihydrocodeine phosphate for assay, (previously determine the loss on drying <2.41> (105°C, 4 hours)), dissolve in water to make exactly 100 mL, then pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dihydrocodeine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of dihydrocodeine phosphate} \\ & (C_{18}H_{23}NO_3 \cdot H_3PO_4) \\ & = W_S \times (Q_T/Q_S) \times 5 \end{aligned}$$

W_S : Amount (mg) of dihydrocodeine phosphate for assay, calculated on the dried basis

Internal standard solution—A solution of ethylefrine hydrochloride (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wave-

length: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of dihydrocodeine is about 9 minutes.

System suitability—

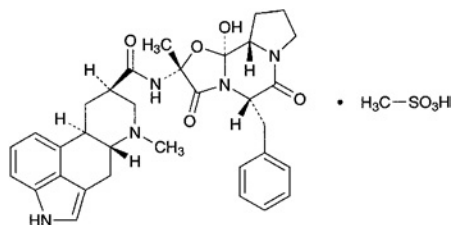
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, dihydrocodeine and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydrocodeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Dihydroergotamine Mesilate

ジヒドロエルゴタミンメシル酸塩



$C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$: 679.78
(5'S,10R)-5'-Benzyl-12'-hydroxy-2'-methyl-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate
[6190-39-2]

Dihydroergotamine Mesilate contains not less than 97.0% of $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$, calculated on the dried basis.

Description Dihydroergotamine Mesilate occurs as a white to yellowish white or grayish white to reddish white powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

It is gradually colored by light.

Melting point: about 214°C (with decomposition).

Identification (1) Dissolve 1 mg of Dihydroergotamine Mesilate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, and shake: a blue color develops.

(2) To 0.1 g of Dihydroergotamine Mesilate add 0.4 g of sodium hydroxide, stir well, and incinerate by gradual ignition. After cooling, add 10 mL of water to the residue, heat to boiling, cool, and filter. To the filtrate add 0.5 mL of hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate. Separately, to 0.1 g of Dihydroergotamine Mesilate add 5 mL of dilute hydrochloric acid, shake for 5 minutes, filter, and to the filtrate add 1 mL of barium chloride TS: the solution is clear.

(3) Determine the absorption spectrum of a solution of Dihydroergotamine Mesilate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dihydroergotamine Mesilate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-16.7 - -22.7^\circ$ [0.5 g, calculated on the dried basis, a mixture of ethanol (99.5), chloroform and ammonia solution (28) (10:10:1), 20 mL, 100 mm].

pH <2.54> Dissolve 0.05 g of Dihydroergotamine Mesilate in 50 mL of water: the pH of this solution is between 4.4 and 5.4.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Dihydroergotamine Mesilate in 0.1 mL of a solution of methanesulfonic acid (7 in 100) and 50 mL of water: the solution is clear, and has no more color than the following control solutions [1] or [2].

Control solution [1]: Pipet 0.6 mL of Ferric Chloride Stock CS and 0.15 mL of Cobaltous Chloride Stock CS, mix, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

Control solution [2]: Pipet 0.6 mL of Ferric Chloride Stock CS, 0.25 mL of Cobaltous Chloride Stock CS and 0.1 mL of copper (II) sulfate CS, mix, and add diluted hydrochloric acid (1 in 40) to make exactly 100 mL.

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Dihydroergotamine Mesilate in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution (1). Pipet 10 mL of the standard solution (1), add a mixture of chloroform and methanol (9:1) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and dry the plate with cold wind within 1 minute. Develop the plate again immediately with a freshly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:6:1) to a distance of about 15 cm, and air-dry

the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and dry the plate with warm wind: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), and the spots, which are more intense than the spot from the standard solution (2), are not more than two.

Loss on drying <2.41> Not more than 4.0% (0.5 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 6 hours).

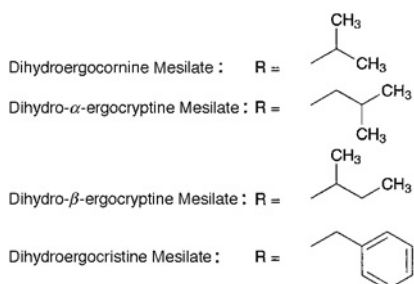
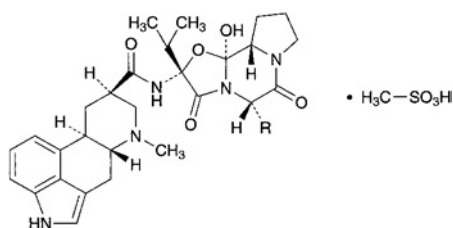
Assay Weigh accurately about 0.2 g of Dihydroergotamine Mesilate, dissolve in 170 mL of a mixture of acetic anhydride and acetic acid (100) (10:1), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 13.60 mg of $C_{33}H_{37}N_5O_5 \cdot CH_4O_3S$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dihydroergotoxine Mesilate

ジヒドロエルゴトキシンメシル酸塩



Dihydroergocornine Mesilate

$C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$: 659.79
(5'S,10R)-12'-Hydroxy-2',5'-bis(1-methylethyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate

Dihydro- α -ergocryptine Mesilate

$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$: 673.82
(5'S,10R)-12'-Hydroxy-2'-(1-methylethyl)-5'-(2-methylpropyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate

Dihydro- β -ergocryptine Mesilate

$C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$: 673.82
(5'S,10R)-12'-Hydroxy-2'-(1-methylethyl)-5'-(1-methylpropyl)-9,10-dihydroergotaman-3',6',18-trione monomethanesulfonate

Dihydroergocristine Mesilate

$C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$: 707.84
(5'S,10R)-5'-Benzyl-12'-hydroxy-2'-(1-methylethyl)-9,10-dihydroergotaman-3',6',18-trione

monomethanesulfonate

[8067-24-1, Dihydroergotoxine Mesilate]

Dihydroergotoxine Mesilate contains not less than 97.0% and not more than 103.0% of dihydroergotoxine mesilate [as a mixture of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$), dihydro- α -ergocryptine mesilate ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$), dihydro- β -ergocryptine mesilate ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$)], calculated on the anhydrous basis. The relative contents of dihydroergocornine mesilate ($C_{31}H_{41}N_5O_5 \cdot CH_4O_3S$), dihydroergocryptine mesilate ($C_{32}H_{43}N_5O_5 \cdot CH_4O_3S$) and dihydroergocristine mesilate ($C_{35}H_{41}N_5O_5 \cdot CH_4O_3S$) are 30.3–36.3% each, and the content ratio of dihydro- α -ergocryptine mesilate and dihydro- β -ergocryptine mesilate is 1.5–2.5:1.

Description Dihydroergotoxine Mesilate occurs as a white to pale yellow powder.

It is soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, in acetonitrile and in chloroform, and practically insoluble in diethyl ether.

Identification Determine the infrared absorption spectrum of Dihydroergotoxine Mesilate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +11.0 – +15.0° (0.2 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Dihydroergotoxine Mesilate in 20 mL of water: the solution is clear and the color of the solution is not more intense than that of the following control solution.

Control solution: To a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 0.4 mL of Cupric Sulfate Stock CS and 2.4 mL of Ferric Chloride Stock CS add diluted hydrochloric acid (1 in 40) to make exactly 200 mL.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Dihydroergotoxine Mesilate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Weigh accurately 0.100 g of Dihydroergotoxine Mesilate, dissolve it in a mixture of chloroform and methanol (9:1) to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately 10 mg of dihydroergocristine mesilate for thin-layer chromatography, and dissolve in a mixture of chloroform and methanol (9:1) to make exactly 100 mL. Pipet 6 mL, 4 mL and 2 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, respectively, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03> without putting the filter paper in the developing vessel. Spot 5 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate with the aid of a cool air

stream. Immediately after that, develop the plate again with a newly prepared mixture of dichloromethane, ethyl acetate, methanol and ammonia solution (28) (50:50:3:1) to a distance of about 15 cm, and dry the plate within 1 minute with the aid of a cool air stream. Spray evenly *p*-dimethylaminobenzaldehyde-hydrochloric acid TS on the plate, dry the plate within 2 minutes with the aid of a cool air stream, and heat it at 40°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1), not more than 2 spots are more intense than that from the standard solution (2), and not more than 4 spots are more intense than that from the standard solution (3).

Water <2.48> Not more than 5.0% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Dihydroergotoxine mesilate—Weigh accurately about 30 mg each of Dihydroergotoxine Mesilate and Dihydroergotoxine Mesilate Reference Standard, and dissolve them separately in a suitable amount of a mixture of water and acetonitrile (3:1). To these solutions add exactly 10 mL of the internal standard solution and an amount of a mixture of water and acetonitrile (3:1) to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L of the sample solution and standard solution as directed under Liquid Chromatography according to the following conditions, and calculate the ratios of the peak areas of dihydroergocornine, dihydro- α -ergocryptine, dihydroergocristine and dihydro- β -ergocryptine to the peak area of the internal standard of these solutions.

$$\begin{aligned} & \text{Amount (mg) of dihydroergotoxine mesilate} \\ & = W_S \times \{(M_{TA} + M_{TB} + M_{TC} + M_{TD}) / \\ & \quad (M_{SA} + M_{SB} + M_{SC} + M_{SD})\} \end{aligned}$$

W_S : Amount (mg) of Dihydroergotoxine Mesilate Reference Standard, calculated on the anhydrous basis

M_{TA} : Ratio of the peak area of dihydroergocornine to that of the internal standard of the sample solution \times 659.80

M_{TB} : Ratio of the peak area of dihydro- α -ergocryptine to that of the internal standard of the sample \times 673.83

M_{TC} : Ratio of the peak area of dihydroergocristine to that of the internal standard of the sample solution \times 707.85

M_{TD} : Ratio of the peak area of dihydro- β -ergocryptine to that of the internal standard of the sample solution \times 673.83

M_{SA} : Ratio of the peak area of dihydroergocornine to that of the internal standard of the standard solution \times 659.80

M_{SB} : Ratio of the peak area of dihydro- α -ergocryptine to that of the internal standard of the standard solution \times 673.83

M_{SC} : Ratio of the peak area of dihydroergocristine to that of the internal standard of the standard solution \times 707.85

M_{SD} : Ratio of the peak area of dihydro- β -ergocryptine to that of the internal standard of the standard solution \times 673.83

Internal standard solution—Dissolve 0.04 g of chloramphenicol in a mixture of water and acetonitrile (3:1) to make 250 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, acetonitrile and triethylamine (30:10:1).

Flow rate: Adjust the flow rate so that the retention time of chloramphenicol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard, dihydroergocornine, dihydro- α -ergocryptine, dihydroergocristine and dihydro- β -ergocryptine are eluted in this order with the resolution between the peaks of dihydro- α -ergocryptine and dihydroergocristine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dihydroergocornine, dihydro- α -ergocryptine, dihydroergocristine and dihydro- β -ergocryptine to that of the internal standard is not more than 0.5%.

(2) Relative contents of dihydroergocornine mesilate, dihydroergocryptine mesilate and dihydroergocristine mesilate—Calculate the relative amounts of dihydroergocornine mesilate, dihydroergocryptine mesilate (dihydro- α -ergocryptine mesilate and dihydro- β -ergocryptine mesilate) and dihydroergocristine mesilate from the chromatogram obtained in Assay (1) for the sample solution using the following equations:

$$\begin{aligned} & \text{Relative amount (\%)} \text{ of dihydroergocornine mesilate} \\ & = \{M_{TA} / (M_{TA} + M_{TB} + M_{TC} + M_{TD})\} \times 100 \end{aligned}$$

$$\begin{aligned} & \text{Relative amount (\%)} \text{ of dihydroergocryptine mesilate} \\ & = \{(M_{TB} + M_{TD}) / (M_{TA} + M_{TB} + M_{TC} + M_{TD})\} \times 100 \end{aligned}$$

$$\begin{aligned} & \text{Relative amount (\%)} \text{ of dihydroergocristine mesilate} \\ & = \{M_{TC} / (M_{TA} + M_{TB} + M_{TC} + M_{TD})\} \times 100 \end{aligned}$$

(3) Ratio of the content of dihydro- α -ergocryptine mesilate to dihydro- β -ergocryptine mesilate—Calculate the ratio of the amount of dihydro- α -ergocryptine mesilate to dihydro- β -ergocryptine mesilate from the chromatogram obtained in the Assay (1) for the sample solution using the following equations:

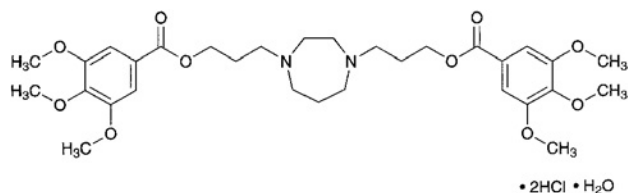
$$\begin{aligned} & \text{Ratio of the content of dihydro-}\alpha\text{-ergocryptine} \\ & \text{mesilate to dihydro-}\beta\text{-ergocryptine mesilate} \\ & = (M_{TB} / M_{TD}) \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dilazep Hydrochloride Hydrate

ジラゼブ塩酸塩水和物



$C_{31}H_{44}N_2O_{10} \cdot 2HCl \cdot H_2O$: 695.63
3,3'-(1,4-Diazepane-1,4-diyl)dipropyl bis(3,4,5-trimethoxybenzoate) dihydrochloride monohydrate [20153-98-4, anhydride]

Dilazep Hydrochloride Hydrate contains not less than 98.0% of dilazep hydrochloride ($C_{31}H_{44}N_2O_{10} \cdot 2HCl$: 677.62), calculated on the dried basis.

Description Dilazep Hydrochloride Hydrate occurs as a white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in water, slightly soluble in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 200 – 204°C Immerse the sample in a bath of 110°C, and raise the temperature at the rate of about 3°C per minute from 140°C to 150°C, about 10°C per minute from 160°C to 195°C and about 1°C per minute from 195°C.

Identification (1) To 1 mL of a solution of Dilazep Hydrochloride Hydrate (1 in 100) add 0.1 mL of a solution of hydroxylammonium chloride (1 in 10) and 0.1 mL of 8 mol/L potassium hydroxide TS, and warm in a water bath of 70°C for 10 minutes. After cooling, add 0.5 mL of dilute hydrochloric acid and 0.1 mL of iron (III) chloride TS: a purple color develops.

(2) To 5 mL of a solution of Dilazep Hydrochloride Hydrate (3 in 500) add 0.3 mL of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Dilazep Hydrochloride Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Dilazep Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dilazep Hydrochloride Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Dilazep Hydrochloride Hydrate. Prepare the control solution with

0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Dilazep Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dilazep Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Dilazep Hydrochloride Hydrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ethyl acetate, dichloromethane and hydrochloric acid (500:200:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 2.0 – 3.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

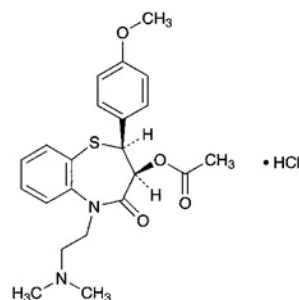
Assay Weigh accurately about 0.3 g of Dilazep Hydrochloride Hydrate, dissolve in 40 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.88 mg of $C_{31}H_{44}N_2O_{10} \cdot 2HCl$

Containers and storage Containers—Tight containers.

Diltiazem Hydrochloride

ジルチアゼム塩酸塩



$C_{22}H_{26}N_2O_4S \cdot HCl$: 450.98
(2*S*,3*S*)-5-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate monohydrochloride [33286-22-5]

Diltiazem Hydrochloride, when dried, contains not less than 98.5% of $C_{22}H_{26}N_2O_4S \cdot HCl$.

Description Diltiazem Hydrochloride occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in formic acid, freely soluble in water, in methanol and in chloroform, sparingly soluble in acetonitrile, slightly soluble in acetic anhydride and in ethanol (99.5), and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.05 g of Diltiazem Hydrochloride in 1 mL of 1 mol/L hydrochloric acid TS, add 2 mL of ammonium thiocyanate-cobaltous nitrate TS and 5 mL of chloroform, shake well, and allow to stand: a blue color develops in the chloroform layer.

(2) Proceed as directed under Oxygen Flask Combustion Method <1.06> with 0.03 g of Diltiazem Hydrochloride, using 20 mL of water as the absorbing liquid, and prepare the test solution: the test solution responds to the Qualitative Tests <1.09> (1) for sulfate.

(3) Dissolve 0.01 g of Diltiazem Hydrochloride in 0.01 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of the solution add 0.01 mol/L hydrochloric acid TS to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Diltiazem Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1741 cm^{-1} , 1678 cm^{-1} , 1252 cm^{-1} and 1025 cm^{-1} .

(5) A solution of Diltiazem Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +115 – +120° (after drying, 0.20 g, water, 20 mL, 100 mm).

Melting point <2.60> 210 – 215°C (with decomposition).

pH <2.54> Dissolve 1.0 g of Diltiazem Hydrochloride in 100 mL of water: the pH of this solution is between 4.3 and 5.3.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Diltiazem Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Diltiazem Hydrochloride. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Diltiazem Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Place 1.0 g of Diltiazem Hydrochloride in a decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the neck of the flask, and heat cautiously until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution becomes colorless to pale yellow. After cooling, add 2 mL of saturated solution of ammonium oxalate monohydrate, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, use this solution as the test solution, and perform the test: the test solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as the test solution without Diltiazem Hydrochloride, add 2.0 mL of

Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 50 mg of Diltiazem Hydrochloride in 50 mL of diluted ethanol (99.5) (4 in 5), and use this solution as the sample solution. Measure exactly 1 mL of the sample solution, add diluted ethanol (99.5) (4 in 5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by automatic integration method: the total peak area of peaks other than the peak of diltiazem obtained from the sample solution is not more than 3/5 times the peak area of diltiazem obtained from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 8 g of sodium acetate trihydrate and 1.5 g of *d*-camphorsulfonic acid in 500 mL of water, and filter using a membrane filter (0.4 μm in pore size). Add 250 mL each of acetonitrile and methanol to the filtrate, and adjust the solution to a pH of 6.6 by adding sodium acetate trihydrate.

Flow rate: Adjust the flow rate so that the retention time of diltiazem is about 9 minutes.

Time span of measurement: About twice as long as the retention time of diltiazem beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add diluted ethanol (99.5) (4 in 5) to make exactly 10 mL. Confirm that the peak area of diltiazem obtained from 20 μL of this solution is equivalent to 15 to 25% of that of diltiazem obtained from 20 μL of the standard solution.

System performance: Dissolve 0.03 g of Diltiazem Hydrochloride, 0.02 g of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one hydrochloride and 0.02 g of phenylbenzoate in 160 mL of ethanol (99.5), and add water to make 200 mL. Perform the test with 20 μL of this solution as directed under Liquid Chromatography under the above operating conditions: *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one, diltiazem and phenyl benzoate are eluted in this order with the resolutions between the peaks of *d*-3-hydroxy-*cis*-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one and diltiazem and between the peaks of diltiazem and phenyl benzoate being not less than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of diltiazem is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

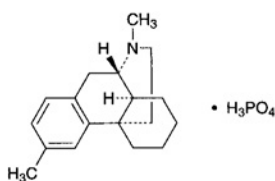
Assay Weigh accurately about 0.7 g of Diltiazem Hydrochloride, previously dried, dissolve in 2.0 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 45.10 mg of $C_{22}H_{26}N_2O_4S \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dimemorfan Phosphate

ジメモルファンリン酸塩



$C_{18}H_{25}N \cdot H_3PO_4$: 353.39
(9*S*,13*S*,14*S*)-3,17-Dimethylmorphinan monophosphate
[36304-84-4]

Dimemorfan Phosphate, when dried, contains not less than 98.5% of $C_{18}H_{25}N \cdot H_3PO_4$.

Description Dimemorfan Phosphate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 265°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Dimemorfan Phosphate (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dimemorfan Phosphate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibits similar intensities of absorption at the same wave numbers.

(3) To 2 mL of a solution of Dimemorfan Phosphate (1 in 100) add 2 to 3 drops of silver nitrate TS: a yellow precipitate is formed, and it dissolves on the addition of dilute nitric acid.

Optical rotation <2.49> $[\alpha]_D^{20}$: +25 – +27° (after drying, 1 g, methanol, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Dimemorfan Phosphate in 100 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Dimemorfan Phosphate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dimemorfan Phosphate according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Dimemorfan Phosphate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (150:150:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly the plate with Dragendorff's TS for spraying: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

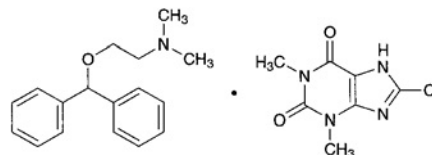
Assay Weigh accurately about 0.6 g of Dimemorfan Phosphate, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 35.34 mg of $C_{18}H_{25}N \cdot H_3PO_4$

Containers and storage Containers—Tight containers.

Dimenhydrinate

ジメンヒドリナート



$C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$: 469.96
2-(Diphenylmethoxy)-*N,N*-dimethylethylamine—
8-chloro-1,3-dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione
(1/1) [523-87-5]

Dimenhydrinate, when dried, contains not less than 53.0% and not more than 55.5% of diphenhydramine ($C_{17}H_{21}NO$: 255.36), and not less than 44.0% and not more than 47.0% of 8-chlorotheophylline ($C_7H_7ClN_4O_2$: 214.61).

Description Dimenhydrinate occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in chloroform, freely soluble in ethanol (95), and slightly soluble in water and in diethyl ether.

Identification (1) Dissolve 0.5 g of Dimenhydrinate in 30 mL of dilute ethanol, add 30 mL of water, and use this solution as the sample solution. Transfer 30 mL of the sample solution to a separator, and add 2 mL of ammonia solution (28). Extract with two 10-mL portions of diethyl ether, com-

bine the diethyl ether extracts, wash the combined extracts with 5 mL of water, and then extract the combined extracts with 15 mL of diluted hydrochloric acid (1 in 100). With this acid extract perform the following tests.

(i) To 5 mL of this acid extract add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(ii) To 10 mL of this acid extract add 10 mL of 2,4,6-trinitrophenol TS dropwise, and allow to stand for 30 minutes. Collect the precipitate by filtrating, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt <2.60> between 128°C and 133°C.

(2) To 30 mL of the sample solution obtained in the Identification (1) add 2 mL of dilute sulfuric acid, and cool for 30 minutes. Scratch the inside wall of the container frequently to facilitate crystallization. Filter, and wash the white crystals with a small amount of ice-cooled water. Dry the crystals for 1 hour at 105°C: the crystals melt <2.60> between 300°C and 305°C with decomposition.

(3) To 0.01 g of the crystals obtained in the Identification (2) add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid, and evaporate on a water bath to dryness: the residue shows a yellow-red color. When the dish containing the residue is held over a vessel containing 2 to 3 drops of ammonia TS, the color changes to red-purple, which is discharged on the addition of 2 to 3 drops of sodium hydroxide TS.

(4) Mix well 0.05 g of the crystals obtained in the Identification (2) with 0.5 g of sodium peroxide in a nickel crucible, and heat until the mass melts. Cool, dissolve the melted mass in 20 mL of water, and acidify with dilute nitric acid: the solution responds to the Qualitative Tests for chloride <1.09>.

Melting point <2.60> 102 – 107°C

Purity (1) Chloride <1.03>—Transfer 50 mL of the filtrate obtained in the Assay (2) to a Nessler tube, add 1 mL of nitric acid, and allow to stand for 5 minutes: the turbidity of the solution is not greater than that of the following control solution.

Control solution: Dilute 0.25 mL of 0.01 mol/L hydrochloric acid VS with 6 mL of dilute nitric acid and with water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes (not more than 0.044%).

(2) Bromide and iodide—Place 0.10 g of Dimenhydrinate in a glass-stoppered test tube, and add 0.05 g of sodium nitrite, 10 mL of chloroform and 10 mL of dilute hydrochloric acid. Stopper, shake well, and allow to stand: the chloroform layer remains colorless.

Loss on drying <2.41> Not more than 0.5% (3 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay (1) Diphenhydramine—Weigh accurately about 0.5 g of Dimenhydrinate, previously dried, transfer to a 250-mL separator, and add 50 mL of water, 3 mL of ammonia TS and 10 g of sodium chloride. Extract with six 15-mL portions of diethyl ether with shaking, combine the diethyl ether extracts, and wash the combined diethyl ether extracts with three 50-mL portions of water. To the diethyl ether extracts add exactly 25 mL of 0.05 mol/L sulfuric acid VS, and add 25 mL of water. Shake thoroughly, and evaporate the diethyl ether gently. Cool, and titrate the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of methyl

red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sulfuric acid VS
= 25.54 mg $C_{17}H_{21}NO$

(2) 8-Chlorotheophylline—Weigh accurately about 0.8 g of Dimenhydrinate, previously dried, transfer to a 200-mL volumetric flask, add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), and heat on a water bath for 5 minutes. Add exactly 25 mL of 0.1 mol/L silver nitrate VS, heat on a water bath for 15 minutes with occasional shaking, cool, and add water to make exactly 200 mL. Allow to stand overnight to settle the precipitate, and filter through a dry filter paper, discarding the first 20 mL of the filtrate. Measure exactly 100 mL of the subsequent filtrate, acidify with nitric acid, add 3 mL of nitric acid, and titrate the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 21.46 mg of $C_7H_7ClN_4O_2$

Containers and storage Containers—Well-closed containers.

Dimenhydrinate Tablets

ジメンヒドリナート錠

Dimenhydrinate Tablets contain not less than 95% and not more than 105% of the labeled amount of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$; 469.96).

Method of preparation Prepare as directed under Tablets, with Dimenhydrinate.

Identification (1) Triturate a quantity of powdered Dimenhydrinate Tablets, equivalent to 0.5 g of Dimenhydrinate according to the labeled amount, with 25 mL of warm ethanol (95), and filter. Dilute the filtrate with 40 mL of water, and filter again. Use the filtrate as the sample solution. Transfer 30 mL of the sample solution to a separator, and proceed as directed in the Identification (1) under Dimenhydrinate.

(2) With 30 mL of the sample solution obtained in (1), proceed as directed in the Identification (2), (3) and (4) under Dimenhydrinate.

Assay Weigh accurately, and powder not less than 20 Dimenhydrinate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of dimenhydrinate ($C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$), transfer to a flask, add 40 mL of ethanol (95), and heat with swirling on a water bath until the solution just boils. Continue to heat for 30 seconds, and filter through a glass filter (G4). Wash the filter with warm ethanol (95), transfer the filtrate and washings to a flask, and evaporate the ethanol on a water bath to make 5 mL. Add 50 mL of water, 3 mL of ammonia TS and 6 mL of a solution of ammonium nitrate (1 in 10), heat the mixture on a water bath for 5 minutes, add exactly 25 mL of 0.1 mol/L silver nitrate VS, and heat on a water bath for 15 minutes with occasional shaking. Transfer the mixture to a 200-mL volumetric flask,

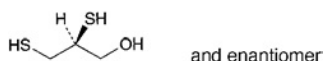
using water to rinse the flask, cool, add water to make exactly 200 mL, and proceed as directed in the Assay (2) under Dimenhydrinate.

Each mL of 0.1 mol/L silver nitrate VS
= 47.00 mg of $C_{17}H_{21}NO \cdot C_7H_7ClN_4O_2$

Containers and storage Containers—Well-closed containers.

Dimercaprol

ジメルカプロール



$C_3H_8OS_2$: 124.23
(2*RS*)-2,3-Disulfanylpropan-1-ol [59-52-9]

Dimercaprol contains not less than 98.5% and not more than 101.5% of $C_3H_8OS_2$.

Description Dimercaprol is a colorless or pale yellow liquid. It has a mercaptan-like, disagreeable odor.

It is miscible with methanol and with ethanol (99.5)

It is soluble in peanut oil, and sparingly soluble in water.

It shows no optical rotation.

Identification (1) Add 1 drop of Dimercaprol to a mixture of 1 drop of a solution of cobalt (II) chloride hexahydrate (1 in 200) and 5 mL of water: a yellow-brown color develops.

(2) Determine the infrared absorption spectrum of Dimercaprol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.570 – 1.575

Specific gravity <2.56> d_{20}^{20} : 1.238 – 1.248

Purity (1) Clarity and color of solution—Dissolve 1.0 mL of Dimercaprol in 20 mL of peanut oil: the solution is clear and colorless to pale yellow.

(2) Bromide—To 2.0 g of Dimercaprol add 25 mL of dilute potassium hydroxide-ethanol TS, and heat in a water bath under a reflux condenser for 2 hours. Evaporate the ethanol in a current of warm air, add 20 mL of water, and cool. Add a mixture of 10 mL of strong hydrogen peroxide and 40 mL of water, boil gently under a reflux condenser for 10 minutes, and filter rapidly after cooling. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, add 10 mL of dilute nitric acid and exactly 5 mL of 0.1 mol/L silver nitrate VS, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination: not more than 1.0 mL of 0.1 mol/L silver nitrate VS is consumed.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dimercaprol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Assay Weigh accurately about 0.15 g of Dimercaprol into a glass-stoppered flask, dissolve in 10 mL of methanol, and ti-

trate <2.50> immediately with 0.05 mol/L iodine VS until a pale yellow color is produced. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 6.211 mg of $C_3H_8OS_2$

Containers and storage Containers—Tight containers.
Storage—Not exceeding 5°C.

Dimercaprol Injection

ジメルカプロール注射液

Dimercaprol Injection is an oily solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dimercaprol ($C_3H_8OS_2$: 124.23).

Method of preparation Prepare as directed under Injections, with Dimercaprol. Benzyl Benzoate or Benzyl Alcohol may be added to increase the solubility.

Description Dimercaprol Injection is a clear, colorless or light yellow liquid. It has an unpleasant odor.

Identification Measure a volume of Dimercaprol Injection, equivalent to 30 mg of Dimercaprol according to the labeled amount, and proceed as directed in the Identification (1).

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 2: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

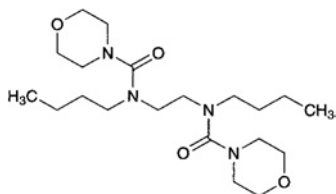
Assay Pipet a volume of Dimercaprol Injection, equivalent to about 0.1 g of dimercaprol ($C_3H_8OS_2$), into a flask, and rinse the pipet several times with a mixture of methanol and diethyl ether (3:1), adding the rinsings to the flask. Add the mixture of methanol and diethyl ether (3:1) to make 50 mL, and titrate <2.50> with 0.05 mol/L iodine VS until a yellow color persists. Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 6.211 mg of $C_3H_8OS_2$

Containers and storage Containers—Hermetic containers.
Storage—In a cold place.

Dimorpholamine

ジモルホラミン



$C_{20}H_{38}N_4O_4$: 398.54

N,N'-Ethylenebis(*N*-butylmorpholine-4-carboxamide)

[119-48-2]

Dimorpholamine, when dried, contains not less than 98.0% and not more than 101.0% of $C_{20}H_{38}N_4O_4$.

Description Dimorpholamine is a white to light yellow, crystalline powder, masses or syrupy liquid.

It is very soluble in ethanol (99.5) and in acetic anhydride, and soluble in water.

The pH of a solution prepared by dissolving 1.0 g of Dimorpholamine in 10 mL of water is between 6.0 and 7.0.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Dimorpholamine (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dimorpholamine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dimorpholamine in 50 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—To 20 mL of the solution obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Sulfate <1.14>—To 10 mL of the solution obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Dimorpholamine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Dimorpholamine in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5) and water (4:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 10 minutes: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 8 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Dimorpholamine, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.85 mg of $C_{20}H_{38}N_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dimorpholamine Injection

ジモルホラミン注射液

Dimorpholamine Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of dimorpholamine ($C_{20}H_{38}N_4O_4$: 398.54).

Method of preparation Prepare as directed under Injections, with Dimorpholamine.

Description Dimorpholamine Injection is a clear, colorless liquid.

pH: 3.0 – 5.5

Identification (1) To a volume of Dimorpholamine Injection, equivalent to 0.1 g of Dimorpholamine according to the labeled amount, add 3 drops of Dragendorff's TS: an orange color develops.

(2) To a volume of Dimorpholamine Injection, equivalent to 50 mg of Dimorpholamine according to the labeled amount, add 1 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of hydrochloric acid, boil for 10 minutes under a reflux condenser, and evaporate to dryness on a water bath. Dissolve the residue with 1 mL of water, neutralize with sodium hydroxide TS, and add 0.2 mL of a solution of acetaldehyde (1 in 20), 0.1 mL of sodium pentacyanonitrosyl ferrate (III) TS and 0.5 mL of sodium carbonate TS: a blue color develops.

Bacterial endotoxins <4.01> Less than 5.0 EU/mg. Perform the test with the sample diluted to 0.15 w/v% with water for bacterial endotoxins test.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a volume of Dimorpholamine Injection, equivalent to about 30 mg of dimorpholamine ($C_{20}H_{38}N_4O_4$), and add water to make exactly 200 mL. Pipet 1 mL of this solution, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of dimorpholamine for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 8 hours, and dissolve in water to make exactly 1000 mL. Pipet 1 mL of this solution, shake with exactly 4 mL of the internal standard solution for 5 minutes, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dimorpholamine to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of dimorpholamine (C}_{20}\text{H}_{38}\text{N}_4\text{O}_4) \\ = W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of dimorpholamine for assay

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 216 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of dimorpholamine is about 4 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, dimorpholamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

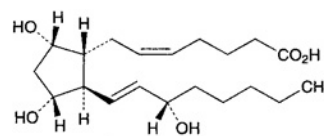
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of dimorpholamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Dinoprost

Prostaglandin $F_{2\alpha}$

ジノプロスト



$C_{20}H_{34}O_5$: 354.48
(5*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-Dihydroxy-2-[(1*E*,3*S*)-3-hydroxyoct-1-en-1-yl]cyclopentyl]hept-5-enoic acid
[551-11-1]

Dinoprost contains not less than 98.5% of $C_{20}H_{34}O_5$, calculated on the anhydrous basis.

Description Dinoprost occurs as white, waxy masses or powder, or a clear, colorless to light yellow and viscous liquid. It is odorless.

It is very soluble in *N,N*-dimethylformamide, freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and very slightly soluble in water.

Identification (1) To 5 mg of Dinoprost add 2 mL of sulfuric acid, and dissolve by shaking for 5 minutes: a dark red color develops. To this solution add 30 mL of sulfuric acid: an orange color develops with a green fluorescence.

(2) Dissolve 1 mg of Dinoprost in 50 mL of diluted sulfuric acid (7 in 10), and warm in a water bath heated at 50°C for 40 minutes. After cooling, determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Warm Dinoprost at 40°C to effect a liquid, and determine the infrared absorption spectrum of the liquid as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +24 – +31° (0.2 g, ethanol (99.5), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Dinoprost in 5 mL of ethanol (99.5): the solution is clear and colorless to pale yellow.

(2) Related substances—Dissolve 10 mg of Dinoprost in 2 mL of methanol, add water to make 10 mL, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of dinoprost from the sample solution is not larger than the peak area of dinoprost from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogenphosphate TS and acetonitrile (5:2).

Flow rate: Adjust the flow rate so that the retention time of dinoprost is about 20 minutes.

Selection of column: Dissolve 0.01 g each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 2 mL of methanol, and add water to make 10 mL. To 1 mL of this solution add diluted methanol (1 in 5) to make 30 mL, proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dinoprost from the standard solution composes 5% to 15% of the full scale.

Time span of measurement: About 1.5 times as long as the retention time of dinoprost beginning after the solvent peak.

Water <2.48> Not more than 0.5% (0.3 g, direct titration).

Assay Weigh accurately about 50 mg of Dinoprost, dissolve in 30 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.02 mol/L tetramethylammonium hydroxide VS under a stream of nitrogen (potentiometric titration). Perform a blank determination, and make any necessary correction.

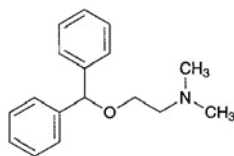
Each mL of 0.02 mol/L tetramethylammonium hydroxide VS
= 7.090 mg of C₂₀H₃₄O₅

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a place not exceeding 5°C.

Diphenhydramine

ジフェンヒドラミン



C₁₇H₂₁NO: 255.35

2-(Diphenylmethoxy)-*N,N*-dimethylethylamine [58-73-1]

Diphenhydramine contains not less than 96.0% of C₁₇H₂₁NO.

Description Diphenhydramine is a clear, light yellow to yellow liquid. It has a characteristic odor, and has a burning taste at first, followed by a slight sensation of numbness on the tongue.

It is miscible with acetic anhydride, with acetic acid (100), with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Boiling point: about 162°C (in vacuum, 0.67 kPa).

Refractive index n_D^{20} : about 1.55

It is gradually affected by light.

Identification (1) To 0.05 g of Diphenhydramine add 2 mL of sulfuric acid: an orange-red precipitate is produced immediately, and its color changes to red-brown on standing. Add carefully 2 mL of water to this solution: the intensity of the color changes, but the color tone does not change.

(2) Dissolve 0.1 g of Diphenhydramine in 10 mL of dilute ethanol, add an excess of a saturated solution of 2,4,6-trinitrophenol in dilute ethanol with stirring, and cool in ice. Collect the produced crystals, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt between 128°C and 133°C.

Specific gravity <2.56> d_{20}^{20} : 1.013 – 1.020

Purity (1) β -Dimethylaminoethanol—Dissolve 1.0 g of Diphenhydramine in 20 mL of diethyl ether, and extract with two 10-mL portions of water with thorough shaking. Combine the water extracts, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.05 mol/L sulfuric acid VS: no red color develops.

(2) Benzohydrol—Transfer 1.0 g of Diphenhydramine to a separator, dissolve in 20 mL of diethyl ether, and extract with two 25-mL portions of diluted hydrochloric acid (1 in 15) with thorough shaking. Separate the diethyl ether layer, evaporate slowly on a water bath, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the mass of the residue is not more than 20 mg.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Diphenhydramine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Diphenhydramine, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.54 mg of C₁₇H₂₁NO

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled.

Diphenhydramine and Bromovalerylurea Powder

ジフェンヒドラミン・バレリル尿素散

Method of preparation

Diphenhydramine Tannate	90 g
Bromovalerylurea	500 g
Starch, Lactose Hydrate, or their mixture	a sufficient quantity

To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Diphenhydramine and Bromovalerylurea Powder occurs as a slightly grayish white powder.

Identification (1) To 0.1 g of Diphenhydramine and Bromovalerylurea Powder add 5 mL of dilute hydrochloric acid, 1 mL of ethanol (95) and 10 mL of water, shake, and filter. To the filtrate add 10 mL of sodium hydroxide TS, and extract with 10 mL of chloroform. Separate the chloroform layer, add 1 mL of bromophenol blue TS, and shake: a yellow color develops in the chloroform layer (diphenhydramine tannate).

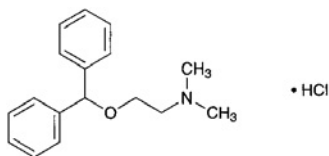
(2) Shake 0.02 g of Diphenhydramine and Bromovalerylurea Powder with 10 mL of diethyl ether, filter, and evaporate the filtrate on a water bath. Dissolve the residue in 2 mL of sodium hydroxide TS, and add 5 mL of dimethylglyoxime-thiosemicarbazide TS, and heat on a water bath for 30 minutes: a red color develops (bromovalerylurea).

(3) Shake 0.3 g of Diphenhydramine and Bromovalerylurea with 5 mL of methanol, filter, and use the filtrate as the sample solution. Dissolve 0.15 g of bromovalerylurea and 0.03 g of diphenhydramine tannate in 5 mL each of methanol, and use the solutions as standard solution (1) and standard solution (2). Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm. Air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): 3 spots from the sample solution and the corresponding spot from standard solutions (1) and (2) show the same R_f value. Spray Dragendorff's TS for spraying evenly on the plate: the spot from the standard solution (2) and the corresponding spot from the sample solution reveal an orange color.

Containers and storage Containers—Well-closed containers.

Diphenhydramine Hydrochloride

ジフェンヒドรามミン塩酸塩



$C_{17}H_{21}NO \cdot HCl$: 291.82

2-(Diphenylmethoxy)-*N,N*-dimethylethylamine monohydrochloride [147-24-0]

Diphenhydramine Hydrochloride, when dried, contains not less than 98.0% of $C_{17}H_{21}NO \cdot HCl$.

Description Diphenhydramine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a

bitter taste, followed by a sensation of numbness on the tongue.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Diphenhydramine Hydrochloride in methanol (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Diphenhydramine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Diphenhydramine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Melting point <2.60> 166 – 170°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Diphenhydramine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Diphenhydramine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Diphenhydramine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate, methanol and ammonia solution (28) (10:4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine TS on the plate: the spots other than the principal spot from the sample solution and the spot on the original point are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Diphenhydramine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.18 mg of $C_{17}H_{21}NO \cdot HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Diphenhydramine, Phenol and Zinc Oxide Liniment

ジフェンヒドラミン・フェノール・亜鉛華リニメント

Method of preparation

Diphenhydramine	20 g
Phenol and Zinc Oxide Liniment	980 g
To make 1000 g	

Dissolve and mix the above ingredients.

Description Diphenhydramine, Phenol and Zinc Oxide Liniment is a white to whitish, pasty mass. It has a slight odor of phenol.

Identification (1) To 3 g of Diphenhydramine, Phenol and Zinc Oxide Liniment add 20 mL of hexane, shake well, and separate the hexane layer. Shake thoroughly the hexane solution with 10 mL of 0.2 mol/L hydrochloric acid. Separate the aqueous layer, and adjust with sodium hydroxide TS to a pH of 4.6. Add 1 mL of bromophenol blue-potassium biphthalate TS and 10 mL of chloroform, and shake: a yellow color develops in the chloroform layer (diphenhydramine).

(2) Place 1 g of Diphenhydramine, Phenol and Zinc Oxide Liniment in a porcelain crucible, gradually raise the temperature by heating until the mass is charred, and ignite strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. Add 2 to 3 drops of potassium hexacyanoferrate (II) TS to the filtrate: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Diphenhydramine, Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.01 g each of diphenhydramine and phenol in 5 mL each of chloroform, and use these solutions as standard solution (1) and standard solution (2). Perform the test as directed under Thin-layer Chromatography <2.03> with the sample solution and the standard solutions. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: two spots from the sample solution and each spot from standard solution (1) and standard solution (2) show the same R_f value. Sublime iodine, and spray Dragendorff's TS evenly upon the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal an orange color.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Diphenhydramine Tannate

タンニン酸ジフェンヒドラミン

Diphenhydramine Tannate is a compound of diphenhydramine and tannic acid, and contains not less than 25.0% and not more than 35.0% of diphenhydramine ($C_{17}H_{21}NO$: 255.35).

Description Diphenhydramine Tannate occurs as a grayish white to light brown powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) To 1 g of Diphenhydramine Tannate add 15 mL of water and 0.3 mL of dilute hydrochloric acid, shake thoroughly for 1 minute, filter, and use this filtrate as the sample solution. Transfer 10 mL of the sample solution to a separator, extract with two 20-mL portions of chloroform, combine the chloroform extracts, and evaporate on a water bath to dryness. To 5 mL of a solution of the residue (1 in 100) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) To 10 mL of a solution of the residue obtained in (1) (1 in 100) add 10 mL of 2,4,6-trinitrophenol TS dropwise, and allow to stand for 30 minutes. Collect the precipitate by filtration, recrystallize from dilute ethanol, and dry at 105°C for 30 minutes: the crystals melt <2.60> between 128°C and 133°C.

(3) To 1 mL of the sample solution obtained in (1) add 1 drop of iron (III) chloride TS: a dark blue-purple color develops.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Diphenhydramine Tannate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 5 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Transfer about 1.7 g of Diphenhydramine Tannate, accurately weighed, to a separator, dissolve in 20 mL of water and 3.0 mL of dilute hydrochloric acid with thorough shaking, add 20 mL of a solution of sodium hydroxide (1 in 10) and exactly 25 mL of isooctane, shake vigorously for 5 minutes, dissolve 2 g of sodium chloride with shaking, and allow to stand. To 20 mL of the isooctane layer add exactly 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 25.54 mg of $C_{17}H_{21}NO$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Freeze-dried Diphtheria Antitoxin, Equine

乾燥ジフテリアウマ抗毒素

Freeze-dried Diphtheria Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains diphtheria antitoxin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Diphtheria Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Diphtheria Antitoxin, Equine, becomes a colorless or light yellow-brown, clear liquid or a slightly whitish turbid liquid on addition of solvent.

Diphtheria Toxoid

ジフテリアトキソイド

Diphtheria Toxoid is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity.

It conforms to the requirements of Diphtheria Toxoid in the Minimum Requirements for Biological Products.

Description Diphtheria Toxoid is a clear, colorless to light yellow-brown liquid.

Adsorbed Diphtheria Toxoid for Adult Use

成人用沈降ジフテリアトキソイド

Adsorbed Diphtheria Toxoid for Adult Use is a liquid for injection containing diphtheria toxoid prepared by treating diphtheria toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and very few antigenic substances other than toxoid, and rendered insoluble with aluminum salt.

It conforms to the requirements of Adsorbed Diphtheria Toxoid for Adult Use in the Minimum Requirements of Biological Products.

Description Adsorbed Diphtheria Toxoid for Adult Use becomes a homogeneous, whitish turbid liquid on shaking.

Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine

沈降精製百日せきジフテリア破傷風混合ワクチン

Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine is a liquid for injection consisting of a liquid containing the protective antigen of *Bordetella pertussis*, Diphtheria Toxoid and a liquid containing tetanus toxoid obtained by detoxifying the tetanus toxin with formaldehyde solution without impairing its immunogenicity, to which aluminum is added to make the antigen and the toxoids insoluble.

It conforms to the requirements of Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine becomes a homogeneous, white turbid liquid on shaking.

Diphtheria-Tetanus Combined Toxoid

ジフテリア破傷風混合トキソイド

Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity.

It conforms to the requirements of Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements of Biological Products.

Description Diphtheria-Tetanus Combined Toxoid is a colorless or light yellow-brown, clear liquid.

Adsorbed Diphtheria-Tetanus Combined Toxoid

沈降ジフテリア破傷風混合トキソイド

Adsorbed Diphtheria-Tetanus Combined Toxoid is a liquid for injection containing diphtheria toxoid and tetanus toxoid which are prepared by treating diphtheria toxin and tetanus toxin, respectively, with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by adding aluminum salt.

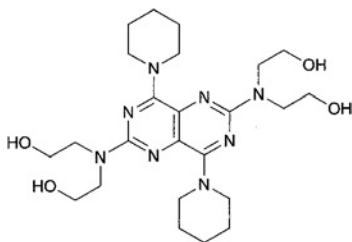
It conforms to the requirements of Adsorbed

Diphtheria-Tetanus Combined Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Diphtheria-Tetanus Combined Toxoid becomes a homogeneous, whitish turbid liquid on shaking.

Dipyridamole

ジピリダモール



$C_{24}H_{40}N_8O_4$: 504.63

2,2',2'',2'''-[4,8-Di(piperidin-1-yl)pyrimido[5,4-d]pyrimidine-2,6-diyl]dinitrilo}tetraethanol [58-32-2]

Dipyridamole, when dried, contains not less than 98.5% of $C_{24}H_{40}N_8O_4$.

Description Dipyridamole occurs as yellow crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 5 mg of Dipyridamole in 2 mL of sulfuric acid, add 2 drops of nitric acid, and shake: a deep purple color develops.

(2) Determine the absorption spectrum of a solution of Dipyridamole in a mixture of methanol and hydrochloric acid (99:1) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dipyridamole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 165 – 169°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Dipyridamole in 10 mL of chloroform: the solution is clear, and shows a yellow color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Dipyridamole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dipyridamole according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Dipyridamole in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add

the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of dipyridamole from the sample solution is not larger than the peak area of dipyridamole from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.2 g of potassium dihydrogen phosphate in 200 mL of water, and add 800 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of dipyridamole is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of dipyridamole.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of dipyridamole obtained from 20 μ L of this solution is equivalent to 15 to 25% of that of dipyridamole obtained from 20 μ L of the standard solution.

System performance: Dissolve 7 mg of Dipyridamole and 3 mg of terphenyl in 50 mL of methanol. When the procedure is run with 20 μ L of this solution under the above operating conditions, dipyridamole and terphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of dipyridamole is not more than 1.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Dipyridamole, previously dried, dissolve in 70 mL of methanol, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.46 mg of $C_{24}H_{40}N_8O_4$

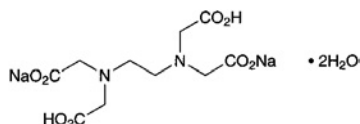
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Disodium Edetate Hydrate

EDTA Sodium Hydrate

エデト酸ナトリウム水和物



$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$: 372.24

Disodium dihydrogen ethylenediaminetetraacetate dihydrate [6381-92-6]

Disodium Edetate Hydrate contains not less than 99.0% of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$.

Description Disodium Edetate Hydrate occurs as white crystals or crystalline powder. It is odorless and has a slight, acid taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 0.01 g of Disodium Edetate Hydrate in 5 mL of water, add 2 mL of a solution of potassium chromate (1 in 200) and 2 mL of arsenic (III) trioxide TS, and heat in a water bath for 2 minutes: a purple color develops.

(2) Dissolve 0.5 g of Disodium Edetate Hydrate in 20 mL of water, and add 1 mL of dilute hydrochloric acid: a white precipitate is produced. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 1 hour: the precipitate melts <2.60> between 240°C and 244°C (with decomposition).

(3) A solution of Disodium Edetate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 1 g of Disodium Edetate Hydrate in 100 mL of water: the pH of this solution is between 4.3 and 4.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Disodium Edetate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Cyanide—Transfer 1.0 g of Disodium Edetate Hydrate to a round-bottomed flask, dissolve in 100 mL of water, add 10 mL of phosphoric acid, and distil. Place 15 mL of 0.5 mol/L sodium hydroxide VS in a 100-mL measuring cylinder, which is used as a receiver, and immerse the bottom end of the condenser into the solution. Distil the mixture until the distillate measures 100 mL, and use this solution as the sample solution. Transfer 20 mL of the sample solution to a glass-stoppered test tube, add 1 drop of phenolphthalein TS, neutralize with dilute acetic acid, and add 5 mL of phosphate buffer solution, pH 6.8, and 1.0 mL of diluted sodium toluensulfonchloramide TS (1 in 5). Immediately stopper the tube, mix gently, and allow to stand for a few minutes. Mix well with 5 mL of pyridine-pyrazolone TS, and allow to stand between 20°C and 30°C for 50 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, add 15 mL of 0.5 mol/L sodium hydroxide VS and water to make exactly 1000 mL, transfer 20 mL of this solution to a glass-stoppered test tube, and proceed as directed

for the sample solution.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Disodium Edetate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Disodium Edetate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Residue on ignition <2.44> 37.0 – 39.0% (1 g).

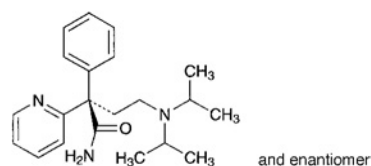
Assay Weigh accurately about 1 g of Disodium Edetate Hydrate, dissolve in 50 mL of water, add 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and 0.04 g of eriochrome black T-sodium chloride indicator, and titrate <2.50> with 0.1 mol/L zinc VS until the color of the solution changes from blue to red.

Each mL of 0.1 mol/L zinc VS
= 37.22 mg of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$

Containers and storage Containers—Well-closed containers.

Disopyramide

ジソピラミド



$C_{21}H_{29}N_3O$: 339.47

(2*RS*)-4-Bis(1-methylethyl)amino-2-phenyl-2-(pyridin-2-yl)butanamide [3737-09-5]

Disopyramide contains not less than 98.5% of $C_{21}H_{29}N_3O$, calculated on the dried basis.

Description Disopyramide occurs as white crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95), freely soluble in acetic anhydride, in acetic acid (100) and in diethyl ether, and slightly soluble in water.

Identification (1) To 1 mL of a solution of Disopyramide in ethanol (95) (1 in 20) add 10 mL of 2,4,6-trinitrophenol TS, and warm: a yellow precipitate is formed. Filter this precipitate, wash with water, and dry at 105°C for 1 hour: the residue melts <2.60> between 172°C and 176°C.

(2) Determine the absorption spectrum of a solution of Disopyramide in 0.05 mol/L sulfuric acid-methanol TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Disopyramide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (269 nm): 194 – 205 (10 mg, 0.05 mol/L sulfuric acid-methanol TS, 500 mL).

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Disopyramide in 10 mL of ethanol (95), and add 2 mL of dilute acetic acid (31) and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 10 mL of ethanol (95), 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Disopyramide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Disopyramide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 400 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and ammonia solution (28) (45:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, 80°C, 2 hours).

Residue on ignition <2.44> Not more than 0.20% (1 g).

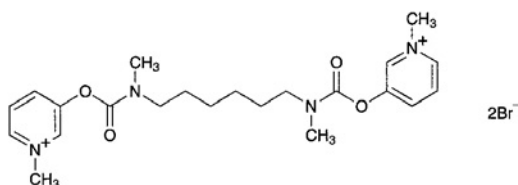
Assay Weigh accurately about 0.25 g of Disopyramide, dissolve in 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.97 mg of $\text{C}_{21}\text{H}_{29}\text{N}_3\text{O}$

Containers and storage Containers—Tight containers.

Distigmine Bromide

ジスチグミン臭化物



$\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_4\text{O}_4$; 576.32

3,3'-[Hexamethylenebis(methyliminocarbonyloxy)]bis(1-methylpyridinium) dibromide [I5876-67-2]

Distigmine Bromide contains not less than 98.5% of $\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_4\text{O}_4$, calculated on the anhydrous basis.

Description Distigmine Bromide occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in methanol, in

ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

The pH of a solution of Distigmine Bromide (1 in 100) is between 5.0 and 5.5.

It is slightly hygroscopic.

It is gradually colored by light.

Melting point: about 150°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Distigmine Bromide (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Distigmine Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Distigmine Bromide (1 in 10) add 2 mL of dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (1) for bromide.

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Distigmine Bromide in 5 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.40 g of Distigmine Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Distigmine Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 40 mg of Distigmine Bromide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, ethanol (99.5) and acetic acid (100) (8:3:2:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 1.0% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Distigmine Bromide, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (8:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration with platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.82 mg of $\text{C}_{22}\text{H}_{32}\text{Br}_2\text{N}_4\text{O}_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Distigmine Bromide Tablets

ジスチグミン臭化物錠

Distigmine Bromide Tablets contain not less than 95% and not more than 105% of the labeled amount of distigmine bromide ($C_{22}H_{32}Br_2N_4O_4$: 576.32).

Method of preparation Prepare as directed under Tablets, with Distigmine Bromide.

Identification Determine the absorption spectrum of the solution obtained in the Assay, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 268 nm and 272 nm, and a minimum between 239 nm and 243 nm.

Assay Weigh accurately and powder not less than 20 tablets of Distigmine Bromide Tablets. Weigh accurately a portion of the powder, equivalent to about 15 mg of Distigmine Bromide ($C_{22}H_{32}Br_2N_4O_4$), add 30 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and filter. Discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of distigmine bromide for assay (previously determine the water), and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 10 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution, A_{T2} and A_{S2} , at 270 nm and, A_{T1} and A_{S1} , at 241 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

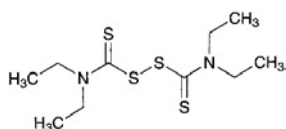
$$\text{Amount (mg) of distigmine bromide } (C_{22}H_{32}Br_2N_4O_4) \\ = W_S \times \{(A_{T2} - A_{T1}) / (A_{S2} - A_{S1})\} \times (1/2)$$

W_S : Amount (mg) of distigmine bromide for assay, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Disulfiram

ジスルフィラム



$C_{10}H_{20}N_2S_4$: 296.54

Tetraethylthiuram disulfide [97-77-8]

Disulfiram, when dried, contains not less than 99.0% of $C_{10}H_{20}N_2S_4$.

Description Disulfiram occurs as white to yellowish white crystals or crystalline powder.

It is freely soluble in acetone and in toluene, sparingly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Disulfiram in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Disulfiram, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 70 – 73°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Disulfiram according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Disulfiram according to Method 4, and perform the test (not more than 2 ppm).

(3) Diethyldithiocarbamic acid—Dissolve 0.10 g of Disulfiram in 10 mL of toluene, and shake with 10 mL of diluted sodium carbonate TS (1 in 20). Discard the toluene layer, wash the water layer with 10 mL of toluene, shake with 5 drops of a solution of cupric sulfate (1 in 250) and 2 mL of toluene, and allow to stand: no light yellow color develops in the toluene layer.

(4) Related substances—Dissolve 50 mg of Disulfiram in 40 mL of methanol, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of disulfiram from the sample solution is not larger than the peak area of disulfiram from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of disulfiram is about 8 minutes.

Selection of column: Dissolve 50 mg of Disulfiram and 50 mg of benzophenone in 40 mL of methanol, and add water to make 50 mL. To 1 mL of this solution add the mobile phase to make 200 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of benzophenone and disulfiram in this order with the resolution between these peaks being

not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of disulfiram obtained from 10 μL of the standard solution is 15 – 30 mm.

Time span of measurement: About 3.5 times of the retention time of disulfiram.

Loss on drying <2.41> Not more than 0.20% (2 g, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

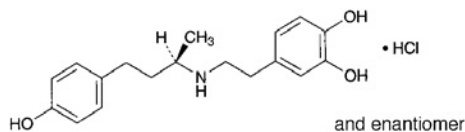
Assay Weigh accurately about 0.2 g of Disulfiram, previously dried, in an iodine bottle, dissolve in 20 mL of acetone, add 1.5 mL of water and 1.0 g of potassium iodide, and dissolve by shaking thoroughly. To this solution add 3.0 mL of hydrochloric acid, stopper the bottle tightly, shake, and allow to stand in a dark place for 3 minutes. Add 70 mL of water, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 14.83 mg of $\text{C}_{10}\text{H}_{20}\text{N}_2\text{S}_4$

Containers and storage Containers—Tight containers.

Dobutamine Hydrochloride

ドブタミン塩酸塩



$\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{HCl}$: 337.84

4-{2-[(1*RS*)-3-(4-Hydroxyphenyl)-1-methylpropylamino]ethyl} benzene-1,2-diol monohydrochloride [49745-95-1]

Dobutamine Hydrochloride, when dried, contains not less than 98.0% of $\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{HCl}$.

Description Dobutamine Hydrochloride occurs as white to very pale orange crystalline powder or grains.

It is freely soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Dobutamine Hydrochloride (1 in 100) shows no optical rotation.

Identification (1) Determine the infrared absorption spectra of Dobutamine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Dobutamine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Dobutamine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Dobutamine Hydrochloride in 100 mL of water: the pH of this solution is between 4.0 and 5.5.

Melting point <2.60> 188 – 191°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Dobutamine Hydrochloride in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Dobutamine Hydrochloride in 40 mL of water by warming, cool, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Dobutamine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and formic acid (78:22:5) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g each of Dobutamine Hydrochloride and Dobutamine Hydrochloride Reference Standard, each previously dried, dissolve separately in exactly 10 mL of the internal standard solution, add diluted methanol (1 in 2) to make 50 mL, and use these solutions as the sample solution and standard solution respectively. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dobutamine to that of the internal standard, respectively.

Amount (mg) of $\text{C}_{18}\text{H}_{23}\text{NO}_3 \cdot \text{HCl}$ = $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of Dobutamine Hydrochloride Reference Standard

Internal standard solution—A solution of salicylamide in diluted methanol (1 in 2) (1 in 125).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of tartrate buffer solution, pH 3.0 and methanol (7:3).

Flow rate: Adjust the flow rate so that the retention time of dobutamine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, dobutamine and internal standard are eluted in this

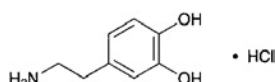
order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of dobutamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Dopamine Hydrochloride

ドパミン塩酸塩



$\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$: 189.64

4-(2-Aminoethyl)benzene-1,2-diol monohydrochloride
[62-31-7]

Dopamine Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$.

Description Dopamine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in formic acid, and sparingly soluble in ethanol (95).

Melting point: about 248°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Dopamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Dopamine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Dopamine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (1) for chloride.

pH <2.54> Dissolve 1.0 g of Dopamine Hydrochloride in 50 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dopamine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.8 g of Dopamine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Dopamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Dopamine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Dopamine Hydrochloride in 10 mL of water, and use this solution as the

sample solution. Pipet 1 mL of the sample solution, add water to make exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of cellulose with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, water and acetic acid (100) (16:8:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Dopamine Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 15 minutes. After cooling, add 50 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 18.96 mg of $\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$

Containers and storage Containers—Tight containers.

Dopamine Hydrochloride Injection

ドパミン塩酸塩注射液

Dopamine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 97% and not more than 103% of the labeled amount of dopamine hydrochloride ($\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$: 189.64).

Method of preparation Prepare as directed under Injections, with Dopamine Hydrochloride.

Description Dopamine Hydrochloride Injection occurs as a clear, colorless liquid.

Identification To a volume of Dopamine Hydrochloride Injection, equivalent to 0.04 g of Dopamine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 5 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 278 nm and 282 nm.

pH <2.54> 3.0 – 5.0

Bacterial endotoxins <4.01> Less than 4.2 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay To an exact volume of Dopamine Hydrochloride Injection, equivalent to about 30 mg of dopamine hydrochloride ($\text{C}_8\text{H}_{11}\text{NO}_2 \cdot \text{HCl}$), add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of

the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of dopamine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add exactly 2.5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of dopamine to that of the internal standard.

$$\text{Amount (mg) of dopamine hydrochloride (C}_8\text{H}_{11}\text{NO}_2\cdot\text{HCl)} \\ = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of dopamine hydrochloride for assay

Internal standard solution—A solution of uracil in the mobile phase (3 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Disodium hydrogen phosphate-citric acid buffer solution, pH 3.0

Flow rate: Adjust the flow rate so that the retention time of dopamine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and dopamine are eluted in this order with the resolution between these peaks being not less than 10.

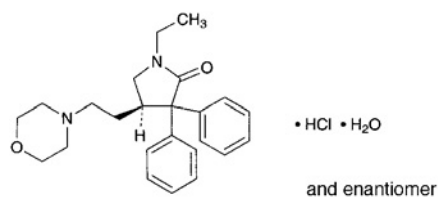
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of peak area of dopamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Plastic containers for aqueous injections may be used.

Doxapram Hydrochloride Hydrate

ドキサプラム塩酸塩水和物



$\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_2\cdot\text{HCl}\cdot\text{H}_2\text{O}$: 432.98
(4*RS*)-1-Ethyl-4-[2-(morpholin-4-yl)ethyl]-3,3-diphenylpyrrolidin-2-one monohydrochloride

monohydrate [7081-53-0]

Doxapram Hydrochloride Hydrate contains not less than 98.0% of doxapram hydrochloride ($\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_2\cdot\text{HCl}$: 414.97), calculated on the anhydrous basis.

Description Doxapram Hydrochloride Hydrate occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water, in ethanol (95) and in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Doxapram Hydrochloride Hydrate (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxapram Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxapram Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point <2.60> 218 – 222°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Doxapram Hydrochloride Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Doxapram Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Doxapram Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Doxapram Hydrochloride Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.5 g of Doxapram Hydrochloride Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 6 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, formic acid, ethyl formate and methanol (8:3:3:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 3.5 – 4.5% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.3% (1 g).

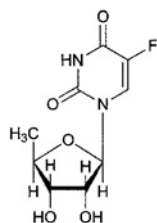
Assay Weigh accurately about 0.8 g of Doxapram Hydrochloride, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.50 mg of $C_{24}H_{30}N_2O_2 \cdot HCl$

Containers and storage Containers—Tight containers.

Doxifluridine

ドキシフルリジン



$C_9H_{11}FN_2O_5$: 246.19
5'-Deoxy-5-fluorouridine
[3094-09-5]

Doxifluridine, when dried, contains not less than 98.5% and not more than 101.0% of $C_9H_{11}FN_2O_5$.

Description Doxifluridine occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in water and in methanol, and slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS and in 0.01 mol/L sodium hydroxide TS.

Melting point: about 191°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Doxifluridine in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxifluridine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +160 – +174° (after drying, 0.1 g, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Doxifluridine in 10 mL of water is between 4.2 and 5.2.

Purity (1) Fluoride—Dissolve 0.10 g of Doxifluridine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution into a 20-mL volumetric flask, add 5 mL of a mixture of acetone and lanthanum-alizarin complexone TS (2:1) and water to make 20 mL, allow to stand for 1 hour, and use this solution as the sample solution. Separately, put 1.0 mL of Standard Fluorine Solution in a

20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) and 5 mL of the mixture of acetone and alizarin complexone TS (2:1), then proceed in the same manner as for preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 620 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained in the same way with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) as a blank: A_T is not larger than A_S .

(2) Chloride <1.03>—Perform the test with 0.30 g of Doxifluridine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.035%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Doxifluridine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 20 mg of Doxifluridine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 25 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot is not more than three, and they are not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible)

Assay Weigh accurately about 0.25 g of Doxifluridine, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 24.62 mg of $C_9H_{11}FN_2O_5$

Containers and storage Containers—Tight containers.

Doxifluridine Capsules

ドキシフルリジンカプセル

Doxifluridine Capsules contain not less than 95.0% and not more than 105.0% of doxifluridine ($C_9H_{11}FN_2O_5$: 246.19).

Method of preparation Prepare as directed under Capsules, with Doxifluridine.

Identification (1) Dissolve an amount of the contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine

according to the labeled amount, in 0.1 mol/L hydrochloric acid TS to make 100 mL, and filter. To 1 mL of the filtrate add 0.1 mol/L hydrochloric acid TS to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 0.1 mol/L hydrochloric acid TS as the blank: it exhibits a maximum between 267 nm and 271 nm.

(2) To an amount of powdered contents of Doxifluridine Capsules, equivalent to 20 mg of Doxifluridine according to the labeled amount, add 2 mL of methanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 20 mg of doxifluridine in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100) and water (17:2:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot with the sample solution and the spot with the standard solution show a dark purple color and these Rf values are the same.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with one capsule of Doxifluridine Capsules at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium, using the sinker. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 13 μ g of doxifluridine ($C_9H_{11}FN_2O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 26 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of these solutions at 269 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 30 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of doxifluridine ($C_9H_{11}FN_2O_5$)

$$= W_S \times (A_T/A_S) \times (V/V') \times (1/C) \times 9$$

W_S : Amount (mg) of doxifluridine for assay

C: Labeled amount (mg) of doxifluridine ($C_9H_{11}FN_2O_5$) in 1 capsule

Assay Weigh accurately the mass and powder the contents of not less than 20 Doxifluridine Capsules. Weigh accurately a portion of the powder, equivalent to about 50 mg of doxifluridine ($C_9H_{11}FN_2O_5$) according to the labeled amount, add 40 mL of water, shake for about 10 minutes, add water to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, then add a mixture of water and methanol (5:3) to make 100 mL, and use this solu-

tion as the sample solution. Separately, weigh accurately about 50 mg of doxifluridine for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mixture of water and methanol (5:3) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak height of doxifluridine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of doxifluridine (C}_9\text{H}_{11}\text{FN}_2\text{O}_5\text{)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of doxifluridine for assay

Internal standard solution—A solution of anhydrous caffeine (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (13:7).

Flow rate: Adjust the flow rate so that the retention time of doxifluridine is about 2.5 minutes.

System suitability—

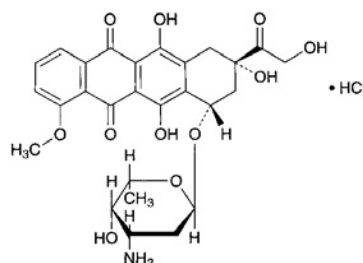
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, doxifluridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of doxifluridine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Doxorubicin Hydrochloride

ドキシロルビシン塩酸塩



$C_{27}H_{29}NO_{11} \cdot HCl$: 579.98

(2*S*,4*S*)-4-(3-Amino-2,3,6-trideoxy- α -L-xylohexopyranosyloxy)-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride [25316-40-9]

Doxorubicin Hydrochloride is the hydrochloride of

a derivative of daunorubicin.

It contains not less than 980 μg (potency) and not more than 1080 μg (potency) per mg, calculated on the anhydrous basis. The potency of Doxorubicin Hydrochloride is expressed as mass (potency) of doxorubicin hydrochloride ($\text{C}_{27}\text{H}_{29}\text{NO}_{11}\cdot\text{HCl}$).

Description Doxorubicin Hydrochloride occurs as a red-orange crystalline powder.

It is sparingly soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the absorption spectrum of a solution of Doxorubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Doxorubicin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Doxorubicin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxorubicin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Doxorubicin Hydrochloride (1 in 200) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +240 – +290° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 50 mg of Doxorubicin Hydrochloride in 10 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 50 mg of Doxorubicin Hydrochloride in 10 mL of water: the solution is clear and red.

(2) Related substances—Dissolve 25 mg of Doxorubicin Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than doxorubicin obtained from the sample solution is not more than 1/4 times the peak area of doxorubicin from the standard solution, and the total area of the peaks other than doxorubicin is not more than the peak area of doxorubicin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3 g of sodium lauryl sulfate in 1000

mL of diluted phosphoric acid (7 in 5000), and add 1000 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of doxorubicin is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of doxorubicin.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of doxorubicin obtained from 20 μL of this solution is equivalent to 3.5 to 6.5% of that from 20 μL of the standard solution.

System performance: Dissolve 5 mg of Doxorubicin Hydrochloride in 20 mL of water, add 1.5 mL of phosphoric acid, and allow to stand at room temperature for 30 minutes. Adjust the pH of this solution to 2.5 with 2 mol/L sodium hydroxide TS. When the procedure is run with 20 μL of this solution under the above operating conditions, doxorubicinone, having the relative retention time of about 0.6 with respect to doxorubicin, and doxorubicin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxorubicin is not more than 2.0%.

Water <2.48> Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Doxorubicin Hydrochloride and Doxorubicin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in water to make exactly 25 mL. Pipet 5 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances at 495 nm, A_{T} and A_{S} , of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

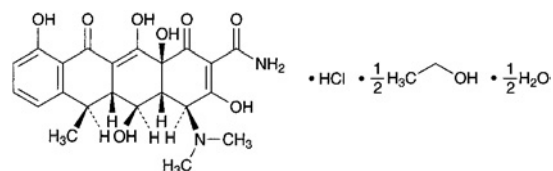
$$\begin{aligned} & \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{27}\text{H}_{29}\text{NO}_{11}\cdot\text{HCl} \\ & = W_{\text{S}} \times (A_{\text{T}}/A_{\text{S}}) \times 1000 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Doxorubicin Hydrochloride Reference Standard

Containers and storage Containers—Tight containers.

Doxycycline Hydrochloride Hydrate

ドキシサイクリン塩酸塩水和物



$\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\cdot\text{HCl}\cdot\frac{1}{2}\text{C}_2\text{H}_6\text{O}\cdot\frac{1}{2}\text{H}_2\text{O}$: 512.94
(4*S*,4*aR*,5*S*,5*aR*,6*R*,12*aS*)-4-Dimethylamino-3,5,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydro-tetracyceno-2-carboxamide monohydrochloride hemimethanolate hemihydrate [564-25-0, Doxycycline]

Doxycycline Hydrochloride Hydrate is the hydrochloride of a derivative of oxytetracycline.

It contains not less than 880 μg (potency) and not more than 943 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of ethanol. The potency of Doxycycline Hydrochloride Hydrate is expressed as mass (potency) of doxycycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$: 444.43).

Description Doxycycline Hydrochloride Hydrate occurs as yellow to dark yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, and slightly soluble in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Doxycycline Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Doxycycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg of Doxycycline Hydrochloride Hydrate in 10 mL of water, and add silver nitrate TS: a white turbidity is produced.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (349 nm): 285 – 315 (10 mg, 0.01 mol/L hydrochloric acid-methanol TS, 500 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: $-105 - -120^\circ$ (0.25 g calculated on the anhydrous basis and corrected by the amount of ethanol, 0.01 mol/L hydrochloric acid-methanol TS, 25 mL, 100 mm). Determine within 5 minutes after the sample solution is prepared.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Doxycycline Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 5.0 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substance—Dissolve 20 mg of Doxycycline Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 6-epidoxycycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 6-epidoxycycline hydrochloride stock solution. Separately, dissolve 20 mg of metacycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as metacycline hydrochloride stock solution. Pipet 2 mL each of 6-epidoxycycline hydrochloride stock solution and metacycline hydrochloride stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of metacycline and 6-epidoxycycline obtained from the sample solution are not more than the peak areas of them obtained from the standard solution, respectively, and the areas of the two peaks, appeared between the solvent peak and metacycline and behind of doxycycline, obtained from the sample solution are not more than 1/4 of the peak area of 6-epidoxycycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Mix 125 mL of 0.2 mol/L potassium dihydrogen phosphate TS, 117 mL of 0.2 mol/L sodium hydroxide TS, and add water to make 500 mL. To 400 mL of this solution add 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 25), 60 g of *t*-butanol and 200 mL of water, adjust the pH to 8.0 with 2 mol/L sodium hydroxide TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 19 minutes.

Time span of measurement: About 2.4 times as long as the retention time of doxycycline beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak areas of 6-epidoxycycline and metacycline obtained from 20 μL of this solution are equivalent to 3.5 to 6.5% of them obtained from 20 μL of the standard solution, respectively.

System performance: To 8 mL of the sample solution, 3 mL of 6-epidoxycycline hydrochloride stock solution and 2 mL of metacycline hydrochloride stock solution add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, metacycline, 6-epidoxycycline and doxycycline are eluted in this order with the resolutions between the peaks, metacycline and 6-epidoxycycline, and 6-epidoxycycline and doxycycline, being not less than 1.3 and not less than 2.0, respectively, and the symmetry factor of the peak of doxycycline is not more than 1.3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of metacycline and 6-epidoxycycline are not more than 3.0% and not more than 2.0%, respectively.

Ethanol Weigh accurately about 0.1 g of Doxycycline Hydrochloride Hydrate, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of ethanol (99.5), and add the internal standard solution to make exactly 100 mL. Pipet 1 mL of this solution, add the internal standard solution to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ethanol to that of the internal standard: the amount of ethanol is not less than 4.3% and not more than 6.0%.

$$\text{Amount (\%)} \text{ of ethanol} = (W_S/W_T) \times (Q_T/Q_S)$$

W_S : Amount (mg) of ethanol (99.5)

W_T : Amount (mg) of the sample

Internal standard solution—A solution of 1-propanol (1 in 2000).

Operating conditions—

Detector: An hydrogen flame-ionization detector.

Column: A glass column 3.2 mm in inside diameter and 1.5 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (0.0075 μm in average pore size, 500 – 600 m^2/g in specific surface area) (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 135°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is about 5 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 5 times with 1 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water <2.48> Not less than 1.4% and not more than 2.8% (0.6 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately an amount of Doxycycline Hydrochloride Hydrate and Doxycycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in water to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of doxycycline.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of doxycycline } (\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Doxycycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 7.0 g of sodium dihydrogen phosphate dihydrate in 450 mL of water, add 553 mL of a mixture of methanol and *N,N*-dimethyl-*n*-octylamine (550:3), and adjust the pH to 8.0 with a solution of sodium hydroxide (43 in 200).

Flow rate: Adjust the flow rate so that the retention time of doxycycline is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the theoretical plates and the symmetry factor of the peak of doxycycline are not less than 1000 and not more than 2.0, respectively.

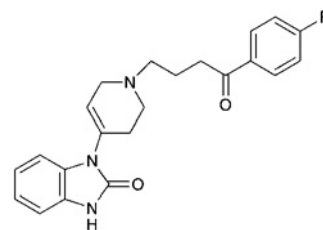
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of doxycycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Droperidol

ドロペリドール



$\text{C}_{22}\text{H}_{22}\text{FN}_3\text{O}_2$: 379.43

1-[1-[4-(4-Fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridin-4-yl]-1,3-dihydro-2*H*-benzimidazol-2-one [548-73-2]

Droperidol, when dried, contains not less than 98.0% of $\text{C}_{22}\text{H}_{22}\text{FN}_3\text{O}_2$.

Description Droperidol occurs as a white to light yellow powder.

It is freely soluble in acetic acid (100), soluble in dichloromethane, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually colored by light.

Identification (1) Put 30 mg of Droperidol in a brown volumetric flask, and dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Transfer 5 mL of the solution to a brown volumetric flask, and add 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Droperidol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Droperidol in acetone, evaporate the acetone, dry the residue in a desiccator (in vacuum, silica gel, 70°C) for 4 hours, and perform the test with the residue.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Droperidol in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0

mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Droperidol in 5 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dichloromethane to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, chloroform, methanol and acetic acid-sodium acetate buffer solution, pH 4.7, (54:23:18:5) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 3.0% (0.5 g, in vacuum, silica gel, 70°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g, platinum crucible).

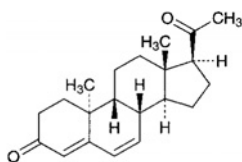
Assay Weigh accurately about 0.5 g of Droperidol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.94 mg of C₂₂H₂₂FN₃O₂

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dydrogesterone

ジドロゲステロン



C₂₁H₂₈O₂: 312.45
9 β ,10 α -Pregna-4,6-diene-3,20-dione [152-62-5]

Dydrogesterone, when dried, contains not less than 98.0% and not more than 102.0% of C₂₁H₂₈O₂.

Description Dydrogesterone occurs as white to light yellowish white crystals or crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetonitrile, sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 5 mg of Dydrogesterone add 5 mL of 4-methoxybenzaldehyde-acetic acid TS and 2 to 3 drops of sulfuric acid, and heat in a water bath for 2 minutes: an orange-red color develops.

(2) Determine the absorption spectrum of a solution of Dydrogesterone in methanol (1 in 200,000) as directed under

Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Dydrogesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -470 - -500° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 167 - 171°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Dydrogesterone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Dydrogesterone in 200 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of dydrogesterone from the sample solution is not larger than the peak area of dydrogesterone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (3 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, ethanol (95) and acetonitrile (53:26:21).

Flow rate: Adjust the flow rate so that the retention time of dydrogesterone is about 12 minutes.

Selection of column: Dissolve 1 mg each of Dydrogesterone and progesterone in 20 mL of the mobile phase. Proceed with 10 μ L each of these solutions under the above operating conditions, and calculate the resolution. Use a column giving elution of dydrogesterone and progesterone in this order with the resolution between these peaks being not less than 8. Wavelength is 265 nm.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of dydrogesterone obtained from 10 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of dydrogesterone beginning after the solvent peak.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg of Dydrogesterone, previously dried, and dissolve in methanol to make exactly

100 mL. Pipet 1 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 286 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_{21}H_{28}O_2 = (A/845) \times 100,000$$

Containers and storage Containers—Tight containers.

Dydrogesterone Tablets

ジドロゲステロン錠

Dydrogesterone Tablets contain not less than 95% and not more than 105% of the labeled amount of dydrogesterone ($C_{21}H_{28}O_2$: 312.45).

Method of preparation Prepare as directed under Tablets, with Dydrogesterone.

Identification (1) To a quantity of powdered Dydrogesterone Tablets, equivalent to 0.05 g of Dydrogesterone according to the labeled amount, add 50 mL of methanol, shake well, and filter. Evaporate 5 mL of the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Dydrogesterone.

(2) To 1 mL of the filtrate obtained in (1) add methanol to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 284 nm and 288 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Dydrogesterone Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter. Discard the first 10 mL of the filtrate, and use the subsequent as the sample solution. Separately, weigh accurately about 50 mg of dydrogesterone for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 296 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate of Dydrogesterone Tablets in 30 minutes is not less than 80%.

$$\begin{aligned} &\text{Dissolution rate (\%)} \text{ with respect to} \\ &\text{the labeled amount of dydrogesterone } (C_{21}H_{28}O_2) \\ &= W_S \times (A_T/A_S) \times (1/C) \times 9 \end{aligned}$$

W_S : Amount (mg) of dydrogesterone for assay

C : Labeled amount (mg) of dydrogesterone ($C_{21}H_{28}O_2$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Dydrogesterone Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of dydrogesterone ($C_{21}H_{28}O_2$), shake well with 50 mL of methanol, and add methanol to make exactly 100 mL. Filter this solution, dis-

card the first 20 mL of the filtrate, pipet the subsequent 5 mL, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of dydrogesterone for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, proceed in the same manner as the preparation of the sample solution, and use the solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 286 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

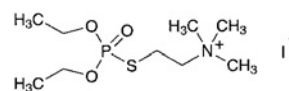
$$\begin{aligned} &\text{Amount (mg) of dydrogesterone } (C_{21}H_{28}O_2) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of dydrogesterone for assay

Containers and storage Containers—Tight containers.

Ecothiopate Iodide

エコチオパートヨウ化物



$C_9H_{23}INO_3PS$: 383.23

2-(Diethoxyphosphorylsulfanyl)-*N,N,N*-trimethylethylaminium iodide [513-10-0]

Ecothiopate Iodide contains not less than 95.0% of $C_9H_{23}INO_3PS$, calculated on the dried basis.

Description Ecothiopate Iodide occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.1 g of Ecothiopate Iodide in 2 mL of water, and add 1 mL of nitric acid: a brown precipitate is formed. To 1 drop of the turbid solution containing this precipitate add 1 mL of hexane, and shake: a light red color develops in the hexane layer.

(2) Heat the suspension of the precipitate obtained in (1) until it becomes colorless, cool, add 10 mL of water, and use this solution as the sample solution. Two mL of the sample solution responds to the Qualitative Tests <1.09> (2) for phosphate.

(3) Two mL of the sample solution obtained in (2) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 0.1 g of Ecothiopate Iodide in 40 mL of water: the pH of this solution is between 3.0 and 5.0.

Melting point <2.60> 116 – 122°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Ecothiopate Iodide in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—To 1.0 g of Ecothiopate Iodide in a Kjeldahl flask add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. Repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat

until the solution becomes colorless, and white fumes are evolved. After cooling, transfer the solution together with a small quantity of water to a Nessler tube, and add water to make about 20 mL. Adjust the solution with ammonia solution (28) and ammonia TS to a pH between 3.0 and 3.5, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: proceed in the same manner as the preparation of the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Ecotioipate Iodide in 10 mL of methanol, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

Assay Weigh accurately about 0.125 g of Ecotioipate Iodide, and dissolve in water to make exactly 100 mL. Pipet 10 mL of of this solution, add 30 mL of water, then add exactly 10 mL of phosphate buffer solution, pH 12, stopper the container, and allow to stand at $25 \pm 3^\circ\text{C}$ for 20 minutes. To this solution add quickly 2 mL of acetic acid (100), and titrate <2.50> with 0.002 mol/L iodine VS (potentiometric titration). Perform the test in the same manner without phosphate buffer solution, pH 12, and make any necessary correction.

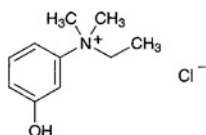
Each mL of 0.002 mol/L iodine VS
= 1.533 mg of $\text{C}_9\text{H}_{23}\text{INO}_3\text{PS}$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 0°C.

Edrophonium Chloride

エドロホニウム塩化物



$\text{C}_{10}\text{H}_{16}\text{ClNO}$: 201.69

N-Ethyl-3-hydroxy-*N,N*-dimethylanilinium chloride
[116-38-1]

Edrophonium Chloride, when dried, contains not less than 98.0% of $\text{C}_{10}\text{H}_{16}\text{ClNO}$.

Description Edrophonium Chloride occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in water, freely soluble in ethanol (95) and

in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

It is hygroscopic.

It is gradually colored by light.

Identification (1) To 5 mL of a solution of Edrophonium Chloride (1 in 100) add 1 drop of iron (III) chloride TS: a light red-purple color develops.

(2) Determine the absorption spectrum of a solution of Edrophonium Chloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Edrophonium Chloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Edrophonium Chloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Melting point <2.60> 166 – 171°C (with decomposition).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Edrophonium Chloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Edrophonium Chloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Edrophonium Chloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 100 mL. Pipet 3 mL of this solution, add ethanol (95) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonia solution (28) (16:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Edrophonium Chloride, previously dried, and dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.17 mg of $\text{C}_{10}\text{H}_{16}\text{ClNO}$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Edrophonium Chloride Injection

エドロホニウム塩化物注射液

Edrophonium Chloride Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of edrophonium chloride ($C_{10}H_{16}ClNO$; 201.69).

Method of preparation Prepare as directed under Injections, with Edrophonium Chloride.

Description Edrophonium Chloride Injection is a clear and colorless liquid.

Identification (1) To a volume of Edrophonium Chloride Injection, equivalent to 0.04 g of Edrophonium Chloride according to the labeled amount, add 4 mL of barium nitrate TS, shake, and filter. Proceed with the filtrate as directed in the Identification (1) under Edrophonium Chloride.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 272 nm and 276 nm.

pH <2.54> 6.5 – 8.0

Extractable volume <6.05> It meets the requirement.

Assay Conduct this procedure without exposure to daylight, using light-resistant containers. Measure exactly a volume of Edrophonium Chloride Injection, equivalent to about 50 mg of edrophonium chloride ($C_{10}H_{16}ClNO$), place in a chromatographic column prepared by pouring 10 mL of weakly basic DEAE-bridged dextran anion exchanger (Cl type) (50 to 150 μ m in particle diameter) into a chromatographic tube about 2 cm in inside diameter and about 10 cm in length, add 25 mL of water, and elute at the flow rate of 1 to 2 mL per minute. Wash the column with two 25-mL portions of water at the flow rate of 1 to 2 mL per minute. Combine the washings with above effluent solutions, and add water to make exactly 100 mL. Measure exactly 10 mL of this solution, and add 10 mL of phosphate buffer solution, pH 8.0, and 5 g of sodium chloride. Wash this solution with four 20-mL portions of a mixture of diethyl ether and hexane (1:1), collect the water layer, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Edrophonium Chloride Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 3 hours, and dissolve in water to make exactly 100 mL. Measure exactly 10 mL of this solution, and prepare the standard solution in the same manner as the sample solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of edrophonium chloride (C}_{10}\text{H}_{16}\text{ClNO)} \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Edrophonium Chloride Reference Standard

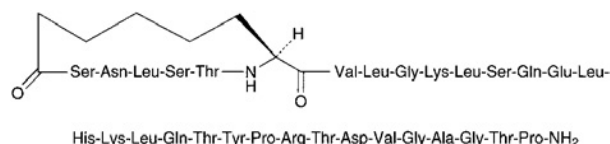
Containers and storage Containers—Hermetic containers,

and colored containers may be used.

Storage—Light-resistant.

Elcatonin

エルカトニン



$C_{148}H_{244}N_{42}O_{47}$; 3363.77
[60731-46-6]

Elcatonin contains not less than 5000 Elcatonin Units and not more than 7000 Elcatonin Units per 1 mg of peptide, calculated on the dehydrated and de-acetic acid basis.

Description Elcatonin is a white powder.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

The pH of its solution (1 in 500) is between 4.5 and 7.0.

Identification Dissolve 5 mg of Elcatonin in 5 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent amino acids Put about 1 mg of Elcatonin in a test tube for hydrolysis, add phenol-hydrochloric acid TS to dissolve, replace the air inside with Nitrogen, seal the tube under reduced pressure, and heat at $110 \pm 2^\circ\text{C}$ for 24 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, dissolve the residue in 1 mL of 0.02 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh exactly 1.33 mg of L-aspartic acid, 1.19 mg of L-threonine, 1.05 mg of L-serine, 1.47 mg of L-glutamic acid, 1.15 mg of L-proline, 0.75 mg of glycine, 0.89 mg of L-alanine, 1.17 mg of L-valine, 1.89 mg of L-2-aminosuberic acid, 1.31 mg of L-leucine, 1.81 mg of L-tyrosine, 1.83 mg of L-lysine hydrochloride, 2.10 mg of L-histidine hydrochloride monohydrate and 2.11 mg of L-arginine hydrochloride, dissolve them in 0.02 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: 14 peaks of amino acids appear on the chromatogram obtained from the sample solution, and their respective molar ratios against alanine are 1.7 – 2.2 for aspartic acid, 3.5 – 4.2 for threonine, 2.4 – 3.0 for serine, 2.7 – 3.2 for glutamic acid, 1.7 – 2.2 for proline, 2.7 – 3.2 for glycine, 1.6 – 2.2 for valine, 0.8 – 1.2 for 2-aminosuberic acid, 4.5 – 5.2 for leucine, 0.7 – 1.2 for tyrosine, 1.7 – 2.2 for lysine, 0.8 – 1.2 for histidine and 0.7 – 1.2 for arginine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440

nm and 570 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated styrene-divinylbenzene copolymer (3 μ m in particle diameter).

Column temperature: Varied between 50°C and 65°C.

Chemical reaction vessel temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Buffer solutions A, B, C and D, with sodium ion concentrations of 0.10 mol/L, 0.135 mol/L, 1.26 mol/L and 0.20 mol/L, respectively. The ion concentration of the mobile phase is changed stepwise from 0.10 mol/L to 1.26 mol/L by using these buffer solutions.

Buffer solution:	Components of buffer solutions			
	A	B	C	D
Citric acid	8.85 g	7.72 g	6.10 g	—
Sodium citrate	3.87 g	10.05 g	26.67 g	—
Sodium hydroxide	—	—	2.50 g	8.00 g
Sodium chloride	3.54 g	1.87 g	54.35 g	—
Ethanol	60.0 mL	—	—	60.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—	—
Purified water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	1000 mL	1000 mL	1000 mL	1000 mL

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for about 20 minutes while passing Nitrogen, and use this solution as solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for about 20 minutes while passing Nitrogen, and use this solution as solution B. Mix solution A and solution B before use.

Flow rate of mobile phase: Adjust the flow rate so that the retention time of arginine is about 75 minutes.

Flow rate of reaction reagent: About 0.2 mL per minute.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions. Use a column from which aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, 2-aminosuberic acid, leucine, tyrosine, lysine, histidine and arginine are eluted in this order, with complete separation of each peak.

Purity (1) Acetic acid—Weigh accurately 3–6 mg of Elcatonin quickly under conditions of 25 \pm 2°C and 50 \pm 5% relative humidity, add exactly 1 mL of the internal standard solution to dissolve it, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of acetic acid (100), and add the internal standard solution to make exactly 100 mL. Pipet 5 mL of this solution, add the internal standard solution to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not more than 7.0%.

Amount (%) of acetic acid (CH₃COOH)

$$= (W_{ST}/W_{SA}) \times (Q_T/Q_S) \times 50$$

W_{ST} : Amount (g) of acetic acid (100)

W_{SA} : Amount (mg) of sample

Internal standard solution—A solution of citric acid (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 13.2 g of diammonium hydrogenphosphate in 900 mL of water, add phosphoric acid to adjust the pH to 2.5, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 4 minutes.

Selection of column: Proceed with 20 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column from which acetic acid and citric acid are eluted in this order with the resolution between their peaks being not less than 2.0.

(2) Related substances—Dissolve 1.0 mg of Elcatonin in 1 mL of a mixture of trifluoroacetic acid TS and acetonitrile (2:1), and use this solution as the sample solution. Take exactly 0.3 mL of the sample solution, add a mixture of trifluoroacetic acid TS and acetonitrile (2:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than the peak of elcatonin of the sample solution is not larger than the peak area of elcatonin of the standard solution, and each peak area other than the peak of elcatonin of the sample solution is not larger than 1/3 of the peak area of elcatonin of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of trifluoroacetic acid TS and acetonitrile (change the ratio linearly from 85:15 to 55:45 in 30 minutes).

Flow rate: Adjust the flow rate so that the retention time of elcatonin is about 25 minutes.

Selection of column: Dissolve 2 mg of Elcatonin in 200 μ L of trypsin TS for test of elcatonin, warm at 37°C for 1 hour, then add 1 drop of acetic acid (100), and heat at 95°C for 1 minute. To 10 μ L of this solution add 50 μ L of the sample solution, and mix. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column such that the resolution between the peak of elcatonin and the peak which appears immediately before the

peak of elcatonin is not less than 2.0, and the retention time of elcatonin is about 25 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of elcatonin from 10 μ L of the standard solution is between 50 mm and 200 mm.

Time span of measurement: Continue measurement until the regularly changing base-line of the chromatogram disappears, beginning after the solvent peak.

Water <2.48> Weigh accurately 1 – 3 mg of Elcatonin quickly under conditions of $25 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity, and perform the test as directed in 2. Coulometric titration: not more than 8.0%.

Nitrogen content Weigh accurately 0.015 – 0.02 g of Elcatonin quickly under conditions of $25 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity, and perform the test as directed under Nitrogen Determination <1.08>: it contains not less than 16.1% and not more than 18.7% of nitrogen (N: 14.01) in the peptide, calculated on the dehydrated and de-acetic acid basis.

Assay (i) Animals: Select healthy male Sprague-Dawley rats each weighing between 90 g and 110 g. Keep the rats for not less than 3 days before use, providing an appropriate uniform diet and water.

(ii) Diluent for elcatonin: Dissolve 2.72 g of sodium acetate in water to make 200 mL, add 0.2 g of bovine serum albumin, and adjust the pH to 6.0 with acetic acid (100). Prepare before use.

(iii) Standard solution: Dissolve Elcatonin Reference Standard in the diluent for elcatonin to make two standard solutions, one to contain exactly 0.075 Unit in each mL which is designated as the high-dose standard solution, S_H , and the other to contain exactly 0.0375 Unit in each mL which is designated as the low-dose standard solution, S_L .

(iv) Sample solution: Weigh accurately 0.5 – 2.0 mg of Elcatonin quickly under conditions of $25 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity, and dissolve in the diluent for elcatonin to make two sample solutions, the high-dose sample solution, T_H , which contains the Units per mL equivalent to S_H and the low-dose sample solution, T_L , which contains the Units per mL equivalent to S_L .

(v) Deproteinizing solution for elcatonin: Dissolve 160 g of trichloroacetic acid and 30.6 g of strontium chloride in water to make 3600 mL.

(vi) Procedure: Divide the animals into 4 equal groups of not less than 10 animals each. Withhold all food, but not water, for 18 to 24 hours before the injections, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject exactly 0.2 mL each of the standard solutions and the sample solutions into the tail vein of each animal as indicated in the following design:

First group	S_H	Third group	T_H
Second group	S_L	Fourth group	T_L

At 1 hour after the injection, take a sufficient blood sample to perform the test from the carotid artery and vein of each animal under ether anesthesia, centrifuge the blood samples to separate serum, and determine the serum calcium according to the following (vii).

(vii) Serum calcium determination: Take exactly 0.3 mL of the serum, add the deproteinizing solution for elcatonin to

make exactly 3 mL, mix well, centrifuge, and use the supernatant liquid as the sample solution for calcium determination. Separately, pipet 1 mL of Standard Calcium Solution for Atomic Absorption Spectrophotometry <2.23>, and add a solution of sodium chloride (17 in 2000) to make exactly 10 mL. Pipet 5 mL of this solution, add the deproteinizing solution for elcatonin to make exactly 50 mL, and use this solution as the standard solution for calcium determination. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions. Determine the absorbance, A_0 , of a solution obtained in the same manner used for preparation of the standard solution, but with 1 mL of water instead of the standard solution.

$$\text{Amount (mg) of calcium (Ca) in 100 mL of the serum} \\ = 0.01 \times \{(A_T - A_0)/(A_S - A_0)\} \times 10 \times 100$$

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: Calcium hollow-cathode lamp

Wavelength: 422.7 nm

(viii) Calculation: Amounts of calcium in 100 mL of the serum obtained with S_H , S_L , T_H and T_L in (vii) are symbolized as y_1 , y_2 , y_3 and y_4 , respectively. Sum up individual y_1 , y_2 , y_3 and y_4 to obtain Y_1 , Y_2 , Y_3 and Y_4 , respectively.

Units per mg of peptide, calculated on the dehydrated and de-acetic acid basis

$$= \text{antilog } M \times (\text{units per mL of } S_H \times (b/a))$$

$$M = 0.3010 \times (Y_a/Y_b)$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a : Amount (mg) of the sample

$$\times \{100 - [\text{water content (\%)} + \text{acetic acid content (\%)}]/100\}$$

b : Total volume (mL) of the high-dose sample solution prepared by dissolving the sample with diluent for elcatonin.

F' computed by the following equation should be smaller than F shown in the table against n with which s^2 is calculated. Calculate L ($P=0.95$) by use of the following equation: L should be not more than 0.20. If F' exceeds F , or if L exceeds 0.20, repeat the test, increasing the number of animals or arranging the assay conditions so that F' is not more than F and L is not more than 0.20.

$$F' = (-Y_1 + Y_2 + Y_3 - Y_4)^2/4fs^2$$

f : Number of the animals of each group.

$$s^2 = \{\sum y^2 - (Y/f)\}/n$$

$\sum y^2$: The sum of squares of y_1 , y_2 , y_3 and y_4 in each group.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f - 1)$$

$$L = 2\sqrt{(C - 1)(CM^2 + 0.09062)}$$

$$C = Y_b^2/(Y_b^2 - 4fs^2t^2)$$

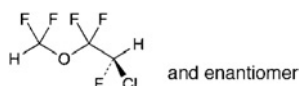
t^2 : Value shown in the following table against n used to calculate s^2 .

<i>n</i>	$t^2 = F$	<i>n</i>	$t^2 = F$	<i>n</i>	$t^2 = F$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Not exceeding 8°C.

Enflurane

エンフルラン



$C_3H_2ClF_5O$: 184.49
(2*RS*)-2-Chloro-1-(difluoromethoxy)-1,1,2-trifluoroethane
[13838-16-9]

Description Enflurane is a clear, colorless liquid.

It is slightly soluble in water.

It is miscible with ethanol (95) and with diethyl ether.

It is a volatile, and not an inflammable.

It shows no optical rotation.

Boiling point: 54 – 57°C

Identification (1) Take 50 μ L of Enflurane, and prepare the test solution as directed to the Oxygen Flask Combustion Method <1.06> using 40 mL of water as the absorbing liquid. The test solution responds to the Qualitative Tests <1.09> for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Enflurane as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.302 – 1.304

Specific gravity <2.56> d_{20}^{20} : 1.520 – 1.540

Purity (1) Acidity or alkalinity—To 60 mL of Enflurane add 60 mL of freshly boiled and cooled water, shake for 3 minutes, separate the water later, and use the layer as the sample solution. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide TS: the color of the solution is purple. To 20 mL of the sample solution add one drop of bromocresol purple TS and 0.06 mL of 0.01 mol/L hydrochloric acid TS: the color of the solution is yellow.

(2) Chloride <1.03>—To 20 g of Enflurane add 20 mL of water, shake well, and separate the water layer. Take 10 mL

of the water layer add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid (not more than 0.001%).

(3) Nonvolatile residue—Evaporate exactly 65 mL of Enflurane on a water bath to dryness, and dry the residue at 105°C for 1 hour: the of the residue is not more than 1.0 mg.

(4) Related substances—Proceed the test with 5 μ L of Enflurane as directed under Gas chromatography <2.02> according to the following conditions. Determine each peak area other than the peak of air which appears soon after injection of the sample by the automatic integration method, and calculate the amount of each peak by the area percentage method: the amount of the substances other than enflurane is not more than 0.10%.

Operating conditions—

Detector: A thermal conductivity detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μ m in particle diameter, coated with diethylene glycol succinate ester for gas chromatography in the ratio of 20%.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of enflurane is about 3 minutes.

Time span of measurement: About three times as long as the retention time of enflurane.

System suitability—

Test for required detection: Pipet exactly 1 mL of enflurane add 2-propanol to make exactly 100 mL. To exactly 2 mL of this solution add 2-propanol to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution, and add 2-propanol to make exactly 10 mL. Confirm that the peak area of enflurane obtained from 5 μ L of this solution is equivalent to 7 to 13% of that of enflurane obtained from 5 μ L of the solution for system suitability test.

System performance: Mix 5 mL of Enflurane and 5 mL of 2-propanol. When the procedure is run with 5 μ L of this mixture under the above operating conditions, enflurane and 2-propanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of enflurane is not more than 2.0%.

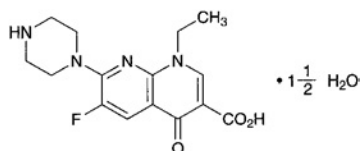
Water <2.48> Not more than 0.10% (10 g, direct titration).

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Enoxacin Hydrate

エノキサシン水和物



$C_{15}H_{17}FN_4O_3 \cdot 1\frac{1}{2}H_2O$: 347.34

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid sesquihydrate
[84294-96-2]

Enoxacin Hydrate, when dried, contains not less than 98.5% of enoxacin ($C_{15}H_{17}FN_4O_3$: 320.32).

Description Enoxacin Hydrate occurs as white to pale yellow-brown crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in methanol, very slightly soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS.

It is gradually colored by light.

Identification (1) Place 0.02 g of Enoxacin Hydrate and 0.05 g of sodium in a test tube, and heat gradually to ignition with precaution. After cooling, add 0.5 mL of methanol and then 5 mL of water, and heat to boiling. To this solution add 2 mL of dilute acetic acid, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Dissolve 0.05 g of Enoxacin Hydrate in dilute sodium hydroxide TS to make 100 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Enoxacin Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 225 – 229°C (after drying).

Purity (1) Sulfate <1.14>—Dissolve 1.0 g of Enoxacin Hydrate in 50 mL of dilute sodium hydroxide TS, shake with 10 mL of dilute hydrochloric acid, and centrifuge. Filter the supernatant liquid, and to 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 25 mL of dilute sodium hydroxide TS, 5 mL of dilute hydrochloric acid TS and water to make 50 mL (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Enoxacin Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Enoxacin Hydrate according to Method 3, and perform

the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Enoxacin Hydrate in 25 mL of a mixture of chloroform and methanol (7:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (7:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 7.0 – 9.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

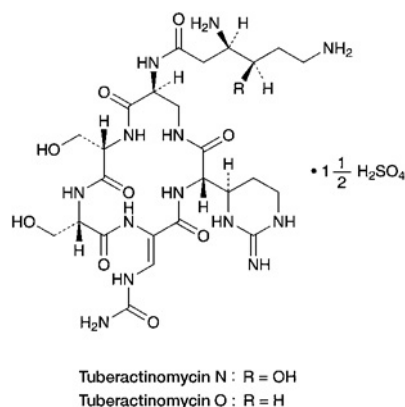
Assay Weigh accurately about 0.3 g of Enoxacin Hydrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.03 mg of $C_{15}H_{17}FN_4O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Enviomycin Sulfate

エンビオマイシン硫酸塩



Tuberactinomycin N Sulfate

$C_{25}H_{43}N_{13}O_{10} \cdot 1\frac{1}{2}H_2SO_4$: 832.81

Tuberactinomycin O Sulfate

$C_{25}H_{43}N_{13}O_9 \cdot 1\frac{1}{2}H_2SO_4$: 816.81

Tuberactinomycin N Sulfate

(3*R*,4*R*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diamino-4-hydroxyhexanamide sesquisulfate

[33103-22-9, Tuberactinomycin N]

Tuberactinomycin O Sulfate
(3*S*)-*N*-[(3*S*,9*S*,12*S*,15*S*)-9,12-Bis(hydroxymethyl)-3-[(4*R*)-2-iminohexahydropyrimidin-4-yl]-2,5,8,11,14-pentaoxo-6-(*Z*)-ureidomethylene-1,4,7,10,13-pentaazacyclohexadec-15-yl]-3,6-diaminohexanamide sesquisulfate
[33137-73-4, Tuberactinomycin O]

Enviomycin Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Streptomyces griseoverticillatus* var. *tuberacticus*.

It contains not less than 770 μg (potency) per mg, calculated on the dried basis. The potency of Enviomycin Sulfate is expressed as mass (potency) of tuberactinomycin N ($\text{C}_{25}\text{H}_{43}\text{N}_{13}\text{O}_{10}$: 685.69).

Description Enviomycin Sulfate occurs as a white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 5 mL of a solution of Enviomycin Sulfate (1 in 200) add 1.5 mL of sodium hydroxide TS, and add 1 drop of a mixture of 0.01 mol/L citric acid TS and copper (II) sulfate TS (97:3): a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Enviomycin Sulfate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 2 mL of a solution of Enviomycin Sulfate (1 in 20) add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-16 - -22^\circ$ (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 2.0 g of Enviomycin Sulfate in 20 mL of water is between 5.5 and 7.5.

Content ratio of the active principle Dissolve 0.1 g of Enviomycin Sulfate in water to make 100 mL, and use this solution as the sample solution. Perform the test with 3 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{T1} and A_{T2} , of tuberactinomycin N and tuberactinomycin O, having the relative retention time, 1.4 ± 0.4 , with respect to tuberactinomycin N, by the automatic integration method: $A_{T2}/(A_{T1} + A_{T2})$ is between 0.090 and 0.150.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of ammonium acetate TS, 1,4-dioxane, tetrahydrofuran, water and ammonia solution (28) (100:75:50:23:2).

Flow rate: Adjust the flow rate so that the retention time of tuberactinomycin N is about 9 minutes.

System suitability—

System performance: When the procedure is run with 3 μL of the sample solution under the above operating conditions, tuberactinomycin N and tuberactinomycin O are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 3 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of tuberactinomycin N is not more than 2.0%.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Enviomycin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Enviomycin Sulfate according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying <2.41> Not more than 4.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

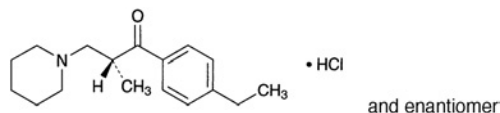
(iii) Standard solutions—Weigh accurately an amount of Enviomycin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Enviomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 400 μg (potency) and 100 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Eperisone Hydrochloride

エペリゾン塩酸塩



$C_{17}H_{25}NO \cdot HCl$: 295.85

(2*RS*)-1-(4-Ethylphenyl)-2-methyl-3-piperidin-1-ylpropan-1-one monohydrochloride [56839-43-1]

Eperisone Hydrochloride contains not less than 98.5% and not more than 101.0% of $C_{17}H_{25}NO \cdot HCl$, calculated on the anhydrous basis.

Description Eperisone Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), and soluble in ethanol (99.5).

Melting point: about 167°C (with decomposition).

A solution of Eperisone Hydrochloride in methanol (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Eperisone Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Eperisone Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Eperisone Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Eperisone Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Piperidine hydrochloride—Dissolve 1.0 g of Eperisone Hydrochloride in 20 mL of water, add 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the sample solution. Separately, to 2.0 mL of a solution of piperidine hydrochloride (1 in 1000) add 18 mL of water, 2.0 mL of diluted hydrochloric acid (1 in 2), 2.0 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 1.5 mL of ammonia solution (28), and use this solution as the standard solution. To each of the sample solution and standard solution add 10 mL of a mixture of isopropylether and carbon disulfide (3:1), shake for 30 seconds, allow them to stand for 2 minutes, and compare the color of the upper layer: the color obtained from the sample solution is not more darker than that from the standard solution.

(3) Related substances—Dissolve 0.1 g of Eperisone

Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of eperisone is not more than 1/5 of the peak area of eperisone from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of methanol, 0.0375 mol/L sodium 1-decanesulfonate TS and perchloric acid (600:400:1).

Flow rate: Adjust the flow rate so that the retention time of eperisone is about 17 minutes.

Time span of measurement: About 2 times as long as the retention time of eperisone.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of eperisone obtained from 10 μ L of this solution is equivalent to 7 to 13% of that obtained from 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eperisone are not less than 4000 steps and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of eperisone is not more than 3.0%.

Water <2.48> Not more than 0.2% (0.1 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

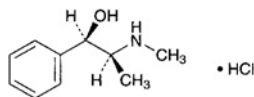
Assay Weigh accurately about 0.6 g of Eperisone Hydrochloride, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.58 mg of $C_{17}H_{25}NO \cdot HCl$

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride

エフェドリン塩酸塩



$C_{10}H_{15}NO \cdot HCl$: 201.69
(1*R*,2*S*)-2-Methylamino-1-phenylpropan-1-ol
monohydrochloride [50-98-6]

Ephedrine Hydrochloride, when dried, contains not less than 99.0% of $C_{10}H_{15}NO \cdot HCl$.

Description Ephedrine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, soluble in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in acetonitrile and in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Ephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ephedrine Hydrochloride (1 in 15) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-33.0 - -36.0^\circ$ (after drying, 1 g, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Ephedrine Hydrochloride in 20 mL of water: the pH of this solution is between 4.5 and 6.5.

Melting point <2.60> 218 – 222°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Ephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Dissolve 0.05 g of Ephedrine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Ephedrine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.05 g of Ephedrine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.07> ac-

ording to the following conditions, and calculate the areas of each peak by the automatic integration method: the total area of the peaks other than ephedrine from the sample solution is not larger than the peak area of ephedrine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 128), acetonitrile and phosphoric acid (640:360:1).

Flow rate: Adjust the flow rate so that the retention time of ephedrine is about 14 minutes.

Time span of measurement: About 3 times as long as the retention time of ephedrine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of ephedrine obtained from 10 μ L of this solution is equivalent to 4 to 6% of that from the standard solution.

System performance: Dissolve 1 mg of ephedrine hydrochloride for assay and 4 mg of atropine sulfate in 100 mL of diluted methanol (1 in 2). When the procedure is run with 10 μ L of this solution under the above operating conditions, ephedrine and atropine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ephedrine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ephedrine Hydrochloride, previously dried, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.17 mg of $C_{10}H_{15}NO \cdot HCl$

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Injection

エフェドリン塩酸塩注射液

Ephedrine Hydrochloride Injection is aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of ephed-

drine hydrochloride (C₁₀H₁₅NO.HCl: 201.69).

Method of preparation Prepare as directed under Injections, with Ephedrine Hydrochloride.

Description Ephedrine Hydrochloride Injection is a clear, colorless liquid.

pH: 4.5 – 6.5

Identification To a volume of Ephedrine Hydrochloride Injection, equivalent to 0.05 g of Ephedrine Hydrochloride according to the labeled amount, add water to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Bacterial endotoxins <4.01> Less than 7.5 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay To an exact volume of Ephedrine Hydrochloride Injection, equivalent to about 40 mg of ephedrine hydrochloride (C₁₀H₁₅NO.HCl) according to the labeled amount, add exactly 10 mL of the internal standard solution and water to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ephedrine to that of the internal standard of each solution.

$$\text{Amount (mg) of ephedrine hydrochloride (C}_{10}\text{H}_{15}\text{NO.HCl)} \\ = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

10% Ephedrine Hydrochloride Powder

Ephedrine Hydrochloride Powder

エフェドリン塩酸塩散 10%

10% Ephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of ephedrine hydrochloride (C₁₀H₁₅NO.HCl: 201.69).

Method of preparation

Ephedrine Hydrochloride	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification To 0.5 g of 10% Ephedrine Hydrochloride Powder add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Assay Weigh accurately about 0.4 g of 10% Ephedrine Hydrochloride Powder, add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ephedrine to that of the internal standard of each solution.

$$\text{Amount (mg) of ephedrine hydrochloride (C}_{10}\text{H}_{15}\text{NO.HCl)} \\ = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Perform as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 6 times with

10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Ephedrine Hydrochloride Tablets

エフェドリン塩酸塩錠

Ephedrine Hydrochloride Tablets contain not less than 93% and not more than 107% of the labeled amount of ephedrine hydrochloride ($\text{C}_{10}\text{H}_{15}\text{NO}\cdot\text{HCl}$; 201.69).

Method of preparation Prepare as directed under Tablets, with Ephedrine Hydrochloride.

Identification To an amount of powdered Ephedrine Hydrochloride Tablets, equivalent to 0.05 g of Ephedrine Hydrochloride, add 100 mL of water, shake for 20 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 249 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Assay Weigh accurately not less than 20 tablets of Ephedrine Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 40 mg of ephedrine hydrochloride ($\text{C}_{10}\text{H}_{15}\text{NO}\cdot\text{HCl}$), add 150 mL of water, and extract with the aid of ultrasonicator for 10 minutes with occasional shaking. Shake more for 10 minutes, then add exactly 10 mL of the internal standard solution and water to make 200 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution to dissolve, add water to make 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ephedrine to that of the internal standard of each solution.

$$\begin{aligned} \text{Amount (mg) of ephedrine hydrochloride } (\text{C}_{10}\text{H}_{15}\text{NO}\cdot\text{HCl}) \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of ephedrine hydrochloride for assay

Internal standard solution—A solution of ephedrine hydrochloride (1 in 500).

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (4) under Ephedrine Hydrochloride.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and ephedrine are eluted in this order with the resolution between these peaks being not less than 15.

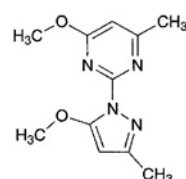
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Epirizole

Mepirizole

エピリゾール



$\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2$: 234.25

4-Methoxy-2-(5-methoxy-3-methyl-1*H*-pyrazol-1-yl)-6-methylpyrimidine [18694-40-1]

Epirizole, when dried, contains not less than 99.0% of $\text{C}_{11}\text{H}_{14}\text{N}_4\text{O}_2$.

Description Epirizole occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (95), and sparingly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid.

The pH of a solution of Epirizole (1 in 100) is between 6.0 and 7.0.

Identification (1) To 0.1 g of Epirizole add 0.1 g of vanillin, 5 mL of water and 2 mL of sulfuric acid, and mix with shaking for a while: a yellow precipitate is formed.

(2) Dissolve 0.1 g of Epirizole in 10 mL of water, and add 10 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Collect the precipitate by filtration, wash with 50 mL of water, and dry at 105°C for 1 hour: it melts <2.60> between 163°C and 169°C.

(3) Determine the absorption spectrum of a solution of Epirizole in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 88 – 91°C

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Epirizole in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Add 0.5 g of Epirizole to a ground mixture of 0.7 g of potassium nitrate and 1.2 g of anhydrous sodium carbonate, mix well, transfer little by little to a platinum crucible, previously heated, and heat until the reaction is completed. After cooling, add 15 mL of dilute sulfuric acid and 5 mL of water to the residue, boil for 5 minutes, filter, wash the insoluble matter with 10 mL of water, and add 6 mL

of dilute nitric acid and water to the combined filtrate and washings to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution as follows: proceed with the same quantities of the same reagents as directed for the preparation of the test solution, and add 0.25 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL (not more than 0.018%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Epirizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Epirizole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 1.0 g of Epirizole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropyl diethyl ether, ethanol (95) and water (23:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Place this plate in a chamber filled with iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Readily carbonizable substances <1.15>—Perform the test with 0.10 g of Epirizole: the solution has no more color than Matching Fluid A.

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Epirizole, previously dried, dissolve in 40 mL of acetic acid (100) and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from purple through blue-green to green.

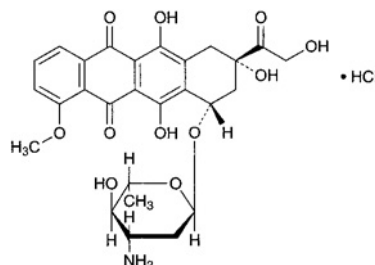
Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 23.43 \text{ mg of } C_{27}H_{29}NO_{11} \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Epirubicin Hydrochloride

エピルビシン塩酸塩



$C_{27}H_{29}NO_{11} \cdot HCl$: 579.98

(2*S*,4*S*)-4-(3-Amino-2,3,6-trideoxy- α -L-arabino-hexopyranosyloxy)-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride [56390-09-1]

Epirubicin Hydrochloride is the hydrochloride of a derivative of daunorubicin.

It contains not less than 970 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvent. The potency of Epirubicin Hydrochloride is expressed as mass (potency) of epirubicin hydrochloride ($C_{27}H_{29}NO_{11} \cdot HCl$).

Description Epirubicin Hydrochloride occurs as a pale yellowish red to brownish red powder.

It is soluble in water and in methanol, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Epirubicin Hydrochloride in methanol (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Epirubicin Hydrochloride and Epirubicin Hydrochloride Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +310 – +340° (10 mg calculated on the anhydrous basis and collected by the amount of the residual solvent, methanol, 20 mL, 100 mm).

pH <2.54> Dissolve 10 mg of Epirubicin Hydrochloride in 2 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 50 mg of Epirubicin Hydrochloride in 5 mL of water: the solution is clear and dark red.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Epirubicin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Perform the test with 10 μ L of

the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the sum amount of the peaks other than epirubicin and 2-naphthalenesulfonic acid by the area percentage method: not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of epirubicin beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add the mobile phase to make 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of epirubicin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that obtained from 10 μ L of the solution for system suitability test.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

(4) Residual solvents <2.46>—Weigh accurately about 0.3 g of Epirubicin Hydrochloride, add exactly 0.6 mL of the internal standard solution, add *N,N*-dimethylformamide to make 6 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, add *N,N*-dimethylformamide to make exactly 25 mL, and use this solution as methanol standard stock solution. Take exactly 125 μ L of acetone, 30 μ L of ethanol (99.5), 32 μ L of 1-propanol and 17 μ L of the methanol standard stock solution, add exactly 10 mL of the internal standard solution and *N,N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following condition, and determine the ratios of the peak areas of acetone, ethanol, 1-propanol and methanol to that of the internal standard, Q_{TA} and Q_{SA} , Q_{TB} and Q_{SB} , Q_{TC} and Q_{SC} , and Q_{TD} and Q_{SD} , respectively. Calculate the amounts of acetone, ethanol, 1-propanol and methanol by the following equations: the amounts of acetone, ethanol, 1-propanol and methanol are not more than 1.5%, not more than 0.5%, not more than 0.5% and not more than 0.1%, respectively.

$$\begin{aligned} \text{Amount (\%)} & \text{ of acetone} \\ & = (1/W_T) \times (Q_{TA}/Q_{SA}) \times 593 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} & \text{ of ethanol} \\ & = (1/W_T) \times (Q_{TB}/Q_{SB}) \times 142 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} & \text{ of 1-propanol} \\ & = (1/W_T) \times (Q_{TC}/Q_{SC}) \times 154 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} & \text{ of methanol} \\ & = (1/W_T) \times (Q_{TD}/Q_{SD}) \times 2.23 \end{aligned}$$

W_T : Amount (mg) of Epirubicin Hydrochloride

Internal standard solution—A solution of 1,4-dioxane in *N,N*-dimethylformamide (1 in 100).

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A fused silica column 0.53 mm in inside diameter

and 30 m in length, coated with polyethylene glycol for gas-chromatography 1 μ m in thickness.

Column temperature: 40°C for 11 minutes after injection of the sample, then rise to 90°C at a rate of 10°C per minute. If necessary, rise to 130°C at a rate of 50°C per minute and maintain the temperature for 30 minutes.

Injection port temperature: A constant temperature of about 120°C.

Detector temperature: A constant temperature of about 150°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 8 minutes.

Split ratio: 1:15

System suitability—

System performance: When the procedure is run with 1 μ L of the standard solution under the above operating conditions, acetone, methanol, ethanol, 1-propanol and the internal standard are eluted in this order with the resolution between the peaks of acetone and the internal standard being not less than 30.

System repeatability: When the test is repeated 6 times with 1 μ L of the standard solution under the above operating conditions, the relative standard deviations of the peak areas of acetone, methanol, ethanol and 1-propanol are not more than 4.0%, respectively.

Water Not more than 8.0% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately an amount of Epirubicin Hydrochloride and Epirubicin Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of epirubicin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] & \text{ of } C_{27}H_{29}NO_{11}.HCl \\ & = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Epirubicin Hydrochloride Reference Standard

Internal standard solution—A solution of sodium 2-naphthalene sulfonate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with trimethylsilylated silica gel for liquid chromatography (6 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in a mixture of water, acetonitrile, methanol and phosphoric acid (540:290:170:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of epirubicin is about 9.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and epirubicin are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epirubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

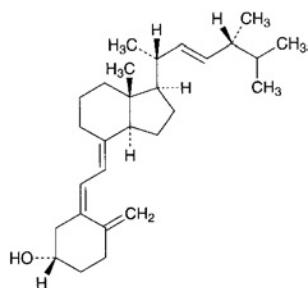
Storage—At a temperature between 0°C and 5°C.

Ergocalciferol

Calciferol

Vitamin D₂

エルゴカルシフェロール



$\text{C}_{28}\text{H}_{44}\text{O}$: 396.65
(3*S*,5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3-ol
[50-14-6]

Ergocalciferol contains not less than 97.0% and not more than 103.0% of $\text{C}_{28}\text{H}_{44}\text{O}$.

Description Ergocalciferol occurs as white crystals. It is odorless, or has a faint, characteristic odor.

It is freely soluble in ethanol (95), in diethyl ether and in chloroform, sparingly soluble in isooctane, and practically insoluble in water.

It is affected by air and by light.

Melting point: 115 – 118°C Transfer Ergocalciferol to a capillary tube, and dry for 3 hours in a desiccator (in vacuum at a pressure not exceeding 2.67 kPa). Immediately fire-seal the capillary tube, put it in a bath fluid, previously heated to a temperature about 10°C below the expected melting point, and heat at a rate of rise of about 3°C per minute, and read the melting point.

Identification (1) Dissolve 0.5 mg of Ergocalciferol in 5 mL of chloroform, add 0.3 mL of acetic anhydride and 0.1 mL of sulfuric acid, and shake: a red color is produced, and rapidly changes through purple and blue to green.

(2) Determine the infrared absorption spectrum of Ergocalciferol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ergocalciferol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (265 nm): 445 – 485 (0.01 g, ethanol (95), 100 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +102 – +107° (0.3 g, ethanol (95), 20 mL, 100 mm). Prepare the solution of Ergocalciferol within 30 minutes after the container has been opened, and determine the rotation within 30 minutes after the solution has been prepared.

Purity Ergosterol—Dissolve 10 mg of Ergocalciferol in 2.0 mL of diluted ethanol (95) (9 in 10), add a solution of 20 mg of digitonin in 2.0 mL of diluted ethanol (95) (9 in 10), and allow the mixture to stand for 18 hours: no precipitate is formed.

Assay Weigh accurately about 30 mg each of Ergocalciferol and Ergocalciferol Reference Standard, and dissolve each in isooctane to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 3 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 to 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{T} and Q_{S} , of the peak area of ergocalciferol to that of the internal standard. Perform the procedure rapidly avoiding contact with air or other oxidizing agents and using light-resistant containers.

$$\text{Amount (mg) of } \text{C}_{28}\text{H}_{44}\text{O} = W_{\text{S}} \times (Q_{\text{T}}/Q_{\text{S}})$$

W_{S} : Amount (mg) of Ergocalciferol Reference Standard

Internal standard solution—A solution of dimethyl phthalate in isooctane (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with a silica gel for liquid chromatography (10 μm particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of hexane and n-amylalcohol (997:3).

Flow rate: Adjust the flow rate so that the retention time of ergocalciferol is about 25 minutes.

System suitability—

System performance: Dissolve 15 mg of Ergocalciferol Reference Standard in 25 mL of isooctane. Transfer this solution to a flask, heat in an oil bath under a reflux condenser for 2 hours, and cool immediately to room temperature. Transfer the solution to a quartz test tube, and irradiate with a short-wave lamp (main wavelength: 254 nm) and a long-wave lamp (main wavelength: 365 nm) for 3 hours. To 10 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μL of this solution under the above operating conditions. Use a column with the ratios of the retention time of previtamin D₂, trans-vitamin D₂ and tachysterol₂ to that of ergocalciferol being about 0.5, about 0.6 and about 1.1, respectively, and with resolution between previtamin D₂ and trans-vitamin D₂ being not less than 0.7, and that between ergocalciferol and tachysterol₂ being not less than 1.0.

System repeatability: When the test is repeated 6 times with

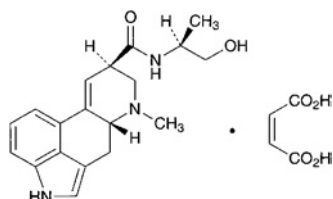
10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ergocalciferol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Ergometrine Maleate

エルゴメトリンマレイン酸塩



$C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$: 441.48

(8*S*)-*N*-[(1*S*)-2-Hydroxy-1-methylethyl]-6-methyl-9,10-didehydroergoline-8-carboxamide monomaleate
[129-51-1]

Ergometrine Maleate, when dried, contains not less than 98.0% of $C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$.

Description Ergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 185°C (with decomposition).

It gradually changes to yellow in color on exposure to light.

Identification (1) Prepare a solution of Ergometrine Maleate (1 in 50): the solution shows a blue fluorescence.

(2) Dissolve 1 mg of Ergometrine Maleate in 5 mL of water. To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-ferric chloride TS, shake, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of a solution of Ergometrine Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the solution disappears immediately.

Optical rotation <2.49> $[\alpha]_D^{20} +48 - +57^\circ$ (after drying 0.25 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water. The pH of the solution is between 3.0 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Ergometrine Maleate in 10 mL of water: the solution is clear and colorless to light yellow.

(2) Ergotamine and ergotamine—To 0.02 g of Ergometrine Maleate add 2 mL of a solution of sodium hydroxide (1 in 10), and heat to boiling: the gas evolved does not change moistened red litmus paper to blue.

(3) Related substances—Dissolve 5 mg each of Ergometrine Maleate and Ergometrine Maleate Reference Standard in 1.0 mL of methanol, and use these solutions as the sample solution and standard solution, respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 10 μ L each of the sample solution and standard solution on a plate, prepared with silica gel for thin-layer chromatography and dilute sodium hydroxide TS. Develop the plate with a mixture of chloroform and methanol (4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots obtained from the sample solution and the standard solution show a red-purple color and the same *R_f* value, and any spot from the sample solution other than that corresponding to the spot from the standard solution does not appear.

Loss on drying <2.41> Not more than 2.0% (0.2 g, silica gel, 4 hours).

Assay Weigh accurately about 10 mg each of Ergometrine Maleate and Ergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 2 mL of each solution into a separate brown glass-stoppered tube. To each tube add 4 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, exactly measured, while cooling in an ice bath, then warm at 45°C for 10 minutes. Allow to stand at room temperature for 20 minutes, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4 \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ergometrine Maleate Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ergometrine Maleate Injection

エルゴメトリンマレイン酸塩注射液

Ergometrine Maleate Injection is an aqueous solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$: 441.48).

Method of preparation Prepare as directed under Injections, with Ergometrine Maleate.

Description Ergometrine Maleate Injection is a clear, colorless to pale yellow liquid.

pH: 2.7 – 3.5

Identification (1) Measure a volume of Ergometrine Maleate Injection, equivalent to 3 mg of Ergometrine Maleate according to the labeled amount, if necessary, dilute with water or evaporate on a water bath to make 15 mL, and use this solution as the sample solution. The sample solution shows a blue fluorescence.

(2) To 1 mL of the sample solution obtained in (1) add 1

mL of ammonia TS, and extract with 20 mL of diethyl ether. To the diethyl ether extract add 1 mL of dilute sulfuric acid, shake, and warm to remove diethyl ether in a water bath. Cool, to the residue obtained add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and allow to stand for 5 to 10 minutes: a deep blue color develops.

(3) To 5 mL of the sample solution obtained in (1) add 1 drop of potassium permanganate TS: a red color disappears immediately.

Extractable volume <6.05> It meets the requirement.

Assay Transfer an exactly measured volume of Ergometrine Maleate Injection, equivalent to about 2 mg of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$), and add sodium chloride in a ratio of 0.3 g to 1 mL of the solution. To this mixture add 20 mL of diethyl ether and 2 mL of ammonia TS, shake, and extract. Further, extract with three 15-mL portions of diethyl ether, combine all the extracts, add 5 g of anhydrous sodium sulfate, filter through a pledget of absorbent cotton, and wash with three 5-mL portions of diethyl ether. Add the washings to the filtrate, shake with 5 mL of dilute sulfuric acid, evaporate the diethyl ether by warming in a current of nitrogen, to the remaining solution add water to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 2 mg of Ergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, add water to make exactly 50 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and standard solution, accurately measured, to separate glass-stoppered test tubes, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$)
 $= W_S \times (A_T/A_S)$

W_S : Amount (mg) of Ergometrine Maleate Reference Standard

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and in a cold place.

Ergometrine Maleate Tablets

エルゴメトリンマレイン酸塩錠

Ergometrine Maleate Tablets contain not less than 90% and not more than 110% of the labeled amount of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$: 441.48).

Method of preparation Prepare as directed under Tablets, with Ergometrine Maleate.

Identification To a quantity of powdered Ergometrine Maleate Tablets, equivalent to 3 mg of Ergometrine Maleate according to the labeled amount, add 15 mL of warm water, shake, and filter: the filtrate shows a blue fluorescence. Proceed with this solution as directed in the Identification (2) and (3) under Ergometrine Maleate.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Ergometrine Maleate Tablets to a

glass-stoppered centrifuge tube, and add a solution of L-tartaric acid (1 in 100) to make exactly V mL of a solution containing about $40 \mu\text{g}$ of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$) per mL. Stopper the tube, shake for 30 minutes vigorously, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 4 mg of Ergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into separate brown glass-stoppered test tubes, add exactly 8 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while cooling in an ice bath, after shaking, and allow to stand for 1 hour at ordinary temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 4 mL of water in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 550 nm, respectively.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$)
 $= W_S \times (A_T/A_S) \times (V/100)$

W_S : Amount (mg) of Ergometrine Maleate Reference Standard

Assay Weigh accurately, and powder not less than 20 Ergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 2 mg of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$), transfer to a glass filter (G4), add 10 mL of a solution of L-tartaric acid (1 in 100), and filter with thorough shaking. Repeat the procedures 3 times, combine the filtrates, add a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 2 mg of Ergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, dissolve in a solution of L-tartaric acid (1 in 100) to make exactly 50 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, and proceed as directed in the Assay under Ergometrine Maleate.

Amount (mg) of ergometrine maleate ($C_{19}H_{23}N_3O_2 \cdot C_4H_4O_4$)
 $= W_S \times (A_T/A_S)$

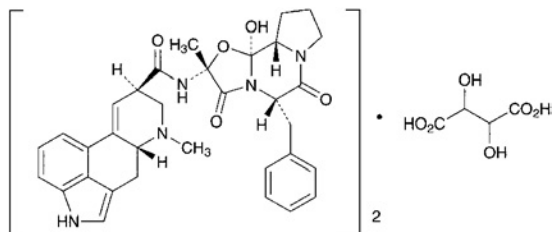
W_S : Amount (mg) of Ergometrine Maleate Reference Standard

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Ergotamine Tartrate

エルゴタミン酒石酸塩



$(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$; 1313.41
(5'S)-5'-Benzyl-12'-hydroxy-2'-methylergotaman-3',6',18-trione hemitartrate [379-79-3]

Ergotamine Tartrate contains not less than 98.0% of $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$, calculated on the dried basis.

Description Ergotamine Tartrate occurs as colorless crystals, or a white to pale yellowish white or grayish white, crystalline powder.

It is slightly soluble in water and in ethanol (95).

Melting point: about 180°C (with decomposition).

Identification (1) Dissolve 1 mg of Ergotamine Tartrate in 10 mL of a mixture of acetic acid (100) and ethyl acetate (1:1). To 0.5 mL of this solution add slowly 0.5 mL of sulfuric acid, with shaking in cold water, and allow to stand: a purple color develops. To this solution add 0.1 mL of diluted iron (III) chloride TS (1 in 12): the color of the solution changes to blue to blue-purple.

(2) Dissolve 1 mg of Ergotamine Tartrate in 5 mL of a solution of L-tartaric acid (1 in 100). To 1 mL of this solution add 2 mL of 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake: a blue color develops.

Optical rotation <2.49> Ergotamine base $[\alpha]_D^{20}$: -155 - -165°. Dissolve 0.35 g of Ergotamine Tartrate in 25 mL of a solution of L-tartaric acid (1 in 100), add 0.5 g of sodium hydrogen carbonate, shake gently and sufficiently, and extract with four 10-mL portions of ethanol-free chloroform. Filter the extracts successively through a small filter paper, moistened with ethanol-free chloroform, into a 50-mL volumetric flask. Allow the flask to stand in a water bath at 20°C for 10 minutes, and determine the optical rotation in a 100-mm cell. Separately, pipet 25 mL of this solution, evaporate to dryness under reduced pressure at a temperature not higher than 45°C, dissolve the residue in 25 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction. Calculate the specific rotation of the ergotamine base from the consumed volume of 0.05 mol/L perchloric acid VS and the optical rotation.

Each mL of 0.05 mol/L perchloric acid VS
= 29.08 mg of $C_{33}H_{35}N_5O_5$

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. To 40 mg of Ergotamine Tartrate add 10 mL of a solution of L-

tartaric acid in diluted methanol (1 in 2) (1 in 1000), dissolve with thorough shaking, and use this solution as the sample solution. Pipet 1 mL of this solution, add a solution of L-tartaric acid in diluted methanol (1 in 2) (1 in 1000) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, 60°C, 4 hours).

Assay Weigh accurately about 0.2 g of Ergotamine Tartrate, dissolve in 15 mL of a mixture of acetic acid (100) and acetic anhydride (50:3), and titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination, and make any necessary correction.

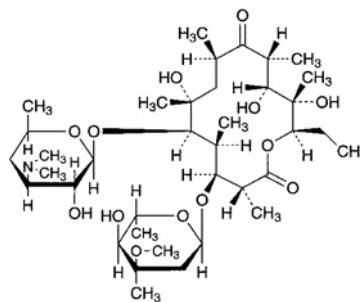
Each mL of 0.05 mol/L perchloric acid VS
= 32.84 mg of $(C_{33}H_{35}N_5O_5)_2 \cdot C_4H_6O_6$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere, and not exceeding 5°C.

Erythromycin

エリスロマイシン



$C_{37}H_{67}NO_{13}$; 733.93
(2R,3S,4S,5R,6R,8R,10R,11R,12S,13R)-5-(3,4,6-Trideoxy-3-dimethylamino-β-D-xylo-hexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide [114-07-8]

Erythromycin is a macrolide substance having antibacterial activity produced by the growth of *Saccharopolyspora erythraea*.

It contains not less than 930 μ g (potency) and not more than 1020 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$).

Description Erythromycin occurs as a white to light yellow-

ish white powder.

It is very soluble in *N,N*-dimethylformamide, freely soluble in methanol and in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the infrared absorption spectrum of Erythromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Erythromycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 10 mg each of Erythromycin and Erythromycin Reference Standard in 1 mL of methanol, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (50:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 100°C for 15 minutes: the principal spot from the sample solution and the spot from the standard solution are dark purple in color, and their *R_f* values are the same.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-71 - -78^\circ$ (1 g calculated on the anhydrous basis, ethanol (95), 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Erythromycin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Erythromycin according to Method 5 using hydrochloric acid instead of diluted hydrochloric acid (1 in 2), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 40 mg of Erythromycin in 2 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 16 mg of Erythromycin Reference Standard in 2 mL of methanol, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 10 mL, and use this solution as the standard stock solution. Dissolve 5 mg each of erythromycin B and erythromycin C in 2 mL of methanol, add exactly 2 mL of the standard stock solution, add a mixture of phosphate buffer solution, pH 7.0 and methanol (15:1) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of erythromycin B and erythromycin C from the sample solution are not more than those of erythromycin B and erythromycin C from the standard solution, respectively, and each area of the peaks other than erythromycin, erythromycin B and erythromycin C is not more than the area of the peak of erythromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μ m in particle diameter).

Column temperature: A constant temperature of about 70°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogen phosphate in water to make 100 mL, and adjust the pH to 9.0 with diluted phosphoric acid (1 in 10). To 50 mL of this solution add 190 mL of *t*-butanol, 30 mL of acetonitrile and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of erythromycin is about 20 minutes.

Time span of measurement: About 4 times as long as the retention time of erythromycin beginning after the solvent peak.

System suitability—

System performance: Dissolve 2 mg of *N*-demethylerythromycin in 10 mL of the standard solution. When the procedure is run with 100 μ L of this solution under the above operating conditions, *N*-demethylerythromycin, erythromycin C, erythromycin and erythromycin B are eluted in this order, with the resolution between the peaks of *N*-demethylerythromycin and erythromycin C being not less than 0.8, and with the resolution between the peaks of *N*-demethylerythromycin and erythromycin being not less than 5.5.

System repeatability: When the test is repeated 3 times with 100 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of erythromycin is not more than 3.0%.

Water <2.48> Not more than 10.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

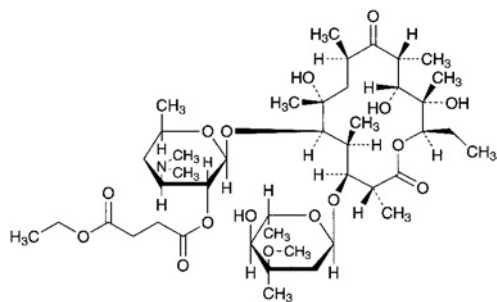
(iv) Sample solutions—Weigh accurately an amount of Erythromycin, equivalent to about 25 mg (potency), dissolve in 25 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration sample solution and low

concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Erythromycin Ethylsuccinate

エリスロマイシンエチルコハク酸エステル



$C_{43}H_{75}NO_{16}$: 862.05
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-[3,4,6-Trideoxy-2-*O*-(3-ethoxycarbonylpropanoyl)-3-dimethylamino- β -*D*-xylo-hexopyranosyloxy]-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide [41342-53-4]

Erythromycin Ethylsuccinate is a derivative of erythromycin.

It contains not less than 780 μ g (potency) and not more than 900 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Ethylsuccinate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Ethylsuccinate occurs as a white powder.

It is freely soluble in methanol and in acetone, soluble in ethanol (95), and practically insoluble in water.

Identification (1) Dissolve 3 mg of Erythromycin Ethylsuccinate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to red to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Ethylsuccinate, previously dried in a desiccator (reduced pressure, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to

8.0 after sterilization.

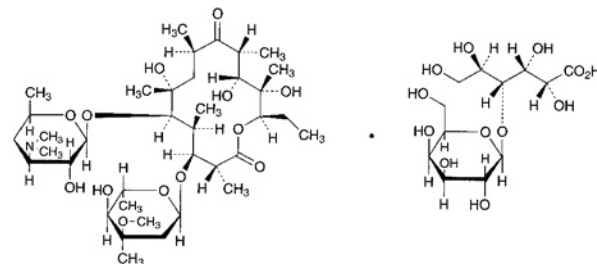
(iii) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Ethylsuccinate equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Erythromycin Lactobionate

エリスロマイシンラクトビオン酸塩



$C_{37}H_{67}NO_{13} \cdot C_{12}H_{22}O_{12}$: 1092.22
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino- β -*D*-xylo-hexopyranosyloxy)-3-(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl- α -*L*-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide mono(4-*O*- β -*D*-galactopyranosyl-*D*-gluconate) [3847-29-8]

Erythromycin Lactobionate is the lactobionate of erythromycin.

It contains not less than 590 μ g (potency) and not more than 700 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Lactobionate is expressed as mass (potency) of erythromycin ($C_{37}H_{67}NO_{13}$: 733.93).

Description Erythromycin Lactobionate occurs as a white powder.

It is freely soluble in water, in methanol and in ethanol (99.5), and very slightly soluble in acetone.

Identification (1) To 3 mg of Erythromycin Lactobionate add 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color is produced, and it changes immediately to red

to deep purple.

(2) Transfer about 0.3 g of Erythromycin Lactobionate to a separator, add 15 mL of ammonia TS and 15 mL of chloroform, shake, and take the separated aqueous layer. Wash the aqueous layer with three 15-mL portions of chloroform, and evaporate the aqueous liquid on a water bath to dryness. Dissolve the residue in 10 mL of a mixture of methanol and water (3:2), and use this solution as the sample solution. Separately, dissolve 0.10 g of lactobionic acid in 10 mL of a mixture of methanol and water (3:2), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatograph. Develop the plate with the upper layer obtained from a mixture of water, 1-butanol and acetic acid (100) (3:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid, and heat at 105°C for 20 minutes: the principal spot obtained from the sample solution shows a deep brown and the *R_f* value which are the same as those of the principal spot from the standard solution.

pH <2.54> The pH of a solution obtained by dissolving 0.5 g of Erythromycin Lactobionate in 10 mL of water is between 5.0 and 7.5.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

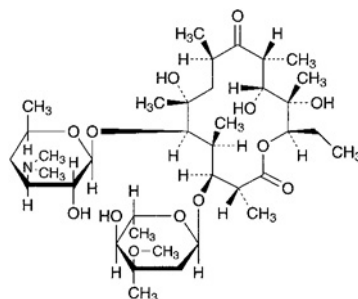
(iii) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Lactobionate, equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Erythromycin Stearate

エリスロマイシンステアリン酸塩



$\text{C}_{37}\text{H}_{67}\text{NO}_{13} \cdot \text{C}_{18}\text{H}_{36}\text{O}_2$: 1018.40

(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-5-(3,4,6-Trideoxy-3-dimethylamino- β -D-xyllo-hexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl- α -L-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-2,4,6,8,10,12-hexamethyl-9-oxopentadecan-13-olide monostearate [643-22-1]

Erythromycin Stearate is the stearate of erythromycin.

It contains not less than 600 μ g (potency) and not more than 720 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Erythromycin Stearate is expressed as mass (potency) of erythromycin ($\text{C}_{37}\text{H}_{67}\text{NO}_{13}$: 733.93).

Description Erythromycin Stearate occurs as a white powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, and practically insoluble in water.

Identification (1) Dissolve 3 mg of Erythromycin Stearate in 2 mL of acetone, and add 2 mL of hydrochloric acid: an orange color develops and is immediately changed to red to deep purple.

(2) Determine the infrared absorption spectrum of Erythromycin Stearate, previously dried in a desiccator (reduced pressure, silica gel) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Erythromycin Reference Standard equivalent to about 50 mg

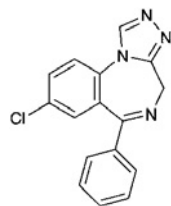
(potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Erythromycin Stearate equivalent to about 50 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Estazolam

エスタゾラム



$C_{16}H_{11}ClN_4$: 294.74
8-Chloro-6-phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine [29975-16-4]

Estazolam, when dried, contains not less than 98.5% of $C_{16}H_{11}ClN_4$.

Description Estazolam occurs as white to pale yellowish white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is soluble in methanol and in acetic anhydride, sparingly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estazolam in 3 mL of sulfuric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Estazolam in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Estazolam as directed under Flame Coloration Test (2) <1.04>: a green color appears.

Melting point <2.60> 229 – 233°C

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Estazolam in 10 mL of ethanol (95): the solution is clear

and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Estazolam in 10 mL of ethanol (95) by heating, add 40 mL of water, cool with shaking in ice water, allow to stand to attain ordinary temperature, and filter. To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of ethanol (95) (not more than 0.015%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Estazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Estazolam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Estazolam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform and methanol (5:3:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the principal spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

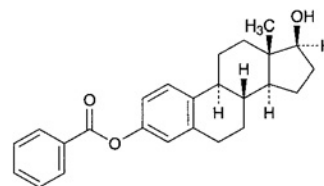
Assay Weigh accurately about 0.25 g of Estazolam, previously dried, dissolve in 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration), until the solution changes to the second equivalence point. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.74 mg of $C_{16}H_{11}ClN_4$

Containers and storage Containers—Well-closed containers.

Estradiol Benzoate

エストラジオール安息香酸エステル



$C_{25}H_{28}O_3$: 376.49
Estra-1,3,5(10)-triene-3,17β-diol 3-benzoate [50-50-0]

Estradiol Benzoate, when dried, contains not less

than 97.0% of $C_{25}H_{28}O_3$.

Description Estradiol Benzoate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in acetone, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) To 2 mg of Estradiol Benzoate add 2 mL of sulfuric acid: a yellowish green color with a blue fluorescence is produced, and the color of the solution changes to light orange on the careful addition of 2 mL of water.

(2) Determine the infrared absorption spectrum of Estradiol Benzoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Estradiol Benzoate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +54 – +58° (after drying, 0.1 g, acetone, 10 mL, 100 mm).

Melting point <2.60> 191 – 198°C

Purity (1) 3,17 α -Estradiol—Dissolve 5.0 mg each of Estradiol Benzoate and Estradiol Benzoate Reference Standard in acetone to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Place exactly 2 mL each of the sample solution and standard solution in separate glass-stoppered test tube, add boiling stones, evaporate the acetone by heating in a water bath, and dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 1 hour. Add 1.0 mL of dilute iron-phenol TS to each test tube. Stopper the test tubes loosely, heat for 30 seconds in a water bath, shake in a water bath for several seconds, and heat for 2 minutes. Cool the solutions in ice for 2 minutes, add 4.0 mL of diluted sulfuric acid (7 in 20), and mix well: the solution obtained from the sample solution has no more color than that from the standard solution.

(2) Related substances—Dissolve 40 mg of Estradiol Benzoate in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (0.1 g).

Assay Weigh accurately about 10 mg each of Estradiol Benzoate and Estradiol Benzoate Reference Standard, previously dried, and dissolve each in methanol to make exactly 20 mL. Pipet 5 mL each of these solutions, add 5 mL of the internal standard solution, then add methanol to make 20 mL, and use these solutions as the sample solution and standard solu-

tion, respectively. Perform the test with 5 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of estradiol benzoate to that of the internal standard.

$$\text{Amount (mg) of } C_{25}H_{28}O_3 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Estradiol Benzoate Reference Standard

Internal standard solution—A solution of progesterone in methanol (13 in 80,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of estradiol benzoate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the internal standard and estradiol benzoate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estradiol benzoate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Estradiol Benzoate Injection

エストラジオール安息香酸エステル注射液

Estradiol Benzoate Injection is an oily solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of estradiol benzoate ($C_{25}H_{28}O_3$; 376.49).

Method of preparation Prepare as directed under Injections, with Estradiol Benzoate.

Description Estradiol Benzoate Injection is a clear, oily liquid.

Identification To a volume of Estradiol Benzoate Injection, equivalent to 1 mg of Estradiol Benzoate according to the labeled amount, add chloroform to make 5 mL, and use this solution as the sample solution. Separately dissolve 1 mg of Estradiol Benzoate Reference Standard in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample so-

lution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with dichloromethane to a distance of about 15 cm, and air-dry the plate. Then develop the plate with a mixture of chloroform and methanol (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R_f* value.

Extractable volume <6.05> It meets the requirement.

Assay Transfer an exactly measured volume of Estradiol Benzoate Injection, equivalent to about 10 mg of estradiol benzoate (C₂₅H₂₈O₃), to a separator, add 30 mL of hexane saturated with diluted methanol (9 in 10), and extract with five 15-mL portions of dilute methanol (9 in 10) saturated with hexane. Filter the extract through a filter paper washed with 10 mL of diluted methanol (9 in 10), to the filtrate add methanol to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estradiol Benzoate Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Transfer 2 mL each of the sample solution and standard solution, exactly measured, to light-resistant 20-mL volumetric flasks, and evaporate to dryness on a water bath with the aid of a current of air. Dissolve the residue in 1 mL of methanol, add 10 mL of boric acid-methanol buffer solution, shake, and boil under a reflux condenser for 30 minutes. Cool, add 5 mL of boric acid-methanol buffer solution, shake, and cool with ice. To each solution add 2 mL of ice-cold diazo TS quickly, shake vigorously, add 2 mL of sodium hydroxide TS, then add water to make 20 mL, and filter after shaking. Discard the first 3 mL of the filtrate, and perform the test with the subsequent filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared with 2 mL of methanol in the same manner, as the blank. Determine the absorbances, *A_T* and *A_S*, of the subsequent solutions obtained from the sample solution and standard solution in a 4-cm cell at 490 nm, respectively.

$$\begin{aligned} &\text{Amount (mg) of estradiol benzoate (C}_{25}\text{H}_{28}\text{O}_3) \\ &= W_S \times (A_T/A_S) \times (2/5) \end{aligned}$$

W_S: Amount (mg) of Estradiol Benzoate Reference Standard

Containers and storage Containers—Hermetic containers.

Estradiol Benzoate Injection (Aqueous Suspension)

エストラジオール安息香酸エステル水性懸濁注射液

Estradiol Benzoate Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled amount of estradiol benzoate (C₂₅H₂₈O₃: 376.49).

Method of preparation Prepare as directed under Injection,

with Estradiol Benzoate.

Description Estradiol Benzoate Injection (Aqueous Suspension) produces a white turbidity on shaking.

Identification Extract a volume of Estradiol Benzoate Injection (Aqueous Suspension), equivalent to 1 mg of Estradiol Benzoate according to the labeled amount, with 5 mL of chloroform, and use this extract as the sample solution. Separately, dissolve 1 mg of Estradiol Benzoate Reference Standard in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot obtained from the sample solution and the spot obtained from the standard solution show the same *R_f* value.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of well-mixed Estradiol Benzoate Injection (Aqueous Suspension), equivalent to about 2 mg of estradiol benzoate (C₂₅H₂₈O₃), dissolve the crystals with an appropriate quantity of methanol, and add methanol to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Estradiol Benzoate Reference Standard, previously dried in desiccator (reduced pressure, phosphorus (V) oxide) for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and methanol to make 100 mL, and use this solution as the standard solution. Proceed with these solutions as directed in the Assay under Estradiol Benzoate.

$$\begin{aligned} &\text{Amount (mg) of estradiol benzoate (C}_{25}\text{H}_{28}\text{O}_3) \\ &= W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

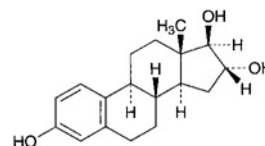
W_S: Amount (mg) of Estradiol Benzoate Reference Standard

Internal standard solution—A solution of progesterone in methanol (13 in 100,000).

Containers and storage Containers—Hermetic containers.

Estriol

エストリオール



C₁₈H₂₄O₃: 288.38

Estra-1,3,5(10)-triene-3,16α,17β-triol [50-27-1]

Estriol, when dried, contains not less than 97.0% and not more than 102.0% of C₁₈H₂₄O₃.

Description Estriol occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in ethanol (95) and in 1,4-dioxane, and practically insoluble in water and in diethyl ether.

Identification (1) Dissolve 0.01 g of Estriol in 100 mL of ethanol (95) by warming, and use this solution as the sample solution. Evaporate 1 mL of this solution on a water bath to dryness, add 5 mL of a solution of sodium *p*-phenolsulfonate in diluted phosphoric acid (1 in 50), heat at 150°C for 10 minutes, and cool: a red-purple color develops.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Estriol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Estriol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Estriol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +54 – +62° (after drying, 40 mg, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 281 – 286°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Estriol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 40 mg of Estriol in 10 mL of ethanol (95) by warming, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and acetic acid (100) (18:1:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 25 mg each of Estriol and Estriol Reference Standard, previously dried, and dissolve each in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of

estriol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{18}H_{24}O_3 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Estriol Reference Standard

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (51:49).

Flow rate: Adjust the flow rate so that the retention time of estriol is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, estriol and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of estriol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Estriol Injection (Aqueous Suspension)

エストリオール水性懸濁注射液

Estriol Injection (Aqueous Suspension) is an aqueous suspension for injection. It contains not less than 90% and not more than 110% of the labeled amount of estriol ($C_{18}H_{24}O_3$; 288.38).

Method of preparation Prepare as directed under Injections, with Estriol.

Description Shake Estriol Injection (Aqueous Suspension): a white turbidity is produced.

Identification (1) Shake well, take a volume of Estriol Injection (Aqueous Suspension), equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 279 nm and 283 nm.

Extractable volume <6.05> It meets the requirement.

Assay Shake well, pipet a volume of Estriol Injection (Aqueous Suspension), equivalent to about 5 mg of estriol

(C₁₈H₂₄O₃), and dissolve in methanol to make exactly 20 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Estriol.

$$\begin{aligned} &\text{Amount (mg) of estriol (C}_{18}\text{H}_{24}\text{O}_3) \\ &= W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Estriol Reference Standard

Internal standard solution—A solution of methyl benzoate for estriol limit test in ethanol (95) (1 in 5000).

Containers and storage Containers—Hermetic containers.

Estriol Tablets

エストリオール錠

Estriol Tablets contain not less than 90% and not more than 110% of the labeled amount of estriol (C₁₈H₂₄O₃: 288.38).

Method of preparation Prepare as directed under Tablets, with Estriol.

Identification (1) Weigh a portion of powdered Estriol Tablets, equivalent to 2 mg of Estriol according to the labeled amount, add ethanol (95) to make 20 mL, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Estriol.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 279 nm and 283 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Estriol Tablets add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, add exactly 15 mL of methanol, and shake for 15 minutes. Centrifuge this solution for 10 minutes, pipet a definite amount of the supernatant liquid, and add methanol to make exactly a definite amount of solution so that each mL of the solution contains about 5 μg of estriol (C₁₈H₂₄O₃). Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Proceed with 20 μL of the sample solution as directed in the Assay under Estriol. Use a solution of methyl benzoate in methanol (1 in 40,000) as the internal standard solution. Calculate the mean value from each ratio of peak areas of 10 samples: the samples conform to the requirements if the deviation (%) of the mean value and each ratio of peak areas is within 15%. If the deviation (%) exceeds 15%, and 1 sample shows deviation within 25%, repeat the test with 20 samples. Calculate the deviation (%) of the mean value from each ratio of peak areas of the 30

samples used in the 2 tests and each ratio of peak areas: the samples conform to the requirements if the deviation exceeds 15%, not more than 1 sample shows deviation within 25%, and no sample shows deviation exceeding 25%.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Estriol Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 0.1 μg of estriol (C₁₈H₂₄O₃) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Estriol Reference Standard, previously dried at 105°C for 3 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 100 μL each of the sample solution and standard solution according to the operating conditions as directed in the Assay under Estriol, and determine the peak areas of estriol, A_T and A_S , from these solutions.

The dissolution rate of Estriol Tablets in 30 minutes is not less than 80%.

$$\begin{aligned} &\text{Dissolution rate (\%)} \text{ with respect to} \\ &\text{the labeled amount of estriol (C}_{18}\text{H}_{24}\text{O}_3) \\ &= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times (9/10) \end{aligned}$$

W_S : Amount (mg) of Estriol Reference Standard

C : Labeled amount (mg) of estriol (C₁₈H₂₄O₃) in 1 tablet

Assay Weigh accurately and powder not less than 20 Estriol Tablets. Weigh accurately a portion of the powder, equivalent to about 1 mg of estriol (C₁₈H₂₄O₃), add exactly 5 mL of water, disperse the fine particles with ultrasonic wave, shake with 25 mL of methanol for 10 minutes, centrifuge, and take the supernatant liquid. Add 25 mL of methanol, repeat the above procedure twice, combine the supernatant liquid, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Estriol Reference Standard, previously dried at 105°C for 3 hours, and dissolve in methanol to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 5 mL of the internal standard solution, then add methanol to make 100 mL, and use this solution as the standard solution. Proceed with 20 μL each of the sample solution and standard solution as directed in the Assay under Estriol.

$$\begin{aligned} &\text{Amount (mg) of estriol (C}_{18}\text{H}_{24}\text{O}_3) \\ &= W_S \times (Q_T/Q_S) \times (1/25) \end{aligned}$$

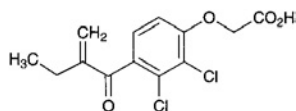
W_S : Amount (mg) of Estriol Reference Standard

Internal standard solution—A solution of methyl benzoate for estriol limit test in methanol (1 in 5000).

Containers and storage Containers—Tight containers.

Etacrynic Acid

エタクリン酸



$C_{13}H_{12}Cl_2O_4$: 303.14
[2,3-Dichloro-4-(2-ethylacryloyl)phenoxy]acetic acid
[58-54-8]

Etacrynic Acid, when dried, contains not less than 98.0% of $C_{13}H_{12}Cl_2O_4$.

Description Etacrynic Acid occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether, and very slightly soluble in water.

Identification (1) Dissolve 0.2 g of Etacrynic Acid in 10 mL of acetic acid (100), and to 5 mL of this solution add 0.1 mL of bromine TS: the color of the test solution disappears. To the remaining 5 mL of the solution add 0.1 mL of potassium permanganate TS: the color of the test solution changes to light orange immediately.

(2) To 0.01 g of Etacrynic Acid add 1 mL of sodium hydroxide TS, and heat in a water bath for 3 minutes. After cooling, add 1 mL of disodium chlorotroponate TS, and heat in a water bath for 10 minutes: a deep purple color develops.

(3) Determine the absorption spectrum of a solution of Etacrynic Acid in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Etacrynic Acid as directed under Flame Coloration Test (2) <1.04>: a green color appears.

Melting point <2.60> 121 – 125°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Etacrynic Acid in 10 mL of methanol: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Etacrynic Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Etacrynic Acid according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Etacrynic Acid in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer

chromatography. Develop the plate with a mixture of chloroform, ethyl acetate and acetic acid (100) (6:5:2) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.25% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Etacrynic Acid, previously dried, place in an iodine bottle, dissolve in 20 mL of acetic acid (100), and add exactly 20 mL of 0.05 mol/L bromine VS. To this solution add 3 mL of hydrochloric acid, stopper tightly at once, shake, and allow to stand in a dark place for 60 minutes. Add carefully 50 mL of water and 15 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 15.16 mg of $C_{13}H_{12}Cl_2O_4$

Containers and storage Containers—Well-closed containers.

Etacrynic Acid Tablets

エタクリン酸錠

Etacrynic Acid Tablets contain not less than 90% and not more than 110% of the labeled amount of etacrynic acid ($C_{13}H_{12}Cl_2O_4$: 303.14).

Method of preparation Prepare as directed under Tablets, with Etacrynic Acid.

Identification (1) Weigh a quantity of powdered Etacrynic Acid Tablets, equivalent to 0.3 g of Etacrynic Acid according to the labeled amount, add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with 50 mL of Dichloromethane. Filter the dichloromethane extract, and evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1), (2) and (4) under Etacrynic Acid.

(2) Prepare a solution of the residue obtained in (1), equivalent to a solution of Etacrynic Acid in methanol (1 in 20,000), and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 268 nm and 272 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Etacrynic Acid Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 45 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, and use the subsequent as the sample solution. Separately, weigh accurately about 55 mg of etacrynic acid for assay, previously dried at 60°C for 2 hours, dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this so-

lution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 277 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate of Etacrylic Acid Tablets in 45 minutes is not less than 70%.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ with respect to} \\ & \text{the labeled amount of etacrylic acid (C}_{13}\text{H}_{12}\text{Cl}_2\text{O}_4) \\ & = W_S \times (A_T/A_S) \times (1/C) \times 45 \end{aligned}$$

W_S : Amount (mg) of etacrylic acid for assay

C : Labeled amount (mg) of etacrylic acid (C₁₃H₁₂Cl₂O₄) in 1 tablet

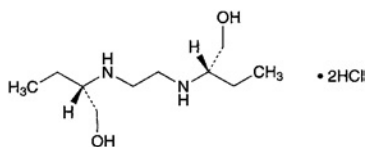
Assay Weigh accurately and powder not less than 20 Etacrylic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of etacrylic acid (C₁₃H₁₂Cl₂O₄), add 25 mL of 0.1 mol/L hydrochloric acid TS, and extract with three 30-mL portions of dichloromethane. Filter the dichloromethane extracts through a pledget of absorbent cotton into an iodine bottle. Wash the pledget of absorbent cotton with a small amount of dichloromethane, and combine the washing with the extracts. Evaporate this solution on a water bath to dryness in a current of air, to the residue add 20 mL of acetic acid (100), and proceed as directed in the Assay under Etacrylic Acid.

$$\begin{aligned} & \text{Each mL of 0.05 mol/L bromine VS} \\ & = 15.16 \text{ mg of C}_{13}\text{H}_{12}\text{Cl}_2\text{O}_4 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Ethambutol Hydrochloride

エタンブトール塩酸塩



C₁₀H₂₄N₂O₂·2HCl: 277.23

2,2'-(Ethylenediimino)bis[(2*S*)-butan-1-ol] dihydrochloride [1070-11-7]

Ethambutol Hydrochloride, when dried, contains not less than 98.5% of C₁₀H₂₄N₂O₂·2HCl.

Description Ethambutol Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, soluble in methanol and in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Ethambutol Hydrochloride (1 in 20) is between 3.4 and 4.0.

Identification (1) To 10 mL of a solution of Ethambutol Hydrochloride (1 in 100) add 0.5 mL of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a deep blue color is produced.

(2) Dissolve 0.1 g of Ethambutol Hydrochloride in 40 mL

of water, add 20 mL of 2,4,6-trinitrophenol TS, and allow to stand for 1 hour. Collect the precipitate, wash with 50 mL of water, and dry at 105°C for 2 hours: the precipitate melts <2.60> between 193°C and 197°C.

(3) A solution of Ethambutol Hydrochloride (1 in 30) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +5.5 – +6.1° (after drying, 5 g, water, 50 mL, 200 mm).

Melting point <2.60> 200 – 204°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ethambutol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g Ethambutol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ethambutol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) 2-Aminobutanol—Dissolve 5.0 g of Ethambutol Hydrochloride in methanol to make exactly 100 mL, and use this solution as the sample solution. Dissolve 0.05 g of 2-amino-1-butanol in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 10 cm, air-dry the plate, and heat at 105°C for 5 minutes. Cool, spray evenly ninhydrin-L-ascorbic acid TS upon the plate, air-dry the plate, and heat at 105°C for 5 minutes: the spot from the sample solution, corresponding to that from the standard solution, has no more color than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ethambutol Hydrochloride, previously dried, dissolve in 20 mL of water, and add 1.8 mL of copper (II) sulfate TS. To the solution add 7 mL of sodium hydroxide TS with shaking, add water to make exactly 50 mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add 10 mL of ammonia-ammonium chloride buffer solution, pH 10.0 and 100 mL of water, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from blue-purple through light red to light yellow (indicator: 0.15 mL of Cu-PAN TS). Perform a blank determination, and make any necessary correction.

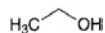
$$\begin{aligned} & \text{Each mL of 0.01 mol/L disodium dihydrogen} \\ & \text{ethylenediamine tetraacetate VS} \\ & = 2.772 \text{ mg of C}_{10}\text{H}_{24}\text{N}_2\text{O}_2\cdot 2\text{HCl} \end{aligned}$$

Containers and storage Containers—Tight containers.

Ethanol

Alcohol

エタノール



$\text{C}_2\text{H}_6\text{O}$: 46.07

Ethanol [64-17-5]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Ethanol contains not less than 95.1 vol% and not more than 96.9 vol% (by specific gravity) of $\text{C}_2\text{H}_6\text{O}$ at 15°C.

♦**Description** Ethanol is a clear, colorless liquid.

It is miscible with water.

It is flammable and burns with a light blue flame on ignition.

It is volatile. ◆

Identification (1) Determine the infrared absorption spectrum of Ethanol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{15}^{15} : 0.809 – 0.816

Purity (1) Clarity and color of solution—Ethanol is clear and colorless. To 1.0 mL of Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water.

(2) Acidity or alkalinity—To 20 mL of Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution prepared by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS: no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: a light red color develops.

(3) Volatile impurities—Pipet 500 mL of Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous methanol add Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 μL each of anhydrous methanol and acetaldehyde add Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 μL of acetal add Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 μL of benzene add Ethanol to make exactly 100 mL. To exactly 100 μL of this solution add Ethanol to make exactly 50 mL, and use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the

peak areas of acetaldehyde, A_E , benzene, B_E and acetal, C_E obtained with Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T : the peak area of methanol is not more than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above and the peak having the area not more than 3% that of 4-methylpentan-2-ol is not larger than the peak area of 4-methylpentan-2-ol.

Total amount (vol ppm) of acetaldehyde and acetal
 $= \{(10 \times A_E)/(A_T - A_E)\} + \{(30 \times C_E)/(C_T - C_E)\}$

Amount (vol ppm) of benzene = $2B_E/(B_T - B_E)$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94% dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium

Flow rate: 35 cm/min.

Split ratio: 1: 20

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Perform the test with Ethanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively. The absorption spectrum determined in a 5-cm cell using water as a blank shows a flat absorption curve between 235 nm and 340 nm.

(5) Residue on evaporation—Evaporate 100 mL of Ethanol, exactly measured, in a tared dish on a water bath, and dry for 1 hour at 105°C: the mass of the residue does not exceed 2.5 mg.

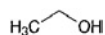
Containers and storage ♦Containers—Tight containers. ◆

Storage—Without exposure to light.

Anhydrous Ethanol

Dehydrated Alcohol

無水エタノール



$\text{C}_2\text{H}_6\text{O}$: 46.07

Ethanol [64-17-5]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Anhydrous Ethanol contains not less than 99.5 vol% (by specific gravity) of $\text{C}_2\text{H}_6\text{O}$ at 15°C.

♦**Description** Anhydrous Ethanol is a clear, colorless liquid.

It is miscible with water.

It is flammable and burns with a light blue flame on ignition.

It is volatile.

Boiling point: 78 – 79°C◆

Identification (1) Determine the infrared absorption spectrum of Anhydrous Ethanol as directed in the solution method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{4}^{15} : 0.794 – 0.797

Purity (1) Clarity and color of solution—Anhydrous Ethanol is clear and colorless. To 1.0 mL of Anhydrous Ethanol add water to make 20 mL, and allow to stand for 5 minutes: the resulting liquid is clear. Control solution: water

(2) Acidity or alkalinity—To 20 mL of Anhydrous Ethanol add 20 mL of freshly boiled and cooled water and 0.1 mL of a solution obtained by addition of 7.0 mL of ethanol (95) and 2.0 mL of water to 1.0 mL of phenolphthalein TS: no color develops. Add 1.0 mL of 0.01 mol/L sodium hydroxide VS to this solution: a light red color develops.

(3) Volatile impurities—Pipet 500 mL of Anhydrous Ethanol, add 150 μL of 4-methylpentan-2-ol, and use this solution as the sample solution. Separately, to 100 μL of anhydrous ethanol add Anhydrous Ethanol to make exactly 50 mL. Pipet 5 mL of this solution, add Anhydrous Ethanol to make exactly 50 mL, and use this solution as the standard solution (1). Separately, to exactly 50 μL each of anhydrous methanol and acetaldehyde add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to exactly 150 μL of acetal add Anhydrous Ethanol to make exactly 50 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 10 mL, and use this solution as the standard solution (3). Separately, to exactly 100 μL of benzene add Anhydrous Ethanol to make exactly 100 mL. To exactly 100 μL of this solution add Anhydrous Ethanol to make exactly 50 mL, and

use this solution as the standard solution (4). Perform the test with exactly 1 μL each of Anhydrous Ethanol, the sample solution and standard solutions (1), (2), (3) and (4) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of acetaldehyde, A_E , benzene, B_E and acetal, C_E obtained with Anhydrous Ethanol, and the peak area of methanol with the standard solution (1), the peak area of acetaldehyde, A_T with the standard solution (2), the peak area of acetal, C_T with the standard solution (3) and the peak area of benzene, B_T : the peak area of methanol is not more than 1/2 times the peak area of methanol with the standard solution (1). When calculate the amounts of the volatile impurities by the following equation, the total amount of acetaldehyde and acetal is not more than 10 vol ppm as acetaldehyde, and the amount of benzene is not more than 2 vol ppm. The total area of the peaks other than the peak mentioned above and the peak having the area not more than 3% that of 4-methylpentan-2-ol is not larger than the peak area of 4-methylpentan-2-ol.

$$\text{Total amount (vol ppm) of acetaldehyde and acetal} \\ = \{(10 \times A_E)/(A_T - A_E)\} + \{(30 \times C_E)/(C_T - C_E)\}$$

$$\text{Amount (vol ppm) of benzene} = 2B_E/(B_T - B_E)$$

If necessary, identify the peak of benzene by using a different stationary liquid phase and suitable chromatographic conditions.

Operating conditions—

Detector: A hydrogen flame-ionization detector

Column: A fused silica tube 0.32 mm in inside diameter and 30 m in length, coated with 6% cyanopropyl phenyl-94% dimethyl silicone polymer for gas chromatography in 1.8 μm thickness.

Column temperature: Inject at a constant temperature of about 40°C, maintain the temperature for 12 minutes, then rise up to 240°C at the rate of 10°C per minute, and maintain at a constant temperature of about 240°C for 10 minutes.

Carrier gas: Helium

Flow rate: 35 cm/min.

Split ratio: 1: 20

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, acetaldehyde and methanol are eluted in this order with the resolution between these peaks being not less than 1.5.

(4) Other impurities (absorbance)—Perform the test with Ethanol as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbances at 240 nm, between 250 nm and 260 nm and between 270 nm and 340 nm are not more than 0.40, 0.30, and 0.10, respectively. The absorption spectrum determined in a 5-cm cell using water as a blank shows a flat absorption curve between 235 nm and 340 nm.

(5) Residue on evaporation—Evaporate 100 mL of Dehydrated Ethanol, exactly measured, in a tared dish on a water bath, and dry for 1 hour at 105°C: the mass of the residue does not exceed 2.5 mg.

Containers and storage ♦Containers—Tight containers.◆

Storage—Without exposure to light.

Ethanol for Disinfection

Alcohol for Disinfection

消毒用エタノール

Ethanol for Disinfection contains not less than 76.9 vol% and not more than 81.4 vol% (by specific gravity) of ethanol (C₂H₆O: 46.07) at 15°C.

Method of preparation

Ethanol	830 mL
Purified Water	a sufficient quantity
To make 1000 mL	

Prepare by mixing the above ingredients.

Description Ethanol for Disinfection is a colorless, clear liquid.

It is miscible with water.

It burns with a light blue flame on ignition.

It is volatile.

Identification (1) To 1 mL of Ethanol for Disinfection add 2 mL of iodine TS and 1 mL of sodium hydroxide TS, and mix: light yellow precipitates appear.

(2) To 1 mL of Ethanol for Disinfection add 1 mL of acetic acid (100) and 3 drops of sulfuric acid, and heat: the odor of ethyl acetate is produced.

Specific gravity <2.56> d_{15}^{15} : 0.860 – 0.873

Purity Proceed as directed in the Purity under Ethanol.

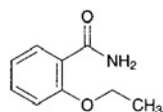
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ethenzamide

Ethoxybenzamide

エテンザミド



C₉H₁₁NO₂: 165.19

2-Ethoxybenzamide [938-73-8]

Ethenzamide, when dried, contains not less than 98.0% of C₉H₁₁NO₂.

Description Ethenzamide occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

Its saturated solution is neutral.

It begins to sublime slightly at about 105°C.

Identification (1) To 0.5 g of Ethenzamide add 5 mL of sodium hydroxide TS, and heat the mixture gently: the gas evolved turns moistened red litmus paper to blue.

(2) To 0.2 g of Ethenzamide add 10 mL of hydrobromic acid, and boil the mixture gently for 1 hour under a reflux condenser. Cool in ice water, collect the separated crystalline precipitate, wash with three 5-mL portions of ice water, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the precipitate melts <2.60> between 158°C and 161°C.

Melting point <2.60> 131 – 134°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.7 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone and 6 mL of dilute nitric acid, and dilute with water to make 50 mL (not more than 0.050%).

(2) Sulfate <1.14>—Dissolve 0.5 g of Ethenzamide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone and 1 mL of dilute hydrochloric acid, and dilute with water to 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Ethenzamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—To 0.40 g of Ethenzamide add 0.3 g of potassium nitrate and 0.5 g of anhydrous sodium carbonate, mix thoroughly, ignite the mixture gradually, and cool. Dissolve the residue in 10 mL of dilute sulfuric acid, and heat the solution until white fumes begin to evolve. After cooling, add water carefully to make 5 mL, use this solution as the test solution, and perform the test (not more than 5 ppm).

(5) Salicylamide—Dissolve 0.20 g of Ethenzamide in 15 mL of diluted ethanol (95) (2 in 3), and add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Loss on drying <2.41> Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Ethenzamide and Ethenzamide Reference Standard, previously dried, and dissolve each in 70 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 100 mL. Pipet 5 mL each of these solutions, add ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.

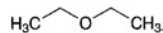
$$\begin{aligned} \text{Amount (mg) of C}_9\text{H}_{11}\text{NO}_2 \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ethenzamide Reference Standard

Containers and storage Containers—Well-closed containers.

Ether

エーテル



$\text{C}_4\text{H}_{10}\text{O}$: 74.12

Diethyl ether [60-29-7]

Ether contains not less than 96% and not more than 98% (by specific gravity) of $\text{C}_4\text{H}_{10}\text{O}$.

It contains a small quantity of ethanol and water. It cannot be used for anesthesia.

Description Ether is a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity <2.56> d_{20}^{20} : 0.718 – 0.721

Purity (1) Foreign odor—Place 10 mL of Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stoppered flask, and add 0.2 mol/L sodium hydroxide dropwise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—Place 10 mL of Ether in a Nessler tube, add 1 mL of potassium hydroxide TS, and allow the mixture to stand for 2 hours, protecting from light, with occasional shaking: no color is produced in the ether layer and the aqueous layer.

(4) Peroxide—Place 10 mL of Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake for 1 minute, then add 1 mL of starch TS, and shake well: no color is produced in the ether layer and in the aqueous layer.

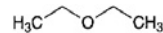
(5) Residue on evaporation—Evaporate 140 mL of Ether, and dry the residue at 105°C for 1 hour: the mass of the residue does not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Anesthetic Ether

麻醉用エーテル



$\text{C}_4\text{H}_{10}\text{O}$: 74.12

Diethyl ether [60-29-7]

Anesthetic Ether contains not less than 96% and not more than 98% (by specific gravity) of $\text{C}_4\text{H}_{10}\text{O}$.

It contains small quantities of ethanol and water. Suitable stabilizers may be added.

It is not to be used for anesthesia if it has been removed from the original container for more than 24 hours.

Description Anesthetic Ether occurs as a colorless, clear, mobile liquid, having a characteristic odor.

It is miscible with ethanol (95).

It is soluble in water.

It is highly volatile and flammable.

It is slowly oxidized by the action of air and light, with the formation of peroxides.

Its vapor, when mixed with air and ignited, may explode violently.

Boiling point: 35 – 37°C

Specific gravity <2.56> d_{20}^{20} : 0.718 – 0.721

Purity (1) Foreign odor—Place 10 mL of Anesthetic Ether in an evaporating dish, and allow it to evaporate spontaneously to a volume of about 1 mL: no foreign odor is perceptible. Drop this residue onto a piece of clean, odorless filter paper to evaporate the ether: no foreign odor is perceptible.

(2) Acidity—Place 10 mL of diluted ethanol (95) (4 in 5) and 0.5 mL of phenolphthalein TS in a 50-mL glass-stoppered flask, and add 0.2 mol/L sodium hydroxide dropwise to produce a red color which persists after shaking for 30 seconds. Add 25 mL of Anesthetic Ether, stopper the flask, shake gently, and add 0.40 mL of 0.02 mol/L sodium hydroxide VS with shaking: a red color develops.

(3) Aldehyde—To 100 mL of water in a 200-mL glass-stoppered flask add 10 mL of Anesthetic Ether and 1 mL of a solution of sodium hydrogen sulfite (1 in 1000), stopper tightly, shake vigorously for 10 seconds, and allow the mixture to stand in a cool place for 30 minutes, protected from light. Add 2 mL of starch TS, and add dropwise 0.01 mol/L iodine VS until a pale blue color develops. Shake with about 2 g of sodium hydrogen carbonate to decolorize the solution, and add 1 mL of diluted 0.01 mol/L iodine VS (9 in 40): a blue color develops. Keep the temperature of the solution below 18°C during the procedure.

(4) Peroxide—Place 10 mL of Anesthetic Ether in a Nessler tube, add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake occasionally for 1 hour, protecting from light, then add 1 mL of starch TS, and shake well: no color is produced and in the aqueous layer and in the ether layer.

(5) Residue on evaporation—Evaporate 50 mL of Anesthetic Ether, and dry the residue at 105°C for 1 hour:

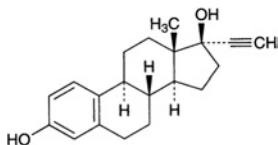
the mass of the residue is not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Storage—Without fill up, light-resistant, remote from fire, and not exceeding 25°C.

Ethinylestradiol

エチニルエストラジオール



$C_{20}H_{24}O_2$: 296.40

17 α -Ethinylestra-1,3,5(10)-triene-3,17 β -diol [57-63-6]

Ethinylestradiol, when dried, contains not less than 98.0% of $C_{20}H_{24}O_2$.

Description Ethinylestradiol occurs as white to pale yellow crystals or crystalline powder. It is odorless.

It is freely soluble in pyridine and in tetrahydrofuran, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 2 mg of Ethinylestradiol in 1 mL of a mixture of ethanol (95) and sulfuric acid (1:1): a purplish red color develops with a yellow-green fluorescence. Add carefully 2 mL of water to this solution: the color of the solution changes to red-purple.

(2) Transfer 0.02 g of Ethinylestradiol to a glass-stoppered test tube, dissolve in 10 mL of a solution of potassium hydroxide (1 in 20), add 0.1 g of benzoyl chloride, and shake. Collect the resulting precipitate, recrystallize from methanol, and dry in a desiccator (in vacuum, phosphorus (V) oxide): the precipitate melts <2.60> between 200°C and 202°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: -26 - -31° (after drying, 0.1 g, pyridine, 25 mL, 200 mm).

Melting point <2.60> 180 - 186°C or 142 - 146°C

Purity Estrone—Dissolve 5 mg of Ethinylestradiol in 0.5 mL of ethanol (95), and add 0.05 g of 1,3-dinitrobenzene. Add 0.5 mL of freshly prepared dilute potassium hydroxide-ethanol TS, allow to stand in a dark place for 1 hour, and add 10 mL of ethanol (95): the solution has no more color than the following control solution.

Control solution: Proceed in the same manner as mentioned above, omitting Ethinylestradiol.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Ethinylestradiol, previously dried, and dissolve in 40 mL of tetrahydrofuran. Add 10 mL of a solution of silver nitrate (1 in 20), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration).

Each mL of 0.1 mol/L sodium hydroxide VS

= 29.64 mg of $C_{20}H_{24}O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ethinylestradiol Tablets

エチニルエストラジオール錠

Ethinylestradiol Tablets contain not less than 90% and not more than 110% of the labeled amount of ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of preparation Prepare as directed under Tablets, with Ethinylestradiol.

Identification (1) Evaporate to dryness 5 mL of the sample solution obtained in Assay, and add 2 mL of a mixture of sulfuric acid and ethanol (95) (2:1) to the residue: a light red color with a yellow fluorescence develops. To the solution add carefully 4 mL of water: the color of the solution changes to red-purple.

(2) Evaporate to dryness 10 mL of the sample solution obtained in Assay, add 0.2 mL of acetic acid (31) and 2 mL of phosphoric acid to the residue, and heat on a water bath for 5 minutes: a red color with a yellow-green fluorescence develops.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Ethinylestradiol Tablets in a separator, add 10 mL of 2nd fluid for disintegration test, and shake until the tablet is disintegrated. Add 10 mL of dilute sulfuric acid and 20 mL of chloroform, shake vigorously for 5 minutes, and filter the chloroform layer into a conical flask through filter paper on which 5 g of anhydrous sodium sulfate is placed. Extract the aqueous layer with two 20-mL portions of chloroform, proceed with the extracts in the same manner as before, and combine the filtrates with the previous one. Evaporate gently the combined filtrate on a water bath with the aid of a current of nitrogen, dissolve the residue in exactly 100 mL of methanol, and centrifuge, if necessary. Pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about 0.04 μ g of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ethinylestradiol Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, dissolve in methanol, dilute to a volume containing about 0.04 μ g of ethinylestradiol ($C_{20}H_{24}O_2$) per mL, and use this solution as the standard solution. Pipet 4 mL each of sulfuric acid-methanol TS into three glass-stoppered test tubes, T, S and B, cool in ice, to each tube add exactly 1 mL each of the sample solution, the standard solution and methanol, shake immediately, and allow to stand in a water bath at 30°C for 40 minutes, then allow to stand in a water bath at 20°C for 5 minutes. Perform the test with these solutions as directed under Fluorometry <2.22>. Determine the fluorescence intensities, F_T , F_S and F_B , of these solutions using the fluorophotometer, at about 460 nm of the excitation and at about 493 nm of the fluorescence.

Amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$)

$$= W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (V/2500) \times (1/x)$$

W_S : Amount (mg) of Ethinylestradiol Reference Standard

Assay (i) Chromatographic tube: Pack a pledget of glass wool in the bottom of a tube 25 mm in inside diameter and 300 mm in length, and place 5 g of anhydrous sodium sulfate on the glass wool.

(ii) Chromatographic column: Place 5 g of siliceous earth for chromatography in a 200-mL beaker, soak well in 4 mL of 1 mol/L hydrochloric acid TS, and mix uniformly. Put the siliceous earth into the chromatographic tube in small portions to make 60 to 80 mm in height in proper hardness with a tamping rod.

(iii) Standard solution: Weigh accurately about 10 mg of Ethinylestradiol Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, and add chloroform to make exactly 100 mL.

(iv) Sample: Weigh accurately not less than 20 Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.5 mg of ethinylestradiol ($C_{20}H_{24}O_2$), place in a 50-mL beaker, add 2 mL of water, shake well, add 3 mL of chloroform, and shake well again. Add 4 g of siliceous earth for chromatography, mix well until the contents do not stick to the inner wall of the beaker, and use the substance as the sample.

(v) Procedure: To the chromatographic column add the sample with a funnel, and pack in proper hardness. Mix well the sample sticking to the beaker with 0.5 g of siliceous earth for chromatography, and place in the chromatographic tube. Wipe off the sample solution sticking to the beaker and the tamping rod with glass wool, and place it in the chromatographic tube. Push down the sample, and press lightly on the chromatographic column to make the height of the column 110 mm to 130 mm. Take 70 mL of chloroform, rinse the inner wall of the chromatographic tube with a portion of the chloroform, and transfer the remaining portion to the chromatographic tube. Collect the effluent solution at a flow rate not more than 0.8 mL per minute. After completing the elution, rinse the lower end of the chromatographic tube with a small quantity of chloroform, add chloroform to make exactly 100 mL, and use this solution as the sample solution. Transfer 6 mL each of the sample solution and standard solution to each separator, and add 20 mL each of iso-octane. Add exactly 10 mL of a mixture of sulfuric acid and methanol (7:3), shake vigorously for 5 minutes, allow to stand in a dark place for 15 minutes, and centrifuge. Perform the test with the resulting color solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 6 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions obtained from the sample solution and standard solution at 540 nm, respectively.

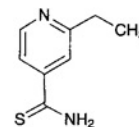
$$\begin{aligned} &\text{Amount (mg) of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2\text{)} \\ &= W_S \times (A_T/A_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Ethinylestradiol Reference Standard

Containers and storage Containers—Well-closed containers.

Ethionamide

エチオナミド



$C_8H_{10}N_2S$: 166.24

2-Ethylpyridine-4-carbothioamide [536-33-4]

Ethionamide, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{10}N_2S$.

Description Ethionamide occurs as yellow crystals or crystalline powder, having a characteristic odor.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Ethionamide in methanol (3 in 160,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethionamide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 161 – 165°C

Purity (1) Acidity—Dissolve 3.0 g of Ethionamide in 30 mL of methanol by warming, add 90 mL of water, allow to stand in ice water for 1 hour, and filter. To 80 mL of the filtrate add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ethionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ethionamide according to Method 3. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.20 g of Ethionamide in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet exactly 0.2 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography, de-

velop with a mixture of ethyl acetate, hexane and methanol (6:2:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained with the sample solution is not more intense than the spot with the standard solution (1), and number of the spot other than the principal spot obtained with the sample solution which is more intense than the spot with the standard solution (2) is not more than one.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

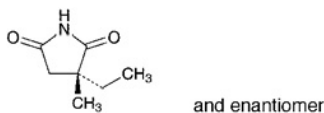
Assay Weigh accurately about 0.3 g of Ethionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.62 mg of C₈H₁₀N₂S

Containers and storage Containers—Well-closed containers.

Ethosuximide

エトスクシミド



C₇H₁₁NO₂: 141.17
(2*RS*)-2-Ethyl-2-methylsuccinimide [77-67-8]

Ethosuximide contains not less than 98.5% of C₇H₁₁NO₂ calculated on the anhydrous basis.

Description Ethosuximide occurs as a white, paraffin-like solid or powder. It is odorless or has a slight, characteristic odor.

It is very soluble in methanol, in ethanol (95), in diethyl ether, and in *N,N*-dimethylformamide, and freely soluble in water.

Melting point: about 48°C

Identification (1) To 0.2 g of Ethosuximide add 10 mL of sodium hydroxide TS, and boil: the gas evolved turns a moistened red litmus paper blue.

(2) Dissolve 0.05 g of Ethosuximide in 1 mL of ethanol (95), add 3 drops of a solution of copper (II) acetate monohydrate (1 in 100), warm slightly, and add 1 to 2 drops of sodium hydroxide TS: a purple color is produced.

(3) Determine the absorption spectrum of a solution of Ethosuximide in ethanol (95) (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Ethosuximide in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—With 1.0 g of Ethosuximide, perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Ethosuximide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ethosuximide, according to Method 1, and perform the test (not more than 2 ppm).

(5) Acid anhydride—Dissolve 0.50 g of Ethosuximide in 1 mL of ethanol (95), add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and allow to stand for 5 minutes. Add 3 mL of water, mix, and allow to stand for 5 minutes: the red to red-purple color of this solution is not more intense than that of the following control solution.

Control solution: Dissolve 0.070 g of succinic anhydride in ethanol (95) to make exactly 100 mL. To 1.0 mL of this solution add 1 mL of hydroxylammonium chloride-iron (III) chloride TS, and proceed in the same manner.

(6) Cyanide—Dissolve 1.0 g of Ethosuximide in 10 mL of ethanol (95), and add 3 drops of iron (II) sulfate TS, 1 mL of sodium hydroxide TS and 2 to 3 drops of iron (III) chloride TS. Warm gently, and acidify with dilute sulfuric acid: not a blue precipitate and a blue color are produced within 15 minutes.

Water <2.48> Not more than 0.5% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Ethosuximide, dissolve in 20 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 14.12 mg of C₇H₁₁NO₂

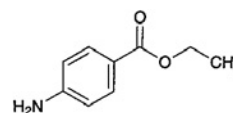
Containers and storage Containers—Tight containers.

Ethyl Aminobenzoate

Anesthamine

Benzocaine

アミノ安息香酸エチル



C₉H₁₁NO₂: 165.19
Ethyl 4-aminobenzoate [94-09-7]

Ethyl Aminobenzoate, when dried, contains not less than 99.0% of C₉H₁₁NO₂.

Description Ethyl Aminobenzoate occurs as white crystals or crystalline powder. It is odorless. It has a slightly bitter

taste, numbing the tongue.

It is freely soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 0.01 g of Ethyl Aminobenzoate in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Dissolve 0.1 g of Ethyl Aminobenzoate in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(3) Warm 0.05 g of Ethyl Aminobenzoate with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

Melting point <2.60> 89 – 91°C

Purity (1) Acidity—Dissolve 1.0 g of Ethyl Aminobenzoate in 10 mL of neutralized ethanol, and add 10 mL of water, 2 drops of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color is produced.

(2) Chloride—Dissolve 0.20 g of Ethyl Aminobenzoate in 5 mL of ethanol (95), add 2 to 3 drops each of dilute nitric acid and of silver nitrate TS: no change occurs immediately.

(3) Heavy metals <1.07>—Dissolve 2.0 g of Ethyl Aminobenzoate in 20 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and sufficient ethanol (95) to make 50 mL (not more than 10 ppm).

(4) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Ethyl Aminobenzoate: the solution has no more color than Matching Fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Ethyl Aminobenzoate, previously dried, dissolve in 10 mL of hydrochloric acid and 70 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), and cool to a temperature below 15°C. Then titrate <2.50> with 0.1 mol/L sodium nitrite VS by the potentiometric titration or the amperometric titration.

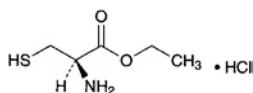
Each mL of 0.1 mol/L sodium nitrite VS
= 16.52 mg of C₅H₁₁NO₂

Containers and storage Containers—Well-closed containers.

Ethyl L-Cysteine Hydrochloride

Ethyl Cysteine Hydrochloride

L-エチルシステイン塩酸塩



C₅H₁₁NO₂S.HCl: 185.67

Ethyl (2*R*)-2-amino-3-sulfanylpropanoate
monohydrochloride
[868-59-7]

Ethyl L-Cysteine Hydrochloride, when dried, contains not less than 98.5% of C₅H₁₁NO₂S.HCl.

Description Ethyl L-Cysteine Hydrochloride occurs as white crystals or crystalline powder. It has a characteristic odor, and has a bitter taste at first with a burning aftertaste.

It is very soluble in water, and freely soluble in ethanol (95).

Melting point: about 126°C (with decomposition).

Identification (1) Determine the infrared absorption spectrum of Ethyl L-Cysteine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ethyl L-Cysteine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation <2.49> [α]_D²⁰: –10.0 – –13.0° (after drying, 2.0 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

Purity (1) Sulfate <1.14>—Perform the test with 0.6 g of Ethyl L-Cysteine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid (not more than 0.028%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ethyl L-Cysteine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure rapidly. Dissolve 0.05 g each of Ethyl L-Cysteine Hydrochloride and *N*-ethylmaleimide in 5 mL of mobile phase, allow to stand for 30 minutes, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: a peak area from the sample solution with the ratio of the retention time to ethyl L-cysteine-*N*-ethylmaleimide complex from the standard solution being about 0.7 is not larger than the peak area of ethyl L-cysteine-*N*-ethylmaleimide complex from the standard solution. Each area of all peaks other than the peaks of ethyl L-cysteine-*N*-ethylmaleimide complex and *N*-ethylmaleimide from the sample solution is not larger than 1/3 of the peak area of ethyl L-cysteine *N*-ethylmaleimide complex from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L monobasic potas-

sium phosphate TS and acetonitrile (2:1).

Flow rate: Adjust the flow rate so that the retention time of ethyl L-cysteine-*N*-ethylmaleimide complex is about 4 minutes.

Selection of column: Dissolve 0.05 g of Ethyl L-Cysteine Hydrochloride, 0.01 g of L-cysteine hydrochloride and 0.05 g of *N*-ethylmaleimide in 25 mL of the mobile phase, and allow to stand for 30 minutes. Proceed with 2 μ L of this solution under the above conditions, and calculate the resolution. Use a column giving elution of L-cysteine-*N*-ethylmaleimide complex, ethyl L-cysteine-*N*-ethylmaleimide complex and *N*-ethylmaleimide in this order, complete resolution of each component, and the resolution of the peaks of L-cysteine-*N*-ethylmaleimide complex and ethyl L-cysteine-*N*-ethylmaleimide complex being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ethyl L-cysteine-*N*-ethylmaleimide complex obtained from 2 μ L of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About 3 times as long as the retention time of ethyl L-cysteine-*N*-ethylmaleimide complex.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus oxide (V), 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

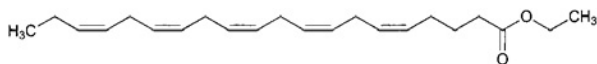
Assay Weigh accurately about 0.25 g of Ethyl L-Cysteine Hydrochloride, previously dried, transfer into a glass-stoppered flask, and dissolve in 10 mL of water previously freshly boiled and cooled to a temperature not exceeding 5°C in a stream of nitrogen. Add exactly 20 mL of 0.05 mol/L iodine VS, previously cooled to a temperature not exceeding 5°C, and allow to stand for 30 seconds, then titrate <2.50> with 0.1 mol/L sodium thiosulfate VS, on cooling below 5°C (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 18.57 mg of C₅H₁₁NO₂SHCl

Containers and storage Containers—Tight containers.

Ethyl Icosapentate

イコサペント酸エチルエステル



C₂₂H₃₄O₂: 330.50

Ethyl (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-icosa-5,8,11,14,17-pentaenoate
[86227-47-6]

Ethyl Icosapentate contains not less than 96.5% and not more than 101.0% of C₂₂H₃₄O₂.

It may contain a suitable antioxidant.

Description Icosapentate is a colorless or pale yellow, clear liquid. It has a faint, characteristic odor.

It is miscible with ethanol (99.5), with acetic acid (100) and with hexane. It is practically insoluble in water and in ethylene glycol.

Identification (1) To 20 mg of Ethyl Icosapentate add 3

mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), stopper tightly while passing a current of nitrogen, and heat at 180°C for 15 minutes. After cooling, add methanol to make 100 mL. To 4 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner as the sample solution with 3 mL of the solution of potassium hydroxide in ethylene glycol (21 in 100), as a control, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ethyl Icosapentate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethyl Icosapentate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ethyl Icosapentate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.481 – 1.491

Specific gravity <2.56> d_{20}^{20} : 0.905 – 0.915

Acid value <1.13> Not more than 0.5

Saponification value <1.13> 165 – 175

Iodine value <1.13> 365 – 395. Perform the test with 20 mg of Ethyl Icosapentate.

Purity (1) Heavy metals <1.07>—Mix 1.0 g of Ethyl Icosapentate with ethanol (99.5), and add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL. Perform the test with this solution as the test solution. Control solution: To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and ethanol (99.5) to make 50 mL (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ethyl Icosapentate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—To 0.40 g of Ethyl Icosapentate add hexane to make 50 mL, and use this solution as the sample solution. Perform the test with 1.5 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of these peaks by the area percentage method: the area of the peak, having the relative retention time of about 0.53 with respect to ethyl icosapentate, is not more than 0.5%, the areas of the peaks other than the principal peak and other than the peak mentioned above are not more than 1.0%, and the total amount of these peaks other than the principal peak is not more than 3.5%.

(the rest is omitted)

(4) Peroxide—Weigh accurately about 1 g of Ethyl Icosapentate, put in a 200-mL glass-stoppered conical flask, add 25 mL of a mixture of acetic acid (100) and chloroform (3:2), and dissolve by gentle shaking. Add 1 mL of saturated potassium iodide solution, immediately stopper tightly, shake gently, and allow to stand in a dark place for 10 minutes. Then add 30 mL of water, shake vigorously for 5 to 10 seconds, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears

after addition of 1 mL of starch TS. Calculate the amount of peroxide by the following equation: not more than 2 meq/kg.

$$\text{Amount (meq/kg) of peroxide} = (V/W) \times 10$$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed

W: Amount (g) of the sample

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Ethyl Icosapentate, and add hexane to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of Ethyl Icosapentate Reference Standard, and add hexane to make exactly 10 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ethyl icosapentate to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of ethyl icosapentate (C}_{22}\text{H}_{34}\text{O}_2) \\ = W_S \times (Q_T/Q_S) \times 5 \end{aligned}$$

W_S: Amount (mg) of Ethyl Icosapentate Reference Standard

Internal standard solution—A solution of methyl docosanate in hexane (1 in 125).

Operating conditions—

Detector: A hydrogen-flame ionization detector

Column: A glass column in 4 mm inside diameter and 1.8 m in length, packed with siliceous earth for gas chromatography (175 to 246 μ m in particle diameter), coated with diethylene glycol succinate polyester for gas chromatography in the ratio of 25%.

Column temperature: A constant temperature of about 190°C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethyl icosapentate is about 30 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating conditions, the internal standard and ethyl icosapentate are eluted in this order with the resolution between these peaks being not less than 3.

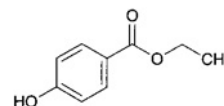
System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethyl icosapentate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Being fully filled, or replacing the air with Nitrogen.

Ethyl Parahydroxybenzoate

パラオキシ安息香酸エチル



$\text{C}_9\text{H}_{10}\text{O}_3$: 166.17

Ethyl 4-hydroxybenzoate [120-47-8]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Ethyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of $\text{C}_9\text{H}_{10}\text{O}_3$.

♦**Description** Ethyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.♦

Identification (1) The melting point <2.60> of Ethyl Parahydroxybenzoate is between 115°C and 118°C.

♦(2) Determine the infrared absorption spectrum of Ethyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.♦

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Ethyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

♦(3) Heavy metals <1.07>—Dissolve 1.0 g of Ethyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).♦

(4) Related substances—Dissolve 0.10 g of Ethyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance

of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

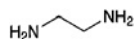
Assay Weigh accurately about 1.0 g of Ethyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point. (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 166.2 mg of C₉H₁₀O₃

◆**Containers and storage** Containers—Well-closed containers. ◆

Ethylenediamine

エチレンジアミン



C₂H₈N₂: 60.10

Ethane-1,2-diamine [107-15-3]

Ethylenediamine contains not less than 97.0% of C₂H₈N₂.

Description Ethylenediamine is a clear, colorless to pale yellow liquid. It has an ammonia-like odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It has a caustic nature and an irritating property.

It is gradually affected by air.

Specific gravity d_{20}^{20} : about 0.898

Identification (1) A solution of Ethylenediamine (1 in 500) is alkaline.

(2) To 2 mL of copper (II) sulfate TS add 2 drops of Ethylenediamine: a blue-purple color develops.

(3) To 0.04 g of Ethylenediamine add 6 drops of benzoyl chloride and 2 mL of a solution of sodium hydroxide (1 in 10), warm for 2 to 3 minutes with occasional shaking, collect the white precipitate formed, and wash with water. Dissolve the precipitate in 8 mL of ethanol (95) by warming, promptly add 8 mL of water, cool, filter the crystals, wash with water, and dry at 105°C for 1 hour: it melts <2.60> between 247°C and 251°C.

Purity (1) Heavy metals <1.07>—Place 1.0 g of Ethylenediamine in a porcelain crucible, evaporate to dryness on a water bath, cover loosely, ignite at a low temperature until charred, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Residue on evaporation—Pipet 5 mL of Ethylenediamine, heat on a water bath to dryness, and dry to constant mass at 105°C: the mass of the residue does not exceed 3.0 mg.

Distilling range <2.57> 114 – 119°C, not less than 95 vol%.

Assay Weigh accurately about 0.7 g of Ethylenediamine in a glass-stoppered conical flask, add 50 mL of water, and titrate <2.50> with 1 mol/L hydrochloric acid VS (indicator: 3 drops of bromophenol blue TS).

Each mL of 1 mol/L hydrochloric acid VS
= 30.05 mg of C₂H₈N₂

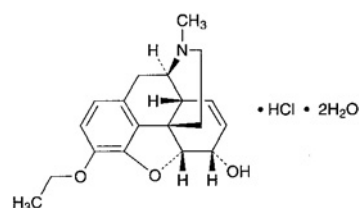
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled.

Ethylmorphine Hydrochloride Hydrate

Dionin

エチルモルヒネ塩酸塩水和物



C₁₉H₂₃NO₃.HCl.2H₂O: 385.88

(5*R*,6*S*)-4,5-Epoxy-3-ethoxy-17-methyl-

7,8-didehydromorphinan-6-ol monohydrochloride dihydrate [125-30-4, anhydride]

Ethylmorphine Hydrochloride Hydrate contains not less than 98.0% of ethylmorphine hydrochloride (C₁₉H₂₃NO₃.HCl: 349.86), calculated on the anhydrous basis.

Description Ethylmorphine Hydrochloride Hydrate occurs as white to pale yellow crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water, soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is affected by light.

Melting point: about 123°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ethylmorphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ethylmorphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ethylmorphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: -103 – -106° (0.4 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Ethylmorphine Hydrochloride

Hydrate in 10 mL of water: the pH of this solution is between 4.0 and 6.0.

Purity Related substances—Dissolve 0.20 g of Ethylmorphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 8.0 – 10.0% (0.25 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

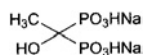
Assay Weigh accurately about 0.5 g of Ethylmorphine Hydrochloride Hydrate, and dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.99 mg of $C_{19}H_{23}NO_3 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etidronate Disodium

エチドロン酸二ナトリウム



$C_2H_6Na_2O_7P_2$: 249.99

Disodium dihydrogen (1-hydroxyethylidene)diphosphate
[7414-83-7]

Etidronate Disodium, when dried, contains not less than 98.0% and not more than 101.0% of $C_2H_6Na_2O_7P_2$.

Description Etidronate Disodium occurs as a white powder. It is freely soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 0.10 g of Etidronate Disodium in 10 mL of water is between 4.4 and 5.4.

It is hygroscopic.

Identification (1) To 5 mL of a solution of Etidronate Disodium (1 in 100) add 1 mL of copper (II) sulfate TS, and mix for 10 minutes: a blue precipitate is formed.

(2) Determine the infrared absorption spectrum of Etidronate Disodium, previously dried, as directed in the potassium bromide disk method under Infrared Spec-

trophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etidronate Disodium (1 in 100) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Etidronate Disodium according to Method 4, and perform the test using the supernatant liquid obtained by centrifuging after addition of 2 mL of dilute acetic acid. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Etidronate Disodium according to Method 1, and perform the test (not more than 2 ppm).

(3) Phosphite—Weigh accurately about 3.5 g of Etidronate Disodium, dissolve in 100 mL of 0.1 mol/L sodium dihydrogen phosphate TS adjusted the pH to 8.0 with sodium hydroxide TS, add exactly 20 mL of 0.05 mol/L iodine VS, and immediately stopper tightly. Allow to stand in a dark place for 30 minutes, add 1 mL of acetic acid (100), and titrate <2.50> the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. The amount of phosphite (NaH_2PO_3) is not more than 1.0%.

Each mL of 0.05 mol/L iodine VS = 5.199 mg of NaH_2PO_3

(4) Methanol—Weigh accurately about 0.5 g of Etidronate Disodium, dissolve in water to make exactly 5 mL, and use this solution as the sample solution. Separately, pipet 1 mL of methanol, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas of methanol, A_T and A_S , and determine the amount of methanol (CH_4O) by the following equation: not more than 0.1%.

$$\text{Amount (\% of methanol (CH}_4\text{O))} \\ = (1/W) \times (A_T/A_S) \times (1/20) \times 0.79$$

W : Amount (g) of sample

0.79: Density (g/mL) of methanol

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous copolymer beads for gas chromatography (180 – 250 μ m in particle diameter).

Column temperature: A constant temperature of about 130°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of methanol is about 2 minutes.

System suitability—

System performance: To 1 mL of methanol and 1 mL of ethanol (99.5) add water to make 100 mL. To 1 mL of this solution add water to make 100 mL. When the procedure is run with 1 μ L of this solution under the above operating conditions, methanol and ethanol are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with

1 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methanol is not more than 5.0%.

Loss on drying <2.41> Not more than 5.0% (0.5 g, 210°C, 2 hours).

Assay Weigh accurately about 0.5 g of Etidronate Disodium, previously dried, and dissolve in water to make exactly 50 mL. Transfer exactly 15 mL of this solution to a chromatographic column of 10 mm in internal diameter containing 5 mL of strongly acidic ion exchange resin for column chromatography (H type), allow to flow at a flow rate of about 1.5 mL per minute, and wash the column with two 25-mL portions of water. Combine the eluate and the washings, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.50 mg of $\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$

Containers and storage Containers—Tight containers.

Etidronate Disodium Tablets

エチドロン酸二ナトリウム錠

Etidronate Disodium Tablets contains not less than 93.0% and not more than 107.0% of the labeled amount of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$; 249.99).

Method of preparation Prepare as directed under Tablets, with Etidronate Disodium.

Identification (1) Shake an amount of pulverized Etidronate Disodium Tablets, equivalent to 0.2 g of Etidronate Disodium according to the labeled amount, with 20 mL of water, and filter. Proceed with the filtrate as directed in the Identification (1) under Etidronate Disodium.

(2) Shake an amount of pulverized Etidronate Disodium Tablets, equivalent to 0.4 g of Etidronate Disodium according to the labeled amount, with 10 mL of water, and filter. Evaporate total amount of the filtrate to dryness under reduced pressure, shake the residue with 15 mL of ethanol (99.5), centrifuge, and dry the precipitate at 150°C for 4 hours. Determine the infrared absorption spectrum of the precipitate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1170 cm^{-1} , 1056 cm^{-1} , 916 cm^{-1} and 811 cm^{-1} .

Uniformity of dosage unit <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Etidronate Disodium Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 60 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.45 μm . Discard the first 10 mL of the filtrate, take exactly V mL of the subse-

quent filtrate, add water to make exactly V' mL so that each mL contains about 0.22 mg of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of etidronate disodium for assay, previously dried at 210°C for 2 hours, and dissolve in water to make exactly 100 mL. Dilute exactly a suitable amount of this solution with water to make solutions so that each mL contains about 0.12 mg, about 0.21 mg and about 0.24 mg of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$), and use these solutions as the standard solutions. Pipet 2 mL each of the sample solution and standard solutions, add exactly 2 mL of a solution of copper (II) sulfate (7 in 10,000) and water to make exactly 10 mL. Determine the absorbances of these solutions at 233 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution prepared by diluting exactly 2 mL of the solution of copper (II) sulfate (7 in 10,000) with water to make exactly 10 mL as the control. From the calibration curve obtained with the standard solutions determine the concentration of etidronate disodium, C_T , in the sample solution. The dissolution rate of Etidronate Disodium Tablets in 60 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$)
= $C_T \times (V'/V) \times (1/C) \times 90$

C_T : Concentration ($\mu\text{g}/\text{mL}$) of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$) in the sample solution

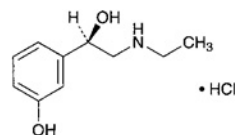
C : Labeled amount (mg) of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Etidronate Disodium Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of etidronate disodium ($\text{C}_2\text{H}_6\text{Na}_2\text{O}_7\text{P}_2$), add 30 mL of water, shake vigorously for 10 minutes, add water to make exactly 50 mL, and filter. Proceed with the filtrate as directed in the Assay under Etidronate Disodium.

Containers and storage Containers—Tight containers.

Etilefrine Hydrochloride

エチレフリン塩酸塩



$\text{C}_{10}\text{H}_{15}\text{NO}_2 \cdot \text{HCl}$: 217.69
(1*S*)-2-Ethylamino-1-(3-hydroxyphenyl)ethanol
monohydrochloride [943-17-9]

Etilefrine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of $\text{C}_{10}\text{H}_{15}\text{NO}_2 \cdot \text{HCl}$.

Description Etilefrine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in ethanol (99.5), and sparingly soluble in acetic acid (100).

It is gradually colored to yellow-brown by light.

A solution of Etilefrine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Dissolve 5 mg of Etilefrine Hydrochloride in 100 mL of diluted hydrochloric acid (1 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etilefrine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Etilefrine Hydrochloride (1 in 1000) responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 118 – 122°C

Purity (1) Acidity or alkalinity—To 10 mL of a solution of Etilefrine Hydrochloride (1 in 50) add 0.1 mL of methyl red TS for acid or alkali test and 0.2 mL of 0.01 mol/L sodium hydroxide VS: a yellow color develops, and the necessary volume of 0.01 mol/L hydrochloric acid VS to change the color to red is not more than 0.4 mL.

(2) Clarity and color of solution—Dissolve 0.5 g of Etilefrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(3) Sulfate <1.14>—Perform the test with 0.85 g of Etilefrine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.020%).

(4) Heavy metals <1.07>—Dissolve 1.0 g of Etilefrine Hydrochloride in 30 mL of water and 2 mL of acetic acid (100), adjust with sodium hydroxide TS to a pH of 3.3, add water to make 50 mL, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Etilefrine Hydrochloride, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.77 mg of $C_{10}H_{15}NO_2 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Etilefrine Hydrochloride Tablets

エチレフリン塩酸塩錠

Etilefrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled

amount of etilefrine hydrochloride ($C_{10}H_{15}NO_2 \cdot HCl$: 217.69).

Method of preparation Prepare as directed under Tablets, with Etilefrine Hydrochloride.

Identification To a quantity of powdered Etilefrine Hydrochloride Tablets, equivalent to 5 mg of Etilefrine Hydrochloride according to the labeled amount, add 60 mL of diluted hydrochloric acid (1 in 1000), shake well, add 40 mL of diluted hydrochloric acid (1 in 1000), and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>, using diluted hydrochloric acid (1 in 1000) as the blank: it exhibits a maximum between 271 nm and 275 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Etilefrine Hydrochloride Tablets add 60 mL of diluted hydrochloric acid (1 in 1000), and proceed as directed in the Assay.

Amount (mg) of etilefrine hydrochloride ($C_{10}H_{15}NO_2 \cdot HCl$)
= $W_s \times (A_T/A_S) \times (1/10)$

W_s : Amount (mg) of etilefrine hydrochloride for assay

Assay Weigh accurately the mass of not less than 20 Etilefrine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of etilefrine hydrochloride ($C_{10}H_{15}NO_2 \cdot HCl$), add 60 mL of diluted hydrochloric acid (1 in 1000), shake for 10 minutes, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of etilefrine hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in diluted hydrochloric acid (1 in 1000) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted hydrochloric acid (1 in 1000) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of etilefrine.

Amount (mg) of etilefrine hydrochloride ($C_{10}H_{15}NO_2 \cdot HCl$)
= $W_s \times (A_T/A_S) \times (1/10)$

W_s : Amount (mg) of etilefrine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 5 g of sodium lauryl sulfate in 940 mL of water and 500 mL of acetonitrile, and adjust the pH to 2.3 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of etilefrine is about 6 minutes.

System suitability—

System performance: Dissolve 4 mg of bamethan sulfate

and 4 mg of etilefrine hydrochloride in the mobile phase to make 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, etilefrine and bamethan are eluted in this order with the resolution between these peaks being not less than 5.

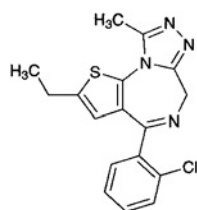
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etilefrine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etizolam

エチゾラム



$C_{17}H_{15}ClN_4S$: 342.85

4-(2-Chlorophenyl)-2-ethyl-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine [40054-69-1]

Etizolam contains not less than 98.5% and not more than 101.0% of etizolam ($C_{17}H_{15}ClN_4S$).

Description Etizolam occurs as a white to pale yellowish white crystalline powder.

It is soluble in ethanol (99.5), sparingly soluble in acetonitrile and in acetic anhydride, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Etizolam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etizolam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 146 – 149°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Etizolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Etizolam in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 20 mL. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed

under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etizolam obtained from the sample solution is not more than the peak area of etizolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). To 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etizolam is about 6 minutes.

Time span of measurement: About 5 times as long as the retention time of etizolam beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of etizolam obtained from 10 μ L of this solution is equivalent to 8 to 12% of that from 10 μ L of the standard solution.

System performance: Dissolve 0.02 g each of Etizolam and ethyl parahydroxybenzoate in the mobile phase to make 50 mL. To 1 mL of this solution add the mobile phase to make 50 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, ethyl parahydroxybenzoate and etizolam are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etizolam is not more than 2%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Etizolam, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

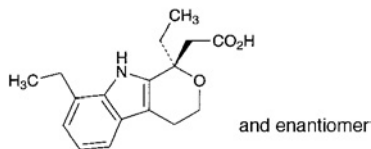
Each mL of 0.1 mol/L perchloric acid VS
= 17.14 mg of $C_{17}H_{15}ClN_4S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Etodolac

エトドラク



$C_{17}H_{21}NO_3$: 287.35

2-[(1*R,S*)-1,8-Diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indol-1-yl]acetic acid

[41340-25-4]

Etodolac, when dried, contains not less than 98.5% and not more than 101.0% of $C_{17}H_{21}NO_3$.

Description Etodolac occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

A solution of Etodolac in methanol (1 in 50) shows no optical rotation.

Melting point: about 147°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Etodolac in ethanol (99.5) (3 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etodolac as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Etodolac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.5 g of Etodolac in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 4 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Previously develop a plate of silica gel with fluorescent indicator for thin-layer chromatography in a developing container containing 2 cm depth of a solution of L-ascorbic acid in a mixture of methanol and water (4:1) (1 in 200 mL) to the distance of 3 cm, and air-dry for 30 minutes. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on the plate 2.5 cm away from the bottom of the plate, then immediately develop with a mixture of toluene, ethanol (95) and acetic acid (100) (140:60:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot

obtained with the sample solution is not more intense than the spot with the standard solution (1), and the number of spots which are more intense than the spot with the standard solution (2) is not more than 2.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Etodolac, previously dried, dissolve in 50 mL of ethanol (99.5), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

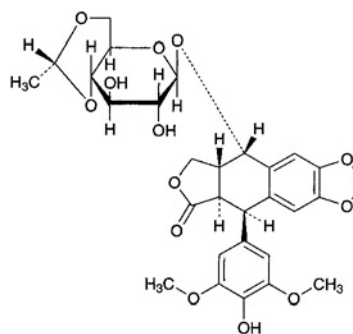
Each mL of 0.1 mol/L sodium hydroxide VS

= 28.74 mg of $C_{17}H_{21}NO_3$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Etoposide

エトポシド



$C_{29}H_{32}O_{13}$: 588.56

(5*R*,5*aR*,8*aR*,9*S*)-9-{[4,6-*O*-(1*R*)-Ethylidene- β -D-glucopyranosyl]oxy}-5-(4-hydroxy-3,5-dimethoxyphenyl)-5,8,8*a*,9-tetrahydrofuro[3',4':6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5*aH*)-one [33419-42-0]

Etoposide contains not less than 98.0% and not more than 102.0% of $C_{29}H_{32}O_{13}$, calculated on the anhydrous basis.

Description Etoposide occurs as white crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Melting point: about 260°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Etoposide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Etoposide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Etoposide as directed in the potassium bromide disk method

under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Etoposide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -100 – -105° (0.1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Etoposide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Etoposide in 10 mL of methanol, add the mobile phase to make 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than etoposide is not larger than 1/5 times the peak area of etoposide with the standard solution, and the total area of the peaks other than the peak of etoposide with the sample solution is not larger than 1/2 times the peak area of etoposide with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of etoposide beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of etoposide obtained with 50 μ L of this solution is equivalent to 7 to 13% of that with 50 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of etoposide is not more than 2.0%.

Water <2.48> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Etoposide and Etoposide Reference Standard (previously determined the water <2.48> in the same manner as Etoposide) dissolve separately in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 5 mL of the internal standard solution and the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of etoposide to that of the internal standard.

$$\text{Amount (mg) of } C_{29}H_{32}O_{13} = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Etoposide Reference Standard,

calculated on the anhydrous basis

Internal standard solution—A solution of 2,6-dichlorophenol in methanol (3 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 290 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with phenylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 6.44 g of sodium sulfate decahydrate in diluted acetic acid (100) (1 in 100) to make 1000 mL, and add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of etoposide is about 20 minutes.

System suitability—

System performance: Dissolve 10 mg of Etoposide in 2 mL of methanol, add 8 mL of the mobile phase, and mix well. Add 0.1 mL of diluted acetic acid (100) (1 in 25) and 0.1 mL of phenolphthalein TS, and add sodium hydroxide TS until the color of the solution changes to faintly red. After allowing to stand for 15 minutes, add 0.1 mL of diluted acetic acid (100) (1 in 25). When the procedure is run with 10 μ L of this solution under the above operating conditions, the resolution between the peak of etoposide and the peak having the relative retention time of about 1.3 with respect to etoposide is not less than 3.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of etoposide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Eucalyptus Oil

Oleum Eucalypti

ユーカリ油

Eucalyptus Oil is the essential oil distilled with steam from the leaves of *Eucalyptus globulus* Labillardière or allied plants (*Myrtaceae*).

It contains not less than 70.0% of cineol ($C_{10}H_{18}O$: 154.25).

Description Eucalyptus Oil is a clear, colorless or pale yellow liquid. It has a characteristic, aromatic odor and a pungent taste.

It is neutral.

Identification Shake 1 mL of Eucalyptus Oil vigorously with 1 mL of phosphoric acid, and allow to stand: the solution congeals within 30 minutes.

Refractive index <2.45> n_D^{20} : 1.458 – 1.470

Specific gravity <1.13> d_{20}^{20} : 0.907 – 0.927

Purity (1) Clarity of solution—Mix 1.0 mL of Eucalyptus

Oil with 5 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Heavy metals <1.07>—Proceed with 1.0 mL of Eucalyptus Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Weigh accurately about 0.1 g of Eucalyptus Oil, and dissolve in hexane to make exactly 25 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add hexane to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of cineol for assay, proceed as directed in the sample solution, and use this solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios, Q_T and Q_S , of the peak area of cineol to that of the internal standard of each solutions, respectively.

$$\begin{aligned} &\text{Amount (mg) of cineol (C}_{10}\text{H}_{18}\text{O)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of cineol for assay

Internal standard solution—A solution of anisol in hexane (1 in 250).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 5 m in length, having alkylene glycol phthalate ester for gas chromatography coated at the ratio of 10% on silanized siliceous earth for gas chromatography (150 to 180 μ m in particle diameter).

Column temperature: A constant temperature of about 120°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of cineol is about 11 minutes.

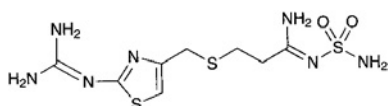
Selection of column: Dissolve 0.1 g each of cineol and limonene in 25 mL of hexane. To 1 mL of this solution add hexane to make 20 mL. Proceed with about 2 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of limonene and cineol in this order with the resolution between these peaks being not less than 1.5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Famotidine

ファミチジン



$\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$: 337.45

N-Aminosulfonyl-3-[(2-diaminomethyleneamino)-1,3-thiazol-4-yl]methylsulfanylpropanimidamide
[76824-35-6]

Famotidine, when dried, contains not less than

98.5% of $\text{C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3$.

Description Famotidine occurs as white to yellowish white crystals.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in 0.5 mol/L hydrochloric acid TS.

It is gradually colored by light.

Melting point: about 164°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Famotidine in 0.05 mol/L potassium dihydrogenphosphate TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Famotidine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Famotidine in 10 mL of 0.5 mol/L hydrochloric acid TS: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Famotidine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.20 g of Famotidine in 10 mL of acetic acid (100), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetic acid (100) to make exactly 100 mL. Pipet 1 mL, 2 mL and 3 mL of this solution, add acetic acid (100) to make exactly 10 mL, respectively, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel (5 to 7 μ m) with fluorescent indicator for thin-layer chromatography, and dry in a stream of nitrogen.

Develop the plate with a mixture of ethyl acetate, methanol, toluene and ammonia solution (28) (40:25:20:2) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and other than the spot of the starting point from the sample solution are not more intense than the spot from the standard solution (3). Total intensity of the spots other than the principal spot and other than the spot of the starting point from the sample solution is not more than 0.5% calculated on the basis of intensities of the spots from the standard solution (1) and the standard solution (2) (each spot is equivalent to 0.1% and 0.2%, respectively).

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Famotidine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.87 mg of $C_8H_{15}N_7O_2S_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Famotidine for Injection

注射用ファモチジン

Famotidine for Injection is a preparation for injection which is dissolved before use.

It contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$; 337.45).

Method of preparation Prepare as directed under Injection, with Famotidine.

Description Famotidine for Injection occurs as white porous masses or powder.

Identification Dissolve an amount of Famotidine for Injection, equivalent to 0.01 g of Famotidine according to the labeled amount, in 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS. To 5 mL of this solution add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>; it exhibits a maximum between 263 nm and 267 nm.

pH <2.54> Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine according to the labeled amount, in 1 mL of water: the pH of this solution is between 4.9 and 5.5.

Purity (1) Clarity and color of solution—Dissolve an amount of Famotidine for Injection, equivalent to 0.02 g of Famotidine according to the labeled amount, in 1 mL of water: the solution is clear and colorless.

(2) Related substances—Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of the container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than peak of famotidine from the sample solution is not larger than peak area of famotidine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of famotidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the water to make exactly 20 mL. Confirm that the peak area of famotidine obtained from 5 μ L of this solution is equivalent to 8 to 12% of that of famotidine obtained from 5 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of famotidine is not more than 2.0%.

Water <2.48> Not more than 1.5% (0.1 g, coulometric titration).

Bacterial endotoxins <4.01> Not more than 15 EU/mg.

Assay Take a number of Famotidine for Injection, equivalent to about 0.1 g of famotidine ($C_8H_{15}N_7O_2S_3$), dissolve each content in water, wash the inside of each container with water, combine the solutions of the contents with the washings, add water to the combined solution to make exactly 100 mL, and use this solution as the sample stock solution. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of famotidine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of famotidine (C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3\text{)} \\ & = W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating condi-

tions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Famotidine Powder

ファミモチジン散

Famotidine Powder contains not less than 94.0% and not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$; 337.45).

Method of preparation Prepare as directed under Powder, with Famotidine.

Identification Weigh a portion of Famotidine Powder, equivalent to 0.01 g of Famotidine according to the labeled amount, add 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Weigh accurately an amount of Famotidine Powder, equivalent to about 20 mg of famotidine ($C_8H_{15}N_7O_2S_3$) according to the labeled amount, and perform the test at 50 revolutions per minute according to the Paddle method, using 900 mL of 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 as the dissolution medium. Withdraw not less than 20 mL of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.5 μ m. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of famotidine for assay, previously dried in vacuum on phosphorus (V) oxide at 80°C for 4 hours, dissolve in 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 to make exactly 100 mL. Pipet 5 mL of this solution, add the same buffer solution to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 266 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of a 20-mg/g powder and a 100-mg/g powder in 15 minutes of Famotidine Powder are not less than 80% and not less than 85%, respectively.

Dissolution rate (%) with respect to the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$)

$$= (W_S/W_T) \times (A_T/A_S) \times (1/C) \times 45$$

W_S : Amount (mg) of famotidine for assay

W_T : Amount (mg) of the sample.

C: Labeled amount (mg) of famotidine ($C_8H_{15}N_7O_2S_3$) in

1 g

Assay Weigh accurately a portion of Famotidine Powder, equivalent to about 20 mg of famotidine ($C_8H_{15}N_7O_2S_3$), add 20 mL of water, and shake well. Add 20 mL of methanol, then shake well, add methanol to make exactly 50 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 50 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of famotidine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of famotidine (} C_8H_{15}N_7O_2S_3 \text{)} \\ &= W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Famotidine Tablets

ファミモチジン錠

Famotidine Tablets contain not less than 94.0% and

not more than 106.0% of the labeled amount of famotidine ($C_8H_{15}N_7O_2S_3$; 337.45).

Method of preparation Prepare as directed under Tablets, with Famotidine.

Identification Weigh a portion of powdered Famotidine Tablets, equivalent to 0.01 g of Famotidine according to the labeled amount, add 50 mL of 0.05 mol/L potassium dihydrogenphosphate TS, shake well, and centrifuge. To 5 mL of the supernatant liquid add 0.05 mol/L potassium dihydrogenphosphate TS to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 263 nm and 267 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Famotidine Tablets add 2 mL of water, shake to disintegrate, then add a suitable amount of methanol, and shake well. Add methanol to make exactly V mL of a solution containing about 0.2 mg of famotidine ($C_8H_{15}N_7O_2S_3$) per mL, and centrifuge. Pipet 10 mL of the supernatant liquid, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 2 mL of the internal standard solution, add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the operating conditions described in the Assay, and calculate the ratios, Q_T and Q_S , of the peak area of famotidine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of famotidine (C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3\text{)} \\ &= W_S \times (Q_T/Q_S) \times (V/500) \end{aligned}$$

W_S : Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Dissolution Being specified separately.

Assay Take a number of Famotidine Tablets, equivalent to 0.2 g of famotidine ($C_8H_{15}N_7O_2S_3$), add 50 mL of water, and disintegrate by shaking well. Add 100 mL of methanol, then shake well, add methanol to make exactly 200 mL, and centrifuge. Pipet 5 mL of the supernatant liquid, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of famotidine for assay, previously dried in vacuum with phosphorus (V) oxide at 80°C for 4 hours, dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios,

Q_T and Q_S , of the peak area of famotidine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of famotidine (C}_8\text{H}_{15}\text{N}_7\text{O}_2\text{S}_3\text{)} \\ &= W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of famotidine for assay

Internal standard solution—To 5 mL of a solution of methyl parahydroxybenzoate in methanol (1 in 500) add water to make 50 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2 g of sodium 1-heptane sulfonate in 900 mL of water, adjust to pH 3.0 with acetic acid (100), and add water to make 1000 mL. To this solution add 240 mL of acetonitrile and 40 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of famotidine is about 6 minutes.

System suitability—

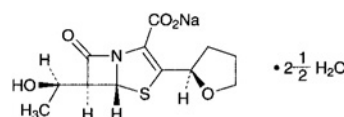
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, famotidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 11.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of famotidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Faropenem Sodium Hydrate

ファロペネムナトリウム水和物



$C_{12}H_{14}NNaO_5S \cdot \frac{1}{2}H_2O$: 352.34

Monosodium (5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-[(2*R*)-tetrahydrofuran-2-yl]-4-thia-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate hemipentahydrate [122547-49-3, anhydride]

Faropenem Sodium Hydrate contains not less than 870 μ g (potency) and not more than 943 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Faropenem Sodium Hydrate is expressed as mass (potency) of faropenem ($C_{12}H_{15}NO_5S$; 285.32).

Description Faropenem Sodium Hydrate occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in water and in methanol, slightly solu-

ble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 5 mg of Faropenem Sodium Hydrate in 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown to brown color develops.

(2) Determine the absorption spectra of solutions of Faropenem Sodium Hydrate and Faropenem Sodium Reference Standard (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Faropenem Sodium Hydrate and Faropenem Sodium Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +145 – +150° (0.5 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Faropenem Sodium Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Being specified separately.

Water <2.48> Not less than 12.6% and not more than 13.1% (20 mg, coulometric titration).

Assay Weigh accurately an amount of Faropenem Sodium Hydrate and Faropenem Sodium Reference Standard, equivalent to about 25 mg (potency), each of these, add exactly 10 mL each of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of faropenem to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of faropenem (C}_{12}\text{H}_{15}\text{NO}_5\text{S)} \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : amount (mg) of Faropenem Sodium Reference Standard

Internal standard solution—Dissolve 0.5 g of *m*-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 305 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.8 g of potassium dihydrogenphosphate, 5.4 g of disodium hydrogen phosphate dodecahydrate and 1.0 g of tetra *n*-butyl ammonium bromide in water

to make 1000 mL. To 870 mL of this solution add 130 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of faropenem is about 11 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and faropenem are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of faropenem to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Faropenem Sodium for Syrup

シロップ用ファロペネムナトリウム

Faropenem Sodium for Syrup is a preparation for syrup, which is dissolved before use. It contains not less than 93.0% and not more than 106.0% of the labeled amount of faropenem (C₁₂H₁₅NO₅S: 285.32).

Method of preparation Prepare as directed under Syrups, with Faropenem Sodium Hydrate.

Identification Dissolve an amount of pulverized Faropenem Sodium for Syrup, equivalent to 25 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, in 50 mL of water. To 5 mL of this solution add water to make 50 mL, centrifuge, if necessary, and determine the absorption spectrum of the solution so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 254 nm and 258 nm, and between 304 nm and 308 nm.

Water <2.48> Not less than 1.5% and not more than 2.1% (80 mg, coulometric titration).

Uniformity of dosage units <6.02> Faropenem Sodium for Syrup in single-unit container meets the requirement of the Mass variation test.

Assay Powder, if necessary, and weigh accurately an amount of Faropenem Sodium for Syrup, equivalent to about 25 mg (potency) of faropenem (C₁₂H₁₅NO₅S), add exactly 10 mL of the internal standard solution and a suitable amount of water, shake well, and add water to make 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium Reference Standard, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Faropenem Sodium Hydrate.

$$\begin{aligned} &\text{Amount [mg (potency)] of faropenem (C}_{12}\text{H}_{15}\text{NO}_5\text{S)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount [mg (potency)] of Faropenem Sodium Reference Standard

Internal standard solution—Dissolve 0.5 g of *m*-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Faropenem Sodium Tablets

ファロペネムナトリウム錠

Faropenem Sodium Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of faropenem ($C_{12}H_{15}NO_5S$; 285.32).

Method of preparation Prepare as directed under Tablets, with Faropenem Sodium Hydrate.

Identification To pulverized Faropenem Sodium Tablets, equivalent to 70 mg (potency) of Faropenem Sodium Hydrate according to the labeled amount, add water to make 100 mL. To 5 mL of this solution add water to make 100 mL, filter, if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 254 nm and 258 nm and between 304 nm and 308 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Faropenem Sodium Tablets add 180 mL of water, shake vigorously until the tablets are disintegrated, and add water to make exactly V mL so that each mL contains about 1 mg (potency) of Faropenem Sodium Hydrate. Pipet 5 mL of this solution, add water to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium Reference Standard, and dissolve in water to make exactly 50 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_{T275} , A_{T305} , A_{T354} , A_{S275} , A_{S305} and A_{S354} , of the sample solution and standard solution at 275 nm, 305 nm and 354 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate A_T and A_S , using the following equations.

$$A_T = A_{T305} - (49 \times A_{T275} + 30 \times A_{T354}) / 79$$

$$A_S = A_{S305} - (49 \times A_{S275} + 30 \times A_{S354}) / 79$$

$$\text{Amount [mg (potency)] of faropenem (C}_{12}\text{H}_{15}\text{NO}_5\text{S)} \\ = W_S \times (A_T / A_S) \times (V / 25)$$

W_S : Amount [mg (potency)] of Faropenem Sodium Reference Standard

Assay Weigh accurately the mass of not less than 5 Faropenem Sodium Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg (potency) of faropenem ($C_{12}H_{15}NO_5S$), add exactly 10 mL of the internal standard solution, shake well, and add water to make exactly 50 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg (potency) of Faropenem Sodium

Hydrate Reference Standard, add exactly 10 mL of the internal standard solution and water to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Faropenem Sodium Hydrate.

$$\text{Amount [mg (potency)] of faropenem (C}_{12}\text{H}_{15}\text{NO}_5\text{S)} \\ = W_S \times (Q_T / Q_S)$$

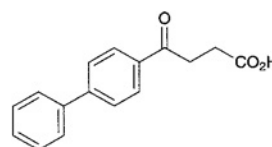
W_S : Amount [mg (potency)] of Faropenem Sodium Hydrate Reference Standard

Internal standard solution—Dissolve 0.5 g of *m*-hydroxyacetophenone in 20 mL of acetonitrile, and add water to make 200 mL.

Containers and storage Containers—Tight containers.

Fenbufen

フェンブフェン



$C_{16}H_{14}O_3$; 254.28
4-(Biphenyl-4-yl)-4-oxobutanoic acid [36330-85-5]

Fenbufen, when dried, contains not less than 98.0% of $C_{16}H_{14}O_3$.

Description Fenbufen occurs as a white crystalline powder. It has a bitter taste.

It is sparingly soluble in acetone, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Melting point: about 188°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Fenbufen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fenbufen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Take 2.0 g of Fenbufen, add 2 mL of sulfuric acid, and carbonize by gentle heating, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fenbufen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.1 g of Fenbufen in 20 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution.

Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (80:20:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

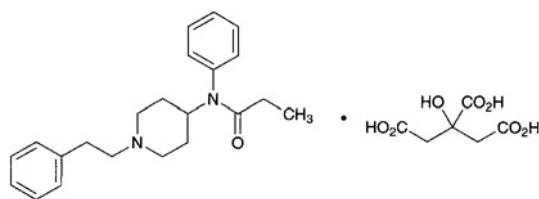
Assay Weigh accurately about 0.2 g of Fenbuten, previously dried, dissolve in 100 mL of ethanol (99.5), and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 25.43 mg of $\text{C}_{16}\text{H}_{14}\text{O}_3$

Containers and storage Containers—Tight containers.

Fentanyl Citrate

フェンタニルクエン酸塩



$\text{C}_{22}\text{H}_{28}\text{N}_2\text{O} \cdot \text{C}_6\text{H}_8\text{O}_7$; 528.59

N-(1-Phenethylpiperidin-4-yl)-*N*-phenylpropanamide monocitrate [990-73-8]

Fentanyl Citrate contains not less than 98.0% of $\text{C}_{22}\text{H}_{28}\text{N}_2\text{O} \cdot \text{C}_6\text{H}_8\text{O}_7$, calculated on the dried basis.

Description Fentanyl Citrate occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in water and in ethanol (95), and very slightly soluble in diethyl ether.

Identification (1) Dissolve 0.05 g of Fentanyl Citrate in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Fentanyl Citrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Fentanyl Citrate (1 in 100) responds to

the Qualitative Tests <1.09> (1) for citrate.

pH <2.54> Dissolve 0.10 g of Fentanyl Citrate in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Melting point <2.60> 150 – 154°C

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Fentanyl Citrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Fentanyl Citrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.2 g, in vacuum, silica gel, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.075 g of Fentanyl Citrate, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS = 10.57 mg of $\text{C}_{22}\text{H}_{28}\text{N}_2 \cdot \text{C}_6\text{H}_8\text{O}_7$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Ferrous Sulfate Hydrate

硫酸鉄水和物

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 278.01

Ferrous Sulfate Hydrate contains not less than 98.0% and not more than 104.0% of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

Description Ferrous Sulfate Hydrate occurs as pale green crystals or crystalline powder. It is odorless, and has an astringent taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is efflorescent in dry air, and its surface becomes yellowish brown in moist air.

Identification A solution of Ferrous Sulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for ferrous salt and for sulfate.

Purity (1) Clarity of solution—Dissolve 1.0 g of Ferrous Sulfate Hydrate in 20 mL of water and 1 mL of dilute sulfuric acid: the solution is clear.

(2) Acidity—To 5.0 g of powdered Ferrous Sulfate Hydrate add 50 mL of ethanol (95), shake well for 2 minutes, and filter the mixture. To 25 mL of the filtrate add 50 mL of water, 3 drops of bromothymol blue TS and 0.5 mL of dilute sodium hydroxide TS: a blue color develops.

(3) Heavy metals <1.07>—Take 1.0 g of Ferrous Sulfate Hydrate in a porcelain dish, add 3 mL of aqua regia, and dissolve. Then evaporate on a water bath to dryness. To the residue add 5 mL of 6 mol/L hydrochloric acid TS, and dissolve. Transfer this solution to a separator. Wash the porcelain dish with two 5-mL portions of 6 mol/L hydrochloric acid TS, and combine the washings and the solution in the separator. Pour two 40-mL portions and one 20-mL portion of diethyl ether in the separator, shaking each time to mix. Allow to stand, and discard each separated diethyl ether layer. To the aqueous layer add 0.05 g of hydroxylammonium chloride, dissolve, and heat on a water bath for 10 minutes. Cool, adjust the solution to a pH of 3 to 4 by dropping strong ammonia solution, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: take 2.5 mL of Standard Lead Solution in a porcelain dish, add 3 mL of aqua regia, and proceed as directed for the preparation of the test solution (not more than 25 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ferrous Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

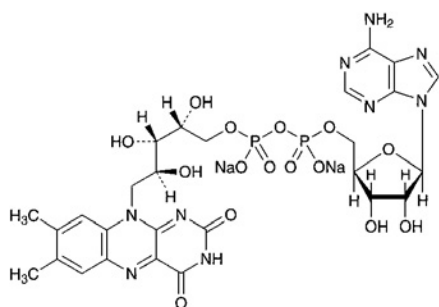
Assay Dissolve about 0.7 g of Ferrous Sulfate Hydrate, accurately weighed, in a mixture of 20 mL of water and 20 mL of dilute sulfuric acid, add 2 mL of phosphoric acid, and immediately titrate <2.50> with 0.02 mol/L potassium permanganate VS.

Each mL of 0.02 mol/L potassium permanganate VS
= 27.80 mg of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

Containers and storage Containers—Tight containers.

Flavin Adenine Dinucleotide Sodium

フラビンアデニンジヌクレオチドナトリウム



$\text{C}_{27}\text{H}_{31}\text{N}_9\text{Na}_2\text{O}_{15}\text{P}_2$: 829.51
Disodium adenosine 5'-[(2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl diphosphate] [84366-81-4]

Flavin Adenine Dinucleotide Sodium contains not less than 93.0% of $\text{C}_{27}\text{H}_{31}\text{N}_9\text{Na}_2\text{O}_{15}\text{P}_2$, calculated on

the anhydrous basis.

Description Flavin Adenine Dinucleotide Sodium occurs as an orange-yellow to light yellow-brown powder. It is odorless or has a slight, characteristic odor, and has a slightly bitter taste.

It is freely soluble in water, and practically insoluble, in methanol, in ethanol (95), in ethyleneglycol and in diethyl ether.

It is hygroscopic.

It is decomposed by light.

Identification (1) A solution of Flavin Adenine Dinucleotide Sodium (1 in 100,000) is light yellow-green in color, and shows a strong yellow-green fluorescence. To 5 mL of the solution add 0.02 g of hydrosulfite sodium: the color and the fluorescence of the solution disappear, and gradually reappear when the solution is shaken in air. Add dilute hydrochloric acid or sodium hydroxide TS dropwise: the fluorescence of the solution disappears.

(2) Determine the infrared absorption spectrum of Flavin Adenine Dinucleotide Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.1 g of Flavin Adenine Dinucleotide Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. To the residue add 10 mL of diluted nitric acid (1 in 50), boil for 5 minutes, and after cooling, neutralize with ammonia TS, then filter the solution if necessary: the solution responds to the Qualitative Tests <1.09> for sodium salt and the Qualitative Tests <1.09> (1) and (3) for phosphate.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: $-21.0 - -25.5^\circ$ (0.3 g, calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Flavin Adenine Dinucleotide Sodium in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Flavin Adenine Dinucleotide Sodium in 10 mL of water: the solution is clear and orange-yellow in color.

(2) Free phosphoric acid—Weigh accurately about 0.02 g of Flavin Adenine Dinucleotide Sodium, dissolve in 10 mL of water, and use this solution as the sample solution. Separately, measure exactly 2 mL of Standard Phosphoric Acid Solution, add 10 mL of water, and use this solution as the standard solution. To each of the sample solution and standard solution add 2 mL of diluted perchloric acid (100 in 117), then add 1 mL of hexaammonium heptamolybdate TS and 2 mL of 2,4-diaminophenol hydrochloride TS, respectively, shake, add water to make exactly 25 mL, and allow to stand at $20 \pm 1^\circ\text{C}$ for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with 2 mL of water, as the blank, and determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution at 730 nm, respectively: the amount of free phosphoric acid is less than 0.25%.

Amount (%) of free phosphoric acid (H_3PO_4)
= $(A_T/A_S) \times (1/W) \times 5.16$

W: Amount (mg) of flavin adenine dinucleotide sodium,

calculated on the anhydrous basis.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Flavin Adenine Dinucleotide Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 2.0 g of Flavin Adenine Dinucleotide Sodium according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.10 g of Flavin Adenine Dinucleotide Sodium in 200 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A , of flavin adenine dinucleotide and the total area, S , of peaks other than the peak of flavin adenine dinucleotide by the automatic integration method: $S/(A + S)$ is not more than 0.10.

Operating conditions—

Column, column temperature, mobile phase, flow rate, and time span of measurement: Proceed as directed in the operating conditions in the Procedure (ii) under Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 20 mL, and use this solution as the solution for system suitability test. Confirm that the peak area of flavin adenine dinucleotide obtained from 20 μ L of this solution is equivalent to 8 to 12% of that of flavin adenine dinucleotide obtained from 20 μ L of the sample solution.

System performance: Proceed as directed in the system suitability in the Procedure (ii) under Assay (1).

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

Water <2.48> Take 50 mL of a mixture of methanol for Karl Fischer method and ethyleneglycol for Karl Fischer method (1:1) into a dry titration flask, and titrate with Karl Fischer TS until end point. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, transfer quickly to the titration flask, add an excess and constant volume of Karl Fischer TS, dissolve by stirring for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay (1) Procedure (i) Total flavin content—Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.1 g of Flavin Adenine Dinucleotide Sodium, and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution, add 5 mL of zinc chloride TS, and heat in a water bath for 30 minutes. After cooling, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, dissolve in 200 mL of diluted acetic acid (100) (1 in 100) by warming, cool, add water to make exactly 500 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 450 nm as

directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\begin{aligned} \text{Total amount (mg) of flavin} \\ = W_S \times (A_T/A_S) \times (4/5) \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

(ii) Peak area ratio of flavin adenine dinucleotide—Dissolve 0.1 g of Flavin Adenine Dinucleotide Sodium in 200 mL of water, and use this solution as the sample solution. Perform the test with 5 μ L of this solution as directed under the Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A of flavin adenine dinucleotide, and the total area, S , of the peaks other than flavin adenine dinucleotide by the automatic integration method.

$$\begin{aligned} \text{Peak area ratio of flavin adenine dinucleotide} \\ = 1.08A/(1.08A + S) \end{aligned}$$

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 450 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (1 in 500) and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of flavin adenine dinucleotide is about 10 minutes.

Time span of measurement: About 4.5 times as long as the retention time of flavin adenine dinucleotide.

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add water to make exactly 20 mL, and use this solution as the solution for system suitability test. Pipet 2 mL of the solution, and add water to make exactly 20 mL. Confirm that the peak area of flavin adenine dinucleotide obtained from 5 μ L of this solution is equivalent to 8 to 12% of that of flavin adenine dinucleotide obtained from 5 μ L of the solution for system suitability test.

System performance: Dissolve 20 mg each of Flavin Adenine Dinucleotide Sodium and riboflavin sodium phosphate in 100 mL of water. When the procedure is run with 5 μ L of this solution under the above operating conditions, flavin adenine dinucleotide and riboflavin phosphate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of flavin adenine dinucleotide is not more than 1.0%.

(2) Calculation

$$\begin{aligned} \text{Amount (mg) of } C_{27}H_{31}N_9Na_2O_{15}P_2 \\ = f_T \times f_R \times 2.2040 \end{aligned}$$

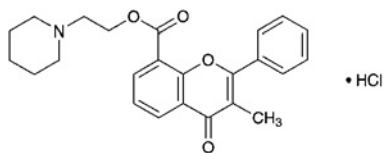
f_T : Total amount (mg) of flavin in Flavin Adenine Dinucleotide Sodium obtained from the procedure (i).

f_R : Peak area ratio of flavin adenine dinucleotide in Flavin Adenine Dinucleotide Sodium obtained from the procedure (ii).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flavoxate Hydrochloride

フラボキサート塩酸塩



$C_{24}H_{25}NO_4 \cdot HCl$: 427.92

2-(Piperidin-1-yl)ethyl 3-methyl-4-oxo-2-phenyl-4*H*-chromene-8-carboxylate monohydrochloride
[3717-88-2]

Flavoxate Hydrochloride, when dried, contains not less than 99.0% of $C_{24}H_{25}NO_4 \cdot HCl$.

Description Flavoxate Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in acetic acid (100) and in chloroform, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Flavoxate Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flavoxate Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flavoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Flavoxate Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Flavoxate Hydrochloride according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 80 mg of Flavoxate Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 20 mL, then pipet 1 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solu-

tion are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, reduced pressure, silica gel, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

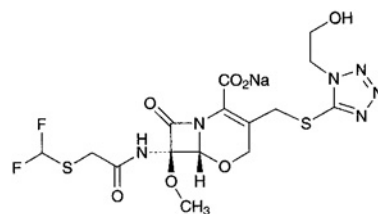
Assay Weigh accurately about 0.6 g of Flavoxate Hydrochloride, previously dried, add 10 mL of acetic acid (100) and 40 mL of acetonitrile to dissolve, add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.79 mg of $C_{24}H_{25}NO_4 \cdot HCl$

Containers and storage Containers—Tight containers.

Flomoxef Sodium

フロモキセフナトリウム



$C_{15}H_{17}F_2N_6NaO_7S_2$: 518.45

Monosodium (6*R*,7*R*)-7-
{[(difluoromethylsulfanyl)acetyl]amino}-
3-[1-(2-hydroxyethyl)-1*H*-tetrazol-5-ylsulfanylmethyl]-
7-methoxy-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-
2-carboxylate [92823-03-5]

Flomoxef Sodium contains not less than 870 μ g (potency) and not more than 985 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Flomoxef Sodium is expressed as mass (potency) of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Description Flomoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and sparingly soluble in ethanol (99.5).

Identification (1) Decompose 0.01 g of Flomoxef Sodium as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. To 2 mL of the test solution so obtained add 1.5 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1): blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Flomoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Flomoxef Sodium as directed in the potassium bromide disk

method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Determine the spectrum of a solution of Flomoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a single signal A at around δ3.5 ppm, a single signal or a sharp multiple signal B at around δ3.7 ppm, and a single signal C at around δ5.2 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 3:2:1.

(5) Flomoxef Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-8 - -13^\circ$ (1 g calculated on the anhydrous basis, a mixture of water and ethanol (99.5) (4:1), 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.5 g of Flomoxef Sodium in 5 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flomoxef Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Flomoxef Sodium in a quartz crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—To 1.0 g of Flomoxef Sodium 5 mL of sulfuric acid and 5 mL of nitric acid, heat carefully until the solution changes to colorless to light yellow with occasional addition of 2 mL of nitric acid. After cooling, add 10 mL of ammonium oxalate TS, heat until white fumes evolve, and concentrate to 2 to 3 mL. After cooling, add water to make 10 mL, and perform the test using this solution as the test solution: the color is not darker than that of the control solution.

Control solution: Proceed to prepare a solution in the same manner as the test solution without Flomoxef Sodium, and transfer 10 mL of the solution so obtained to the generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test solution (not more than 2 ppm).

(4) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution obtained in the Assay and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard: the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol is not more than 1.0% of the amount of Flomoxef Sodium calculated on the anhydrous basis.

Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol
(C₃H₆N₄OS)

$$= W_S \times (Q_T/Q_S) \times (1/10)$$

W_S : Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-

thiol

Internal standard solution—A solution of *m*-cresol (3 in 1000)

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 1.5% (0.5 g, volumetric titration, back titration).

Assay Weigh accurately an amount of Flomoxef Sodium and Flomoxef Triethylammonium Reference Standard, equivalent to about 50 mg (potency), and dissolve each in exactly 50 mL of the internal standard solution, add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of flomoxef to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of flomoxef (C}_{15}\text{H}_{18}\text{F}_2\text{N}_6\text{O}_7\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Flomoxef Triethylammonium Reference Standard

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 246 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5–10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra-*n*-butylammonium bromide in water to make 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of flomoxef is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, flomoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 3 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of flomoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At 5°C or below.

Flomoxef Sodium for Injection

注射用フロモキシセフナトリウム

Flomoxef Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of flomoxef ($C_{15}H_{18}F_2N_6O_7S_2$: 496.47).

Method of preparation Prepare as directed under Injections, with Flomoxef Sodium.

Description Flomoxef Sodium for Injection occurs as white to light yellowish white, friable masses or powder.

Identification Proceed as directed in the Identification (3) under Flomoxef Sodium.

pH <2.54> The pH of a solution obtained by dissolving an amount of Flomoxef Sodium for Injection, equivalent to 0.5 g (potency) of Flomoxef Sodium according to the labeled amount, in 5 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve an amount of Flomoxef Sodium for Injection, equivalent to 1.0 g (potency) of Flomoxef Sodium according to the labeled amount, in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) 1-(2-Hydroxyethyl)-1*H*-tetrazol-5-thiol—Use the sample solution obtained in the Assay as the sample solution. Weigh accurately about 20 mg of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard. Calculate the amount of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol per 1 g (potency) of Flomoxef Sodium for Injection by the following formula: not more than 10 mg.

$$\begin{aligned} &\text{Amount (mg) of 1-(2-hydroxyethyl)-1H-tetrazol-5-thiol} \\ & \quad (C_3H_6N_4OS) \\ &= W_S \times (Q_T/Q_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol

Operating conditions—

Proceed as directed in the Assay.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add water to make exactly 20 mL. Confirm that the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol obtained from 5 μ L of this solution is equivalent to 3.5 – 6.5% of that obtained from 5 μ L of the standard solution.

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

System repeatability: When the test is repeated 3 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of 1-(2-hydroxyethyl)-1*H*-tetrazol-5-thiol to that of the internal standard is not more than 1.0%.

Water <2.48> Not more than 1.5% (0.5 g, volumetric titration, back titration).

Bacterial endotoxins <4.01> Less than 0.025 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Flomoxef Sodium for Injection, and calculate the average mass of the content. Spread out thinly about 1 g of the content in a petri dish, allow the dish to stand in a desiccator containing a saturated solution of magnesium bromide without light exposure to equilibrate the sample to constant water content. Determine the water content, separately, with about 0.1 g of the sample according to the method described in Water. Weigh accurately an amount of the sample, equivalent to about 50 mg (potency) of Flomoxef Sodium according to the labeled amount, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the sample solution. Separately weigh accurately about 50 mg (potency) of Flomoxef Triethylammonium Reference Standard, add exactly 50 mL of the internal standard solution to dissolve, add water to make 100 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Flomoxef Sodium.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of flomoxef } (C_{15}H_{18}F_2N_6O_7S_2) \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

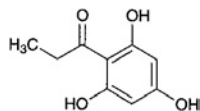
W_S : Amount [mg (potency)] of Flomoxef Triethylammonium Reference Standard

Internal standard solution—A solution of *m*-cresol (3 in 1000).

Containers and storage Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injection may be used.

Flopropione

フロプロピオン



$C_9H_{10}O_4$: 182.17

1-(2,4,6-Trihydroxyphenyl)propan-1-one [2295-58-1]

Flopropione contains not less than 98.0% of $C_9H_{10}O_4$, calculated on the anhydrous basis.

Description Flopropione occurs as a white to pale yellow-brown, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in *N,N*-dimethylformamide, freely soluble in methanol, in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) To 1 mL of a solution of Flopropione in ethanol (99.5) (1 in 200) add 4 mL of water and 1 mL of iron (III) nitrate TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Flopropione in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 177 – 181°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flopropione in 10 mL of ethanol (99.5): the solution is clear, and has no more color than Matching Fluid H.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Flopropione according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Flopropione according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Flopropione in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, ethanol (99.5) and acetic acid (100) (40:20:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly *p*-nitrobenzenediazonium TS for spraying on the plate, and dry in cold wind for about 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 4.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Flopropione, dis-

solve in 30 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 18.22 mg of $C_9H_{10}O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flopropione Capsules

フロプロピオンカプセル

Flopropione Capsules contain not less than 93.0% and not more than 107.0% of the labeled amount of flopropione ($C_9H_{10}O_4$: 182.17).

Method of preparation Prepare as directed under the Capsules, with Flopropione.

Identification (1) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 60 mg of Flopropione according to the labeled amount, add 40 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of iron (III) nitrate TS: a red-purple color appears.

(2) Powder the contents of Flopropione Capsules. To a portion of the powder, equivalent to 90 mg of Flopropione according to the labeled amount, add 100 mL of ethanol (99.5), shake well, and filter. To 5 mL of the filtrate add ethanol (99.5) to make 50 mL. To 5 mL of this solution add ethanol (99.5) to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Flopropione Capsules add 43 mL of a mixture of water and phosphoric acid (86:1), and disintegrate the capsule in a water bath at 50°C. After cooling, add a suitable amount of acetonitrile to make exactly *V* mL of a solution containing about 0.4 mg of flopropione ($C_9H_{10}O_4$) per mL. Stir the solution for 10 minutes, centrifuge a part of the solution at 3000 rpm for 5 minutes, and use the supernatant liquid as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount (mg) of flopropione } (C_9H_{10}O_4) \\ &= W_S \times (A_T/A_S) \times (V/100) \end{aligned}$$

W_S : Amount (mg) of flopropione for assay, calculated on the anhydrous basis

Assay Take out the contents of not less than 20 Flopropione Capsules, weigh accurately the mass of the contents, and power. Weigh accurately a part of the powder, equivalent to about 40 mg of flopropione ($C_9H_{10}O_4$), and add the mobile phase to make exactly 100 mL. Stir the solution for 10 minutes, centrifuge a part of this solution for 5 minutes at 3000 rpm, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 40 mg of

flopropione for assay (previously determine the water <2.48> in the same manner as Flopropione), add 70 mL of the mobile phase, and dissolve by exposure for 10 minutes to ultrasonic vibration. Add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of flopropione.

Amount (mg) of flopropione ($C_9H_{10}O_4$) = $W_S \times (A_T/A_S)$

W_S : Amount (mg) of flopropione for assay, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 267 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, water and phosphoric acid (114:86:1)

Flow rate: Adjust the flow rate so that the retention time of flopropione is about 3 minutes.

System suitability—

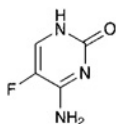
System performance: Dissolve 50 mg of flopropione in 50 mL of the mobile phase. To 20 mL of the solution add 25 mL of a solution prepared by dissolving 25 mg of ethyl parahydroxybenzoate in 30 mL of acetonitrile and add water to make 50 mL, and then add the mobile phase to make 50 mL. When the procedure is run with 5 μ L of this solution under the above operating conditions, Flopropione and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flopropione is not more than 1.0%.

Containers and storage Containers—Tight containers.

Flucytosine

フルシトシン



$C_4H_4FN_3O$: 129.09

5-Fluorocytosine [2022-85-7]

Flucytosine, when dried, contains not less than 98.5% of $C_4H_4FN_3O$, and not less than 14.0% and not more than 15.5% of fluorine (F: 19.00).

Description Flucytosine occurs as a white, crystalline powder.

It is odorless.

It is sparingly soluble in water, slightly soluble in methanol, in ethanol (95), in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

It dissolves in 0.1 mol/L hydrochloric acid TS.

The pH of a solution of Flucytosine (1 in 100) is between 5.5 and 7.5.

It is slightly hygroscopic.

Melting point: about 295°C (with decomposition).

Identification (1) Add 0.2 mL of bromine TS to 5 mL of a solution of Flucytosine (1 in 500): a yellow-brown color of bromine TS is immediately discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Proceed with 0.1 g of Flucytosine as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. The solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Flucytosine in 0.1 mol/L hydrochloric acid TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flucytosine in 100 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Flucytosine in 80 mL of water by heating on a water bath. After cooling, to 40 mL of this solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Fluoride—Dissolve 0.10 g of Flucytosine in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerrous nitrate TS (1:1:1), and add water to make 20 mL. Allow the mixture to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 4.0 mL of Standard Fluorine Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerrous nitrate TS (1:1:1). Proceed in the same manner as directed in the preparation of the sample solution, and use this solution as the standard solution. Transfer 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) to a 20-mL volumetric flask, proceed in the same manner as directed in the preparation of the standard solution, and use this solution as the blank solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 600 nm, using the blank solution as the control as directed under Ultraviolet-visible Spectrophotometry <2.24>: A_T is not larger than A_S (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Flucytosine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Flucytosine according to Method 2, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 50 mg of Flucytosine in 5 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Measure accurately 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 25 mL. Measure accurately 1 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of ethyl acetate, methanol and water (5:3:2) to a distance of about 12 cm, air-dry the plate, and observe the spots under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Flucytosine—Weigh accurately about 0.2 g of Flucytosine, previously dried, dissolve in 40 mL of acetic acid (100), add 100 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 12.91 mg of C₄H₄FN₃O

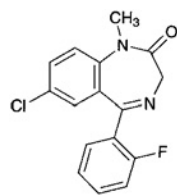
(2) Fluorine—Weigh accurately about 0.01 g of Flucytosine, previously dried, and proceed as directed in the determination of fluorine under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide VS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fludiazepam

フルジアゼパム



C₁₆H₁₂ClFN₂O: 302.73

7-Chloro-5-(2-fluorophenyl)-1-methyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one [3900-31-0]

Fludiazepam, when dried, contains not less than 99.0% of C₁₆H₁₂ClFN₂O.

Description Fludiazepam occurs as white to light yellow crystals or crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

Identification (1) Prepare the test solution with 0.01 g of

Fludiazepam as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Fludiazepam in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fludiazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Fludiazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 91 – 94°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Fludiazepam in 50 mL of diethyl ether, add 50 mL of water, and shake. Separate the water layer, wash it with two 20-mL portions of diethyl ether, and filter the water layer. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Fludiazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Fludiazepam in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethyl acetate (10:7) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Fludiazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any

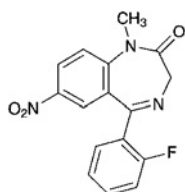
necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.27 mg of C₁₆H₁₂ClFN₂O

Containers and storage Containers—Tight containers.

Flunitrazepam

フルニトラゼパム



C₁₆H₁₂FN₃O₃: 313.28
5-(2-Fluorophenyl)-1-methyl-7-nitro-1,3-dihydro-2H-1,4-benzodiazepin-2-one [I622-62-4]

Flunitrazepam, when dried, contains not less than 99.0% of C₁₆H₁₂FN₃O₃.

Description Flunitrazepam occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetic anhydride and in acetone, slightly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Flunitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flunitrazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 168 – 172°C

Purity (1) Chloride <1.03>—To 1.0 g of Flunitrazepam add 50 mL of water, allow to stand for 1 hour with occasional stirring, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Flunitrazepam according to Method 4 using a platinum crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Flunitrazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10

μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, diethyl ether and ammonia solution (28) (200:100:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): number of the spots other than the principal spot from the sample solution is not more than 2, and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

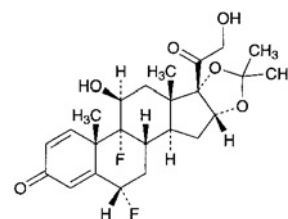
Assay Weigh accurately about 0.5 g of Flunitrazepam, previously dried, dissolve in 20 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 31.33 mg of C₁₆H₁₂FN₃O₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Fluocinolone Acetonide

フルオシノロンアセトニド



C₂₄H₃₀F₂O₆: 452.49
6α,9-Difluoro-11β,21-dihydroxy-16α,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione [67-73-2]

Fluocinolone Acetonide, when dried, contains not less than 97.0% and not more than 102.0% of C₂₄H₃₀F₂O₆.

Description Fluocinolone Acetonide occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (95) and ethanol (99.5), sparingly soluble in methanol and in chloroform, slightly soluble in acetonitrile, very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: 266 – 274°C (with decomposition).

Identification (1) To 2 mg of Fluocinolone Acetonide add 2 mL of sulfuric acid: a yellow color is produced.

(2) Dissolve 0.01 g of Fluocinolone Acetonide in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Proceed as directed under Oxygen Flask Combustion Method <1.06> with 0.01 g of Fluocinolone Acetonide, using

a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When the process is completed, shake well, and force the combustion gas into the absorbing liquid: this liquid responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the infrared absorption spectrum of Fluocinolone Acetonide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluocinolone Acetonide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluocinolone Acetonide and Fluocinolone Acetonide Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +98 – +108° (after drying, 0.1 g, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 15 mg of Fluocinolone Acetonide in 25 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of fluocinolone acetonide from the sample solution is not larger than the peak area of fluocinolone acetonide from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water-saturated chloroform, methanol and acetic acid (100) (200:3:2).

Flow rate: Adjust the flow rate so that the retention time of fluocinolone acetonide is about 12 minutes.

Time span of measurement: About twice as long as the retention time of fluocinolone acetonide beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of fluocinolone acetonide obtained from 20 μ L of this solution is equivalent to 4 to 6% of that of fluocinolone acetonide obtained from 20 μ L of the standard solution.

System performance: Dissolve 15 mg each of Fluocinolone Acetonide and triamcinolone acetonide in 25 mL of the mobile phase. To 5 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, triamcinolone acetonide and fluocinolone acetonide are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with

20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of fluocinolone acetonide is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (0.2 g, in vacuum, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.2 g, platinum crucible).

Assay Dissolve about 20 mg each of Fluocinolone Acetonide and Fluocinolone Acetonide Reference Standard, previously dried and accurately weighed, in 40 mL each of methanol, add exactly 10 mL each of the internal standard solution, then add water to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of fluocinolone acetonide to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of fluocinolone acetonide (C}_{24}\text{H}_{30}\text{F}_2\text{O}_6) \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Fluocinolone Acetonide Reference Standard

Internal standard solution—A solution of ethyl parahydroxybenzoate (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of fluocinolone acetonide is about 20 minutes.

System suitability—

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

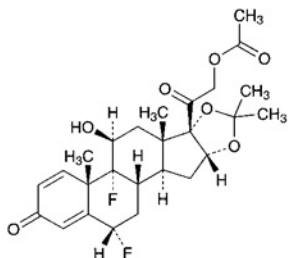
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fluocinonide

フルオシノニド



$C_{26}H_{32}F_2O_7$: 494.52
 6 α ,9-Difluoro-11 β ,21-dihydroxy-16 α ,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione 21-acetate [356-12-7]

Fluocinonide, when dried, contains not less than 97.0% and not more than 103.0% of $C_{26}H_{32}F_2O_7$.

Description Fluocinonide occurs as white crystals or crystalline powder.

It is sparingly soluble in chloroform, slightly soluble in acetonitrile, in methanol, in ethanol (95) and in ethyl acetate, very slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 0.01 g of Fluocinonide add 4 mL of water and 1 mL of Fehling's TS, and heat: a red precipitate is formed.

(2) Prepare the test solution with 0.01 g of Fluocinonide as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for fluoride.

(3) Determine the absorption spectrum of a solution of Fluocinonide in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluocinonide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Fluocinonide and Fluocinonide Reference Standard, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare both spectra: both the sample and the Reference Standard exhibit similar intensities of absorption at the same wave numbers. If any difference appears in the absorption spectra, dissolve the sample and the Reference Standard in ethyl acetate, respectively, evaporate the ethyl acetate, and perform the test with the residue in the same manner.

Optical rotation <2.49> $[\alpha]_D^{20}$: +81 – +89° (after drying, 0.2 g, chloroform, 20 mL, 100 mm).

Purity Related substances—Dissolve 10 mg of Fluocinonide in 2 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100, and use this solution as the standard solution. Perform the test with these solutions as

directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (97:3) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g, platinum crucible).

Assay Weigh accurately about 20 mg of Fluocinonide and Fluocinonide Reference Standard, previously dried, dissolve each in 50 mL of acetonitrile, to each add exactly 8 mL of the internal standard solution and water to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of fluocinonide to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of fluocinonide (C}_{26}\text{H}_{32}\text{F}_2\text{O}_7) \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Fluocinonide Reference Standard

Internal standard solution—A solution of propyl benzoate in acetonitrile (1 in 100).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and water (1:1).

Flow rate: Adjust the flow rate so that the retention time of fluocinonide is about 8 minutes.

System suitability—

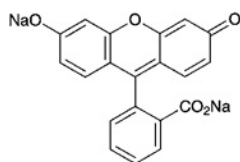
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, fluocinonide and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluocinonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Fluorescein Sodium

フルオレセインナトリウム



$C_{20}H_{10}Na_2O_5$: 376.27

Disodium 2-(6-oxido-3-oxo-3*H*-xanthen-9-yl)benzoate
[518-47-8]

Fluorescein Sodium contains not less than 98.5% of $C_{20}H_{10}Na_2O_5$, calculated on the dried basis.

Description Fluorescein Sodium occurs as an orange powder. It is odorless, and tasteless.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To a solution of Fluorescein Sodium (1 in 100) having a strong green fluorescence, add a large quantity of water: the fluorescence remains. Acidify the solution with hydrochloric acid: the fluorescence disappears. Then render the solution alkaline with sodium hydroxide TS: the fluorescence reappears.

(2) Place 1 drop of a solution of Fluorescein Sodium (1 in 2000) on a piece of filter paper: a yellow spot develops. Expose the spot, while moist, to the vapor of bromine for 1 minute and then to ammonia vapor: the yellow color of the spot changes to red.

(3) Char 0.5 g of Fluorescein Sodium by ignition, cool, mix the residue with 20 mL of water, and filter: the filtrate responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1 g of Fluorescein Sodium in 10 mL of water: the solution is clear, and shows a red color.

(2) Chloride <1.03>—Dissolve 0.15 g of Fluorescein Sodium in 20 mL of water, add 6 mL of dilute nitric acid and water to make 30 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.355%).

(3) Sulfate <1.14>—Dissolve 0.20 g of Fluorescein Sodium in 30 mL of water, add 2.5 mL of dilute hydrochloric acid and water to make 40 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(4) Zinc—Dissolve 0.10 g of Fluorescein Sodium in 10 mL of water, add 2 mL of hydrochloric acid, and filter. To the filtrate add 0.1 mL of potassium hexacyanoferrate (II) TS: no turbidity is produced immediately.

(5) Related substances—Dissolve 0.20 g of Fluorescein Sodium in 10 mL of methanol, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of

the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30:15:1) to a distance of about 10 cm, and air-dry the plate: any colored spot other than the principal spot does not appear.

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, constant mass).

Assay Transfer about 0.5 g of Fluorescein Sodium, accurately weighed, to a separator. Dissolve in 20 mL of water, add 5 mL of dilute hydrochloric acid, and extract the solution with four 20-mL portions of a mixture of 2-methyl-1-propanol and chloroform (1:1). Wash each extract successively with the same 10 mL of water. Evaporate the combined extracts on a water bath with the aid of a current of air. Dissolve the residue in 10 mL of ethanol (99.5), evaporate the solution on a water bath to dryness, dry the residue at 105°C for 1 hour, and weigh as fluorescein ($C_{20}H_{12}O_5$: 332.31).

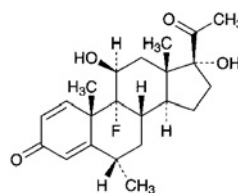
Amount (mg) of $C_{20}H_{10}Na_2O_5$

= amount (mg) of fluorescein ($C_{20}H_{12}O_5$) \times 1.1323

Containers and storage Containers—Tight containers.

Fluorometholone

フルオロメトロロン



$C_{22}H_{29}FO_4$: 376.46

9-Fluoro-11 β ,17-dihydroxy-6 α -methylpregna-1,4-diene-3,20-dione [426-13-1]

Fluorometholone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{29}FO_4$.

Description Fluorometholone occurs as a white to light yellowish white, odorless, crystalline powder.

It is freely soluble in pyridine, slightly soluble in methanol, in ethanol (99.5) and in tetrahydrofuran, and practically insoluble in water and in diethyl ether.

Identification (1) Proceed with 7 mg of Fluorometholone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the liquid responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Fluorometholone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluorometholone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluorometholone, previously dried, as directed in the potassi-

um bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluorometholone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +52 – +60° (after drying, 0.1 g, pyridine, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Fluorometholone according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 20 mg of Fluorometholone in 10 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add tetrahydrofuran to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 25 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, acetone and methanol (45:5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2 % (0.2 g, platinum crucible).

Assay Weigh accurately about 0.1 g each of Fluorometholone and Fluorometholone Reference Standard, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, and add diluted methanol (7 in 10) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution and diluted methanol (7 in 10) to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions and determine the ratios, Q_T and Q_S , of the peak area of fluorometholone to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of fluorometholone (C}_{22}\text{H}_{29}\text{FO}_4) \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Fluorometholone Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Diluted methanol (7 in 10).

Flow rate: Adjust the flow rate so that the retention time of

fluorometholone is about 8 minutes.

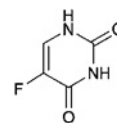
Selection of column: Proceed with 20 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of fluorometholone and the internal standard in this order with the resolution between these peaks being not less than 4.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Fluorouracil

フルオロウラシル



$C_4H_3FN_2O_2$: 130.08
5-Fluorouracil [51-21-8]

Fluorouracil, when dried, contains not less than 98.5% of $C_4H_3FN_2O_2$, and not less than 13.1% and not more than 16.1% of fluorine (F: 19.00).

Description Fluorouracil occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 282°C (with decomposition).

Identification (1) Add 0.2 mL of bromine TS to 5 mL of a solution of Fluorouracil (1 in 500): the color of bromine TS is discharged. Further add 2 mL of barium hydroxide TS: a purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Fluorouracil in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Proceed with 0.01 g of Fluorouracil as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. After combustion is completed, shake well to absorb the gas evolved: the solution responds to the Qualitative Tests <1.09> for fluoride.

Purity (1) Clarity and color of solution—Add 20 mL of water to 0.20 g of Fluorouracil, and dissolve by warming: the solution is clear and colorless.

(2) Fluoride—Dissolve 0.10 g of Fluorouracil in 10.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20). Transfer 5.0 mL of this solution to a 20-mL volumetric flask, add 10 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1), and add water to make 20 mL. Allow to stand for 1 hour, and use this solution as the sample solution. Separately, transfer 1.0 mL of Standard Fluorine Solution to a 20-mL volumetric flask, add 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and add 10 mL of a mixture

of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3, and cerium (III) nitrate TS (1:1:1). Proceed in the same manner as directed for the preparation of the sample solution, and use this solution as the standard solution. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 5.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in the same manner, as the blank: the absorbance of the sample solution at 600 nm is not larger than that of the standard solution (not more than 0.012%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Fluorouracil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—To 1.0 g of Fluorouracil in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol to burn, and incinerate by strong heating at 750°C to 850°C. If a carbonized substance remains in this method, moisten with a small amount of nitric acid, and incinerate by strong heating. Cool, add 10 mL of dilute hydrochloric acid to the residue, dissolve it by warming on a water bath, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Fluorouracil in 10 mL of water, and use this solution as the sample solution. Measure exactly 1 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (7:4:1) to a distance of about 12 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Fluorouracil—Weigh accurately about 0.2 g of Fluorouracil, previously dried, dissolve in 20 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through blue-green to blue (indicator: 3 drops of thymol blue-dimethylformamide TS). Perform a blank determination.

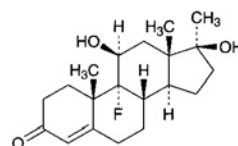
Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 13.01 mg of C₄H₃FN₂O₂

(2) Fluorine—Weigh accurately about 4 mg of Fluorouracil, previously dried, and proceed as directed in the determination of fluorine under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Fluoxymesterone

フルオキシメステロン



C₂₀H₂₉FO₃; 336.44

9-Fluoro-11 β ,17 β -dihydroxy-17-methylandrosta-4-en-3-one
[76-43-7]

Fluoxymesterone, when dried, contains not less than 97.0% and not more than 102.0% of C₂₀H₂₉FO₃.

Description Fluoxymesterone occurs as white crystals or crystalline powder. It is odorless.

It is sparingly soluble in methanol, slightly soluble in ethanol (95) and in chloroform, very slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 5 mg of Fluoxymesterone in 2 mL of sulfuric acid: a yellow color develops.

(2) Prepare the test solution with 0.01 g of Fluoxymesterone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(3) Determine the absorption spectrum of a solution of Fluoxymesterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Fluoxymesterone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Fluoxymesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Fluoxymesterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Fluoxymesterone and Fluoxymesterone Reference Standard in ethanol (99.5), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> [α]_D²⁰: +104 – +112° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Fluoxymesterone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.03 g of Fluoxymesterone in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed

under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, ethanol (95) and ethyl acetate (3:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g, platinum crucible).

Assay Weigh accurately about 25 mg each of Fluoxymesterone and Fluoxymesterone Reference Standard, previously dried, dissolve each in the internal standard solution to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of fluoxymesterone to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of } C_{20}H_{29}FO_3 \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Fluoxymesterone Reference Standard

Internal standard solution—A solution of methylprednisolone in a mixture of chloroform and methanol (19:1) (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of *n*-butyl chloride, water-saturated *n*-butyl chloride, tetrahydrofuran, methanol and acetic acid (100) (95:95:14:7:6).

Flow rate: Adjust the flow rate so that the retention time of fluoxymesterone is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, fluoxymesterone and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

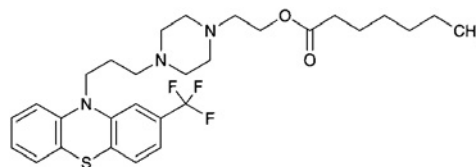
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of fluoxymesterone to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Fluphenazine Enanthate

フルフェナジンエナント酸エステル



$C_{29}H_{38}F_3N_3O_2S$: 549.69

2-(4-{3-[2-(Trifluoromethyl)-10*H*-phenothiazin-10-yl]propyl} piperazin-1-yl)ethyl heptanoate [2746-81-8]

Fluphenazine Enanthate, when dried, contains not less than 98.5% of $C_{29}H_{38}F_3N_3O_2S$.

Description Fluphenazine Enanthate is a light yellow to yellowish orange viscous liquid. It is generally clear, and can be opaque by producing crystals.

It is freely soluble in methanol and in diethyl ether, soluble in acetic acid (100) and in ethanol (95), and practically insoluble in water.

Identification (1) Prepare the test solution with 0.01 g of Fluphenazine Enanthate as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for fluoride.

(2) Dissolve 2 mg of Fluphenazine Enanthate in 200 mL of a solution of hydrochloric acid in methanol (17 in 2000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Fluphenazine Enanthate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Fluphenazine Enanthate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 0.25 g of Fluphenazine Enanthate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, hexane and ammonia solution (28) (16:6:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Then spray evenly diluted sulfuric acid (1 in 2) on the plate: the spots

other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

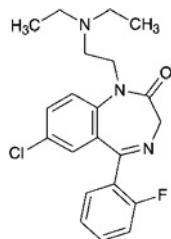
Assay Weigh accurately about 0.5 g of Flurazepam Enanthate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.49 mg of C₂₉H₃₈F₃N₃O₂S

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Flurazepam

フルラゼパム



C₂₁H₂₃ClFN₃O: 387.88

7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one [17617-23-1]

Flurazepam, when dried, contains not less than 99.0% of C₂₁H₂₃ClFN₃O.

Description Flurazepam occurs as white to light yellow crystals or crystalline powder.

It is very soluble in chloroform, freely soluble in methanol, in ethanol (95), in acetic anhydride and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 0.01 g of Flurazepam in 3 mL of sulfuric acid: the solution shows a greenish yellow fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Dissolve 0.01 g of Flurazepam in 3 mL of citric acid-acetic acid TS, and heat in a water bath for 4 minutes: a dark red color develops.

(3) Prepare the test solution with 0.01 g of Flurazepam as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(4) Determine the absorption spectrum of a solution of Flurazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Flurazepam in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>,

and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Perform the test with Flurazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 79 – 83°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flurazepam in 10 mL of ethanol (95): the solution is clear and colorless to light yellow.

(2) Chloride <1.03>—Dissolve 1.0 g of Flurazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, separate the water layer, wash with two 20-mL portions of diethyl ether, and filter the water layer. Neutralize 20 mL of the filtrate with dilute nitric acid, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Sulfate <1.14>—Neutralize 20 mL of the filtrate obtained in (2) with dilute hydrochloric acid, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Flurazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Flurazepam according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Flurazepam in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60:40:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.3 g of Flurazepam, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS to the second equivalence point (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 19.39 mg of C₂₁H₂₃ClFN₃O

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Flurazepam Capsules

フルラゼパムカプセル

Flurazepam Capsules contain not less than 93% and not more than 107% of the labeled amount of flurazepam ($C_{21}H_{23}ClFN_3O$: 387.88).

Method of preparation Prepare as directed under Capsules, with Flurazepam.

Identification (1) Powder the contents of Flurazepam Capsules. To a quantity of the powder, equivalent to 0.1 g of Flurazepam according to the labeled amount, add 100 mL of 0.1 mol/L hydrochloric acid TS, stir, and filter. To 40 mL of the filtrate add 80 mL of a solution of sodium hydroxide (1 in 250) and 100 mL of hexane, extract by shaking well, and use the hexane layer as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness. Dissolve the residue in 3 mL of sulfuric acid: the solution shows a greenish yellow fluorescence under ultraviolet light.

(2) Evaporate 25 mL of the sample solution obtained in (1) on a water bath to dryness. Dissolve the residue in 3 mL of citric acid-acetic acid TS, and heat in a water bath for 4 minutes: a dark red color develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 315 nm and 319 nm, and a minimum between 297 nm and 301 nm.

Assay Weigh accurately the contents of not less than 20 Flurazepam Capsules, and powder the combined contents. Weigh accurately a portion of the powder, equivalent to about 0.05 g of flurazepam ($C_{21}H_{23}ClFN_3O$), add 30 mL of methanol, stir well for 10 minutes, and add methanol to make exactly 50 mL. Filter this solution, discard the first 20 mL of the filtrate, pipet 6 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of flurazepam for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in methanol to make exactly 50 mL. Pipet 6 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 317 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

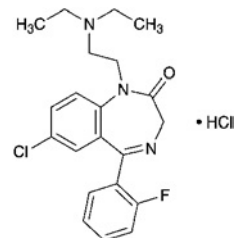
$$\begin{aligned} &\text{Amount (mg) of flurazepam (C}_{21}\text{H}_{23}\text{ClFN}_3\text{O)} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of flurazepam for assay

Containers and storage Containers—Tight containers.

Flurazepam Hydrochloride

フルラゼパム塩酸塩



$C_{21}H_{23}ClFN_3O \cdot HCl$: 424.34
7-Chloro-1-[2-(diethylamino)ethyl]-5-(2-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one monohydrochloride [36105-20-1]

Flurazepam Hydrochloride, when dried, contains not less than 99.0% of $C_{21}H_{23}ClFN_3O \cdot HCl$.

Description Flurazepam Hydrochloride occurs as white to yellowish white crystals or crystalline powder.

It is freely soluble in water, in ethanol (95), in ethanol (99.5) and in acetic acid (100).

Melting point: about 197°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Flurazepam Hydrochloride in sulfuric acid-ethanol TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurazepam Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Flurazepam Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Flurazepam Hydrochloride in 20 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Flurazepam Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate <1.14>—Perform the test with 1.5 g of Flurazepam Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Flurazepam Hydrochloride in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.05 g of Flurazepam Hydrochloride in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 10 mL, and use

this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Place the plate in a chamber filled with ammonia vapor, allow to stand for about 15 minutes, and immediately develop the plate with a mixture of diethyl ether and diethylamine (39:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 3 spots other than the principal spot and the spot on the starting point from the sample solution appear, and are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

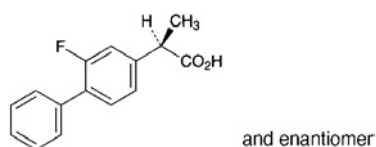
Assay Weigh accurately about 0.3 g of Flurbiprofen Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.22 mg of $\text{C}_{21}\text{H}_{23}\text{ClFN}_3\text{O}\cdot\text{HCl}$

Containers and storage Containers—Tight containers.

Flurbiprofen

フルルビプロフェン



$\text{C}_{15}\text{H}_{13}\text{FO}_2$: 244.26
(2*S*)-2-(2-Fluorobiphenyl-4-yl)propanoic acid
[5104-49-4]

Flurbiprofen, when dried, contains not less than 98.0% of $\text{C}_{15}\text{H}_{13}\text{FO}_2$.

Description Flurbiprofen occurs as a white, crystalline powder. It has a slightly irritating odor.

It is freely soluble in methanol, in ethanol (95), in acetone and in diethyl ether, soluble in acetonitrile, and practically insoluble in water.

A solution of Flurbiprofen in ethanol (95) (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Flurbiprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Flurbiprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry

<2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 114 – 117°C

Purity (1) Chloride <1.03>—Dissolve 0.6 g of Flurbiprofen in 40 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals <1.07>—Dissolve 2.0 g of Flurbiprofen in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Flurbiprofen in 10 mL of a mixture of water and acetonitrile (11:9), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (11:9) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each area of the peaks other than the peak of flurbiprofen from the sample solution is not larger than the peak area of flurbiprofen from the standard solution, and the total area of these peaks is not larger than twice the peak area of flurbiprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (12:7:1).

Flow rate: Adjust the flow rate so that the retention time of flurbiprofen is about 20 minutes.

Time span of measurement: About twice as long as the retention time of flurbiprofen beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add a mixture of water and acetonitrile (11:9) to make exactly 25 mL. Confirm that the peak area of flurbiprofen obtained from 20 μL of this solution is equivalent to 16 to 24% of that of flurbiprofen obtained from 20 μL of the standard solution.

System performance: Dissolve 0.04 g of flurbiprofen and 0.02 g of butyl parahydroxybenzoate in 100 mL of a mixture of water and acetonitrile (11:9). To 5 mL of this solution add a mixture of water and acetonitrile (11:9) to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, butyl parahydroxybenzoate

and flurbiprofen are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of flurbiprofen is not more than 2.0%.

Loss on drying <2.41> Not more than 0.10% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

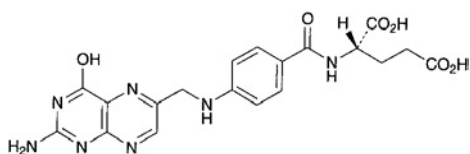
Assay Weigh accurately about 0.6 g of Flurbiprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.43 mg of $\text{C}_{15}\text{H}_{13}\text{FO}_2$

Containers and storage Containers—Well-closed containers.

Folic Acid

葉酸



$\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$; 441.40
N-[4-[(2-Amino-4-hydroxypteridin-6-ylmethyl)amino]benzoyl]-*L*-glutamic acid [59-30-3]

Folic Acid contains not less than 98.0% and not more than 102.0% of $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$, calculated on the anhydrous basis.

Description Folic Acid occurs as a yellow to orange-yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in methanol, in ethanol (95), in pyridine and in diethyl ether.

It dissolves in hydrochloric acid, in sulfuric acid, in dilute sodium hydroxide TS and in a solution of sodium carbonate decahydrate (1 in 100), and these solutions are yellow in color.

It is slowly affected by light.

Identification (1) Dissolve 1.5 mg of Folic Acid in dilute sodium hydroxide TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Folic Acid Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) To 10 mL of the solution obtained in (1) add 1 drop of potassium permanganate TS, and mix well until the color changes to blue, and immediately observe under ultraviolet light (main wavelength: 365 nm): a blue fluorescence is

produced.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Folic Acid in 10 mL of dilute sodium hydroxide TS: the solution is clear and yellow in color.

(2) Free amines—Pipet 30 mL of the sample solution obtained in the Assay, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of *p*-Aminobenzoylglutamic Acid Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in diluted ethanol (95) (2 in 5) to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and the standard solution, proceed as directed in the Assay, and perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>. Determine the absorbances, A_T and A_S , of subsequent solutions of the sample solution and the standard solution at 550 nm: the content of free amines is not more than 1.0%.

Content (%) of free amines = $(A_T/A_S) \times (W'/W)$

W : Amount (mg) of Folic Acid, calculated on the anhydrous basis

W' : Amount (mg) of *p*-Aminobenzoylglutamic Acid Reference Standard

Water <2.48> Not more than 8.5% (10 mg, coulometric titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately about 50 mg each of Folic Acid and Folic Acid Reference Standard. To each add 50 mL of dilute sodium hydroxide TS, mix well to dissolve, add dilute sodium hydroxide TS to make exactly 100 mL, and use these solutions as the sample solution and standard solution. To 30 mL each of these solutions, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. To 60 mL each of these solutions add 0.5 g of zinc powder, and allow to stand with frequent shaking for 20 minutes. Filter each mixture through a dry filter paper, and discard the first 10 mL of the filtrate. Pipet 10 mL each of the subsequent filtrate, and add water to make exactly 100 mL. To 4 mL each of solutions, accurately measured, add 1 mL of water, 1 mL of dilute hydrochloric acid and 1 mL of a solution of sodium nitrite (1 in 1000), mix well, and allow to stand for 2 minutes. To each solution add 1 mL of a solution of ammonium amidosulfate (1 in 200), mix thoroughly, and allow to stand for 2 minutes. To each of these solutions, add 1 mL of a solution of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate (1 in 1000), shake, allow to stand for 10 minutes, and add water to make exactly 20 mL. Separately, to 30 mL of the sample solution, accurately measured, add 20 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 10 mL of this solution, add 18 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 4 mL of this solution, and prepare the blank solution in the same manner as the sample solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 4 mL of water in the same manner as a blank. Determine the absorbances, A_T , A_S and A_C , of the subsequent solution of the sample solution, the standard solution and the blank solution at 550 nm.

Amount (mg) of $\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$

$$= W_S \times \{(A_T - A_C)/A_S\}$$

W_S : Amount (mg) of Folic Acid Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Folic Acid Injection

葉酸注射液

Folic Acid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 115% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$: 441.40).

Method of preparation Dissolve Folic Acid in water with the aid of Sodium Hydroxide or Sodium Carbonate, and prepare as directed under Injections.

Description Folic Acid Injection is a yellow to orange-yellow, clear liquid.

pH: 8.0 – 11.0

Identification (1) To a volume of Folic Acid Injection, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add dilute sodium hydroxide TS to make 100 mL. Proceed as directed in the Identification (2) under Folic Acid, using this solution as the sample solution.

(2) Determine the absorption spectrum of the sample solution obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the sample solution, A_1 and A_2 , between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of A_1/A_2 is between 2.80 and 3.00.

(3) Folic Acid Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Folic Acid Injection, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$) add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid Reference Standard, dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Proceed with 30 mL each of the sample solution and standard solution, exactly measured, as directed in the Assay under Folic Acid.

$$\begin{aligned} &\text{Amount (mg) of folic acid (C}_{19}\text{H}_{19}\text{N}_7\text{O}_6) \\ &= W_S \times \{(A_T - A_C)/A_S\} \end{aligned}$$

W_S : Amount (mg) of Folic Acid Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Folic Acid Tablets

葉酸錠

Folic Acid Tablets contain not less than 90% and not more than 115% of the labeled amount of folic acid ($C_{19}H_{19}N_7O_6$: 441.40).

Method of preparation Prepare as directed under Tablets, with Folic Acid.

Identification (1) Take a quantity of powdered Folic Acid Tablets, equivalent to 1.5 mg of Folic Acid according to the labeled amount, add 100 mL of dilute sodium hydroxide TS, shake, and filter. Discard the first 10 mL of the filtrate, use the subsequent filtrate as the sample solution, and proceed as directed in the Identification (2) under Folic Acid.

(2) Determine the absorption spectrum of the filtrate obtained in (1) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 255 nm and 257 nm, between 281 nm and 285 nm and between 361 nm and 369 nm. Separately, determine the maximal absorbances of the filtrate, A_1 and A_2 , between 255 nm and 257 nm and between 361 nm and 369 nm, respectively: the ratio of A_1/A_2 is between 2.80 and 3.00.

Assay Weigh accurately and powder not less than 20 Folic Acid Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of folic acid ($C_{19}H_{19}N_7O_6$). Add 50 mL of dilute sodium hydroxide TS, shake frequently, then filter into a 100-mL volumetric flask, and wash with dilute sodium hydroxide TS. To the combined filtrate and washings add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Folic Acid Reference Standard, dissolve in dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Take 30 mL each of the sample solution and standard solution, exactly measured, and proceed as directed in the Assay under Folic Acid.

$$\begin{aligned} &\text{Amount (mg) of folic acid (C}_{19}\text{H}_{19}\text{N}_7\text{O}_6) \\ &= W_S \times \{(A_T - A_C)/A_S\} \end{aligned}$$

W_S : Amount (mg) of Folic Acid Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Formalin

ホルマリン

Formalin contains not less than 35.0% and not more than 38.0% of formaldehyde (CH_2O : 30.03.)

It contains 5% to 13% of methanol to prevent polymerization.

Description Formalin is a clear, colorless liquid. Its vapor is

irritating to the mucous membrane.

It is miscible with water and with ethanol (95).

When stored for a long time, especially in a cold place, it may become cloudy.

Identification (1) Dilute 2 mL of Formalin with 10 mL of water in a test tube, and add 1 mL of silver nitrate-ammonia TS: a gray precipitate is produced, or a silver mirror is formed on the wall of the test tube.

(2) To 5 mL of sulfuric acid in which 0.1 g of salicylic acid has been dissolved add 2 drops of Formalin, and warm the solution: a persistent, dark red color develops.

Purity Acidity—Dilute 20 mL of Formalin with 20 mL of water, and add 5.0 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of bromothymol blue TS: a blue color develops.

Residue on ignition <2.44> Not more than 0.06 w/v% (5 mL, after evaporation).

Assay Weigh accurately a weighing bottle containing 5 mL of water, add about 1 g of Formalin, and weigh accurately again. Add water to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 50 mL of 0.05 mol/L iodine VS and 20 mL of potassium hydroxide TS, and allow to stand for 15 minutes at an ordinary temperature. To this mixture add 15 mL of dilute sulfuric acid, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH₂O

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Formalin Water

ホルマリン水

Formalin Water contains not less than 0.9 w/v% and not more than 1.1 w/v% of formaldehyde (CH₂O: 30.03).

Method of preparation

Formalin	30 mL
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Prepare by mixing the above ingredients.

Description Formalin Water is a clear, colorless liquid. It has a slight odor of formaldehyde.

It is almost neutral.

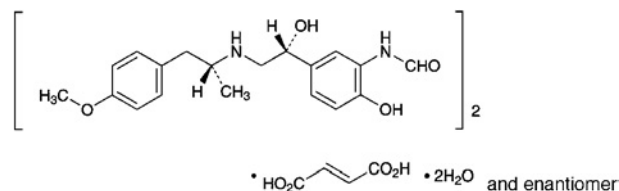
Assay Transfer 20 mL of Formalin Water, measured exactly, to a 100-mL volumetric flask containing 2.5 mL of 1 mol/L sodium hydroxide VS, and add water to make 100 mL. Pipet 10 mL of this solution, and proceed as directed in the Assay under Formalin.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH₂O

Containers and storage Containers—Tight containers.

Formoterol Fumarate Hydrate

ホルモテロール fumarate 水和物



(C₁₉H₂₄N₂O₄)₂·C₄H₄O₄·2H₂O: 840.91

N-(2-Hydroxy-5-((1*RS*)-1-hydroxy-

2-[(1*RS*)-2-(4-methoxyphenyl)-

1-methylethylamino]ethyl) phenyl)formamide

hemifumarate monohydrate [43229-80-7, anhydride]

Formoterol Fumarate Hydrate contains not less than 98.5% of formoterol fumarate [(C₁₉H₂₄N₂O₄)₂·C₄H₄O₄: 804.88], calculated on the anhydrous basis.

Description Formoterol Fumarate Hydrate occurs as a white to yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

A solution of Formoterol Fumarate in methanol (1 in 100) shows no optical rotation.

Melting point: about 138°C (with decomposition).

Identification (1) Dissolve 0.5 g of Formoterol Fumarate Hydrate in 20 mL of 0.5 mol/L sulfuric acid TS, and extract with three 25-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10 mL of 0.5 mol/L sulfuric acid TS, and evaporate the ether layer under reduced pressure, and dry the residue at 105°C for 3 hours: the residue melts <2.60> at about 290°C (with decomposition, in a sealed tube).

(2) Determine the absorption spectrum of a solution of Formoterol Fumarate Hydrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Formoterol Fumarate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Formoterol Fumarate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related Substances—Dissolve 0.20 g of Formoterol Fumarate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard

solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane, ethanol (99.5) and ammonia solution (28) (20:20:10:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 5 minutes in iodine vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 4.0 – 5.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Formoterol Fumarate Hydrate, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

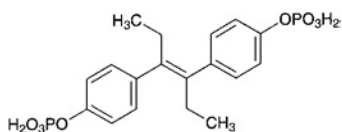
Each mL of 0.1 mol/L perchloric acid VS
= 40.24 mg of $(C_{19}H_{24}N_2O_4)_2 \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.

Fosfestrol

Diethylstilbestrol Diphosphate

ホスフェストロール



$C_{18}H_{22}O_8P_2$: 428.31
(*E*)-4,4'-(Hex-3-ene-3,4-diyl)bis(phenyl dihydrogen phosphate) [522-40-7]

Fosfestrol, when dried, contains not less than 98.5% of $C_{18}H_{22}O_8P_2$.

Description Fosfestrol occurs as a white, crystalline powder. It is odorless.

It is freely soluble in ethanol (95), soluble in formamide, slightly soluble in water, and practically insoluble in acetonitrile and in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 234°C (with decomposition).

Identification (1) Dissolve 15 mg of Fosfestrol in 1 mL of sulfuric acid: a yellow to orange color develops. To this solution add 10 mL of water: the color of the solution disappears.

(2) Determine the infrared absorption spectrum of Fosfestrol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or with the spectrum of Fosfestrol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Place 0.4 g of Fosfestrol in a crucible, wet by adding 0.1 mL of sulfuric acid, and heat to carbonize. Add 10 mL of water to the residue, stir well, and filter. Add 0.1 mL of nitric acid to the filtrate, and heat in a water bath for 15 minutes: this solution responds to the Qualitative Tests <1.09> for phosphate.

pH <2.54> Dissolve 0.10 g of Fosfestrol in 30 mL of water: the pH of this solution is between 1.0 and 2.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Fosfestrol in 15 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.10 g of Fosfestrol in 30 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid, 30 mL of ethanol (95) and water to make 50 mL (not more than 0.248%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Fosfestrol according to Method 4, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5). Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fosfestrol according to Method 3, and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 5) (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.4 g of Fosfestrol, dissolve in a mixture of water and formamide (1:1) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately 0.112 g of monobasic potassium phosphate, previously dried in a desiccator (silica gel) to constant mass, dissolve in 10 mL of diluted sulfuric acid (1 in 10) and water to make exactly 1000 mL. Measure exactly 10 mL of this solution, add 100 mL of formamide and water to make exactly 200 mL, and use this solution as the standard solution. Measure exactly 10 mL each of the sample solution and standard solution, and place in a 25-mL volumetric flask, respectively. To each of these solutions add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make 25 mL, and allow to stand at $20 \pm 1^\circ\text{C}$ for 30 minutes. Perform the test with these solutions, using a solution obtained in the same manner with 10 mL of a mixture of water and formamide (1:1) as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>. Determine the absorbances, A_T and A_S , of the solutions obtained from the sample solution and standard solution at 740 nm: the amount of free phosphoric acid is not more than 0.2%.

Amount (%) of free phosphoric acid (H_3PO_4)
= $(A_T/A_S) \times (1/W) \times 80.65$

W: Amount (mg) of Fosfestrol

(6) Related substances—Dissolve 20 mg of Fosfestrol in 100 mL of the mobile phase, and use this solution as the sample solution. Measure exactly 5 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 3 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the peak of fosfestrol from the sample solution is not larger than the peak area of fosfestrol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of potassium dihydrogen phosphate (1 in 500), acetonitrile and tetrabutylammonium hydroxide TS (70:30:1).

Flow rate: Adjust the flow rate so that the retention time of fosfestrol is about 8 minutes.

Selection of column: Dissolve 0.02 g of Fosfestrol and 8 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of methyl parahydroxybenzoate and fosfestrol in this order with the resolution between these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of fosfestrol obtained from 10 μ L of the standard solution is between 5 mm and 15 mm.

Time span of measurement: Three times as long as the retention time of fosfestrol.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.2 g of Fosfestrol, previously dried, dissolve in 60 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). The end point is the second equivalent point. Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 10.71 \text{ mg of } C_{18}H_{22}O_8P_2 \end{aligned}$$

Containers and storage Containers—Tight containers.

Fosfestrol Tablets

Diethylstilbestrol Diphosphate Tablets

ホスフェストロール錠

Fosfestrol Tablets contain not less than 93% and not more than 107% of the labeled amount of fosfestrol ($C_{18}H_{22}O_8P_2$; 428.31).

Method of preparation Prepare as directed under Tablets, with Fosfestrol.

Identification (1) To a quantity of powdered Fosfestrol Tablets, equivalent to 0.5 g of Fosfestrol according to the labeled amount, add 50 mL of 0.1 mol/L hydrochloric acid TS, shake well, and filter. To the filtrate add 100 mL of diethyl ether, extract, and evaporate carefully the diethyl ether extract on a water bath to dryness. Proceed with 0.015 g of the residue as directed in the Identification (1) under Fosfestrol.

(2) Dry 0.01 g of the residue obtained in (1) at 105°C for 4 hours, and determine the infrared absorption spectrum as

directed in the potassium bromide disk method under Infrared Spectrometry <2.25>: it exhibits absorption at the wave numbers of about 2970 cm^{-1} , 1605 cm^{-1} , 1505 cm^{-1} , 1207 cm^{-1} and 1006 cm^{-1} .

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Fosfestrol Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water. Take 20 mL or more of the dissolved solution 20 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent, add a solution of sodium hydroxide (1 in 250) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Fosfestrol Reference Standard, previously dried at 105°C for 4 hours, and dissolve in a solution of sodium hydroxide (1 in 250) to make exactly 100 mL. Pipet 2 mL of this solution, add a solution of sodium hydroxide (1 in 250) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

The dissolution rate of Fosfestrol Tablets in 20 minutes is not less than 80%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ \text{of fosfestrol } (C_{18}H_{22}O_8P_2) \\ = W_S \times (A_T/A_S) \times (1/C) \times 180 \end{aligned}$$

W_S : Amount (mg) of Fosfestrol Reference Standard

C : Labeled amount (mg) of fosfestrol ($C_{18}H_{22}O_8P_2$) in 1 tablet

Assay Weigh accurately not less than 20 Fosfestrol Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 1 g of fosfestrol ($C_{18}H_{22}O_8P_2$) according to the labeled amount, add 100 mL of a solution of sodium hydroxide (1 in 125), shake well, add water to make exactly 500 mL. Filter this solution, discard the first 30 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add 30 mL of a solution of sodium hydroxide (1 in 125) and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of Fosfestrol Reference Standard, previously dried at 105°C for 4 hours, and dissolve in a solution of sodium hydroxide (1 in 125) to make exactly 50 mL. Pipet 1 mL of this solution, add 10 mL of a solution of sodium hydroxide (1 in 125) and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

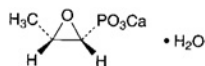
$$\begin{aligned} \text{Amount (mg) of fosfestrol } (C_{18}H_{22}O_8P_2) \\ = W_S \times (A_T/A_S) \times (25/2) \end{aligned}$$

W_S : Amount (mg) of Fosfestrol Reference Standard

Containers and storage Containers—Tight containers.

Fosfomycin Calcium Hydrate

ホスホマイシンカルシウム水和物



$C_3H_5CaO_4P \cdot H_2O$: 194.14

Monocalcium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate monohydrate [26016-98-8]

Fosfomycin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.

It contains not less than 725 μ g (potency) and not more than 805 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Calcium Hydrate is expressed as mass (potency) of fosfomycin ($C_3H_7O_4P$: 138.06).

Description Fosfomycin Calcium Hydrate occurs as a white crystalline powder.

It is slightly soluble in water, and practically insoluble in methanol and in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Fosfomycin Calcium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the spectrum of a solution of Fosfomycin Calcium Hydrate in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits a double signal at around δ 1.5 ppm, a double signal at around δ 2.9 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.4 ppm.

(3) A solution of Fosfomycin Calcium Hydrate (1 in 500) responds to the Qualitative Tests <1.09> (3) for calcium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-2.5 - -5.4^\circ$ (0.5 g calculated on the anhydrous bases, 0.4 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, pH 8.5, 10 mL, 100 mm).

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Calcium Hydrate, add 40 mL of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogenphosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Calcium, and use the solution so

obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at $20 \pm 1^\circ C$, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as a blank, and determine the absorbances at 740 nm, A_T , A_S and A_B , of the sample solution, the standard solution and the blank solution: the content of phosphorus is 15.2 – 16.7%.

$$\begin{aligned} & \text{Amount (mg) of phosphorus (P)} \\ & = W_S \times \{(A_T - A_B)/(A_S - A_B)\} \times 0.22760 \end{aligned}$$

W_S : Amount (mg) of potassium dihydrogenphosphate

Calcium Content Weigh accurately about 0.2 g of Fosfomycin Calcium Hydrate, add 4 mL of 1 mol/L Hydrochloric acid TS, and shake well until the sample is completely dissolved. To this solution add 100 mL of water, 9 mL of sodium hydroxide TS and 0.1 g of methylthymol blue-sodium chloride indicator, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from clear blue to gray or gray-purple: calcium content is 19.6 – 21.7%. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS = 2.004 mg of Ca

Purity (1) Heavy metals <1.07>—To 1.0 g of Fosfomycin Calcium Hydrate add 40 mL of 0.25 mol/L acetic acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fosfomycin Calcium Hydrate according to Method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 12.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Proteus* sp. (MB838)

(ii) Culture medium—Dissolve 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.

(iii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at $37^\circ C$ for 40 – 48 hours. Subcultures at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at $37^\circ C$ for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organ-

ism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10°C or below and use within 7 days. Add 1.0–2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the deeded agar layer.

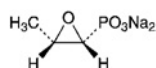
(iv) Standard solutions—Weigh accurately an amount of Fosfomycin Phenethylammonium Reference Standard equivalent to about 20 mg (potency), dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Fosfomycin Calcium Hydrate equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Fosfomycin Sodium

ホスホマイシンナトリウム



$C_3H_5Na_2O_4P$: 182.02

Disodium (2*R*,3*S*)-3-methyloxiran-2-ylphosphonate
[26016-99-9]

Fosfomycin Sodium is the sodium salt of a substance having antibacterial activity produced by the growth of *Streptomyces fradiae* or by the chemical synthesis.

It contains not less than 725 µg (potency) and not more than 770 µg (potency) per mg, calculated on the anhydrous basis. The potency of Fosfomycin Sodium is expressed as mass (potency) of fosfomycin ($C_3H_7O_4P$: 138.06).

Description Fosfomycin Sodium occurs as a white crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

Identification (1) Determine the infrared absorption spectrum of Fosfomycin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at

the same wave numbers.

(2) Determine the spectrum of a solution of Fosfomycin Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 300), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H): it exhibits a double signal at around δ 1.5 ppm, a duple double signal at around δ 2.8 ppm, a multiple signal at around δ 3.3 ppm, and no signal at around δ 1.3 ppm.

(3) A solution of Fosfomycin Sodium (1 in 500) responds to the Quantitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-3.5 - -5.5^\circ$ (0.5 g calculated on the anhydrous bases, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.70 g of Fosfomycin Sodium in 10 mL of water: the pH of the solution is between 8.5 and 10.5.

Phosphorus Content Weigh accurately about 0.1 g of Fosfomycin Sodium, add 40 mL of a solution of sodium periodate (107 in 10,000) and 2 mL of perchloric acid, and heat in a water bath for 1 hour. After cooling, add water to make exactly 200 mL. Pipet 10 mL of this solution, and add 1 mL of potassium iodide TS. To this solution add sodium thiosulfate TS until the solution is colorless, add water to make exactly 100 mL, and use this solution as the sample stock solution. Separately, weigh accurately about 70 mg of potassium dihydrogenphosphate, proceed with this solution in the same manner as directed for the preparation of the sample stock solution, and use the solution so obtained as the standard stock solution. Proceed and prepare a solution in the same manner for the preparation of the sample stock solution without using Fosfomycin Sodium, and use the solution so obtained as the blank stock solution. Pipet 5 mL each of the sample stock solution, the standard stock solution, and the blank stock solution, add 2.5 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, mix, and add water to make exactly 25 mL, and use these solutions as the sample solution, the standard solution, and the blank solution, respectively. After allowing these solutions to stand for 30 minutes at $20 \pm 1^\circ C$, perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as a blank, and determine the absorbances at 740 nm, A_T , A_S and A_B , of the sample solution, the standard solution and the blank solution: the content of phosphorus is 16.2–17.9%.

$$\begin{aligned} &\text{Amount (mg) of phosphorus (P)} \\ &= W \times \{(A_T - A_B)/(A_S - A_B)\} \times 0.22760 \end{aligned}$$

W : Amount (mg) of potassium dihydrogenphosphate

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Fosfomycin Sodium in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Fosfomycin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fosfomycin Sodium according to Method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 3.0% (0.2 g, volumetric titra-

tion, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- (i) Test organism—*Proteus* sp. (MB838)
- (ii) Culture medium—Mix 5.0 g of peptone, 3.0 g of meat extract, 2.0 g of yeast extract, and 15 g of agar in 1000 mL of water, sterilize, and use as the agar media for base layer and seed layer with the pH of between 6.5 and 6.6 after sterilization.
- (iii) Seeded agar layer—Incubate the test organism on the slant of the agar medium for transferring test organisms at 37 °C for 40 – 48 hours. Subcultures at least three times. Inoculate the grown organisms onto the surface of 300 mL of the agar medium for transferring test organisms in a Roux bottle, incubate at 37 °C for 40 – 48 hours, and suspend the grown organisms in about 30 mL of water. To the suspension add water, and use this as the stock suspension of test organism. The amount of the water to be added is adjust so that the percent transmission at 560 nm of the suspension diluted ten times with water is 17%. Keep the stock suspension at 10 °C or below and use within 7 days. Add 1.0 – 2.0 mL of the stock suspension of test organism to 100 mL of the agar medium for seed layer previously kept at 48 °C, mix thoroughly, and use this as the deeded agar layer.

(iv) Standard solutions—Weigh accurately an amount of Fosfomycin Phenethylammonium Reference Standard equivalent to about 20 mg (potency), dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 °C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Fosfomycin Sodium equivalent to about 20 mg (potency), and dissolve in 0.05 mol/L Tris buffer solution, pH 7.0 to make exactly 50 mL. To exactly a suitable amount of this solution add 0.05 mol/L Tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Fosfomycin Sodium for Injection

注射用ホスホマイシシナトリウム

Fosfomycin Sodium for Injection is a preparation for injection which is dissolved before use. It contains not less than 90.0% and not more than 110.0% of the labeled amount of fosfomycin (C₃H₇O₄P: 138.06).

Method of preparation Prepare as directed under Injections, with Fosfomycin Sodium.

Description Fosfomycin Sodium for Injection occurs as a

white crystalline powder.

Identification (1) Dissolve about 0.1 g of Fosfomycin Sodium for Injection in 3 mL of a solution of perchloric acid (1 in 4), add 1 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath at 60 °C for 30 minutes. After cooling, add 50 mL of water, neutralize with saturated sodium hydrogen carbonate solution, and add 1 mL of potassium iodide TS; the solution does not reveal a red color, while the blank solution reveals a red color.

(2) To 2 mL of a solution of Fosfomycin Sodium for Injection (1 in 250) add 1 mL of perchloric acid and 2 mL of 0.1 mol/L sodium periodate solution, and heat in a water bath for 10 minutes. After cooling, add 1 mL of ammonium molybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, and allow to stand for 30 minutes: a blue color develops.

(3) Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 0.1 g (potency) of Fosfomycin Sodium, in 50 mL of water. Perform the test with this solution as directed in the Identification (3) under Fosfomycin Sodium.

pH <2.54> The pH of a solution prepared by dissolving an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium according to the labeled amount, in 20 mL of water is between 6.5 and 8.5.

Purity Clarity and color of solution—Dissolve an amount of Fosfomycin Sodium for Injection, equivalent to 1.0 g (potency) of Fosfomycin Sodium according to the labeled amount, in 10 mL of water: the solution is clear and colorless.

Water <2.48> Not more than 4.0% (25 mg, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.025 EU/mg (potency).

Uniformity of dosage unit <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, seeded agar layer, and standard solutions—Proceed as directed in the Assay under Fosfomycin Sodium.

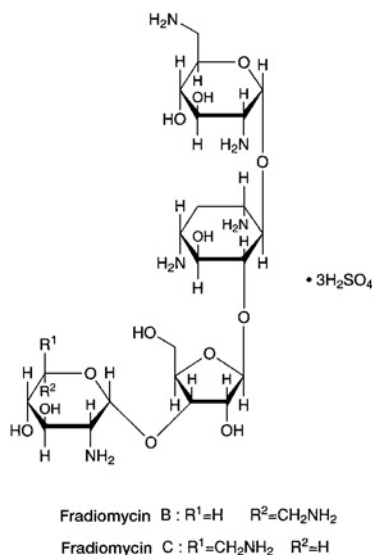
(ii) Sample solutions—Weigh accurately the mass of the contents of not less than 10 Fosfomycin Sodium for Injection. Weigh accurately an amount of the content, equivalent to about 20 mg (potency) of Fosfomycin Sodium according to the labeled amount, and dissolve in 0.05 mol/L tris buffer solution, pH 7.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.05 mol/L tris buffer solution, pH 7.0 to make solutions so that each mL contains 10 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injections may be used.

Fradiomycin Sulfate

Neomycin Sulfate

フラジオマイシン硫酸塩



C₂₃H₄₆N₆O₁₃·3H₂SO₄: 908.88

Fradiomycin Sulfate B

2,6-Diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-[2,6-diamino-2,6-dideoxy- β -L-idopyranosyl-(1 \rightarrow 3)- β -D-ribofuranosyl-(1 \rightarrow 5)]-2-deoxy-D-streptomine trisulfate [119-04-0, Neomycin B]

Fradiomycin Sulfate C

2,6-Diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-[2,6-diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 3)- β -D-ribofuranosyl-(1 \rightarrow 5)]-2-deoxy-D-streptomine trisulfate [66-86-4, Neomycin C] [1405-10-3, Neomycin Sulfate]

Fradiomycin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Streptomyces fradiae*.

It, when dried, contains not less than 623 μ g (potency) and not more than 740 μ g (potency) per mg. The potency of Fradiomycin Sulfate is expressed as mass (potency) of fradiomycin (C₂₃H₄₆N₆O₁₃: 614.64).

Description Fradiomycin Sulfate occurs as a white to light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 50 mg each of Fradiomycin Sulfate and Fradiomycin Sulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the

plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the R_f values of the principal spots from the sample solution and the standard solution are not different each other.

(2) A solution of Fradiomycin Sulfate (1 in 20) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> [α]_D²⁰: +53.5 – +59.0° (1 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Fradiomycin Sulfate in 10 mL of water is between 5.0 and 7.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Fradiomycin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Fradiomycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.63 g of Fradiomycin Sulfate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, ammonia solution (28) and dichloromethane (3:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 110°C for 15 minutes: the spot at around R_f 0.4 from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 8.0% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- | | |
|---|---------|
| (i) Test organism— <i>Staphylococcus aureus</i> ATCC 6538 P | |
| (ii) Agar medium for seed and base layer | |
| Glucose | 1.0 g |
| Peptone | 6.0 g |
| Meat extract | 1.5 g |
| Yeast extract | 3.0 g |
| Sodium chloride | 2.5 g |
| Agar | 15.0 g |
| Water | 1000 mL |

Mix all the ingredients and sterilize. Adjust the pH after sterilization to 7.8 – 8.0 with sodium hydroxide TS.

(iii) Standard solutions—Weigh accurately an amount of Fradiomycin Sulfate Reference Standard, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so

that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

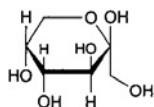
(iv) Sample solutions—Weigh accurately an amount of Fradiomycin Sulfate, previously dried, equivalent to about 50 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fructose

果糖



$\text{C}_6\text{H}_{12}\text{O}_6$: 180.16
 β -D-Fructopyranose [57-48-7]

Fructose, when dried, contains not less than 98.0% of $\text{C}_6\text{H}_{12}\text{O}_6$.

Description Fructose occurs as colorless to white crystals or crystalline powder. It is odorless and has a sweet taste.

It is very soluble in water, sparingly soluble in ethanol (95) and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Add 2 to 3 drops of a solution of Fructose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) Determine the infrared absorption spectrum of Fructose as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 4.0 g of Fructose in 20 mL of water: the pH of the solution is between 4.0 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 25.0 g of Fructose in 50 mL of water: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 3.0 mL of Ferric Chloride Stock CS and 2.0 mL of Cupric Sulfate Stock CS, and add water to make 10.0 mL. To 3.0 mL of the solution add water to make 50 mL.

(2) Acidity—Dissolve 5.0 g of Fructose in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.01 mol/L

hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <1.14>—Perform the test with 2.0 g of Fructose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Sulfite—Dissolve 0.5 g of Fructose in 5 mL of water, and add 0.25 mL of 0.02 mol/L iodine: the color of the solution is yellow.

(6) Heavy metals <1.07>—Proceed with 5.0 g of Fructose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(7) Calcium—Dissolve 0.5 g of Fructose in 5 mL of water, add 2 to 3 drops of ammonia TS and 1 mL of ammonium oxalate TS, and allow to stand for 1 minute: the solution is clear.

(8) Arsenic <1.11>—Dissolve 1.5 g of Fructose in 5 mL of water, heat with 5 mL of dilute sulfuric acid and 1 mL of bromine TS on a water bath for 5 minutes, concentrate to 5 mL, and cool. Perform the test with this solution as the test solution (not more than 1.3 ppm).

(9) 5-Hydroxymethylfurfurals—Dissolve 5.0 g of Fructose in 100 mL of water, and read the absorbance at 284 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance is not more than 0.32.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 4 g of Fructose, previously dried, dissolve in 0.2 mL of ammonia TS and 80 mL of water, and after standing for 30 minutes add water to make exactly 100 mL, and determine the optical rotation, α_D , in a 100-mm cell at $20 \pm 1^\circ\text{C}$ as directed under Optical Rotation Determination <2.49>.

$$\text{Amount (mg) of } \text{C}_6\text{H}_{12}\text{O}_6 = |\alpha_D| \times 1087.0$$

Containers and storage Containers—Tight containers.

Fructose Injection

果糖注射液

Fructose Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of fructose ($\text{C}_6\text{H}_{12}\text{O}_6$: 180.16).

Method of preparation Prepare as directed under Injections, with Fructose. No preservative is added.

Description Fructose Injection is a colorless to pale yellow, clear liquid. It has a sweet taste.

Identification (1) Take a volume of Fructose Injection, equivalent to 1 g of Fructose according to the labeled amount, dilute with water or concentrate on a water bath to 20 mL, if necessary, and use this solution as the sample solution. Add 2 to 3 drops of the sample solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

(2) To 10 mL of the sample solution obtained in (1) add 0.1 g of resorcinol and 1 mL of hydrochloric acid, and warm

in a water bath for 3 minutes: a red color develops.

pH <2.54> 3.0 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

Purity (1) Heavy metals <1.07>—Take a volume of Fructose Injection, equivalent to 5.0 g of Fructose, according to the labeled amount, and evaporate on a water bath to dryness. With the residue, proceed according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution.

(2) Arsenic <1.11>—Take a volume of Fructose Injection, equivalent to 1.5 g of Fructose, according to the labeled amount, dilute with water or concentrate on a water bath to 5 mL, if necessary, and add 5 mL of dilute sulfuric acid and 1 mL of bromine TS. Proceed as directed in the purity (8) under Fructose.

Residue on ignition <2.44> Measure exactly a volume of Fructose Injection, equivalent to 2 g of Fructose according to the labeled amount, evaporate on a water bath to dryness, and perform the test: the residue weighs not more than 2 mg.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Perform the test with Fructose Injection stored in a container in a volume exceeding 10 mL: it meets the requirement.

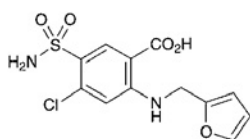
Assay Measure exactly a volume of Fructose Injection equivalent to about 4 g of fructose (C₆H₁₂O₆), add 0.2 mL of ammonia TS, dilute with water to make exactly 100 mL, shake well, and after allowing to stand for 30 minutes, determine the optical rotation, α_D, in a 100-mm cell at 20 ± 1°C as directed under Optical Rotation Determination <2.49>.

$$\text{Amount (mg) of fructose (C}_6\text{H}_{12}\text{O}_6) = |\alpha_D| \times 1087.0$$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Furosemide

フロゼミド



C₁₂H₁₁ClN₂O₅S: 330.74

4-Chloro-2-[(furan-2-ylmethyl)amino]-5-sulfamoylbenzoic acid [54-31-9]

Furosemide, when dried, contains not less than 98.0% and not more than 101.0% of C₁₂H₁₁ClN₂O₅S.

Description Furosemide occurs as white, crystals or crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

It is gradually colored by light.

Melting point: about 205°C (with decomposition).

Identification (1) Dissolve 25 mg of Furosemide in 10 mL of methanol. To 1 mL of this solution add 10 mL of 2 mol/L hydrochloric acid TS. Heat the solution under a reflux condenser on a water bath for 15 minutes, cool, and add 18 mL of sodium hydroxide TS to make weakly acidic: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of a solution of Furosemide in dilute sodium hydroxide TS (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Furosemide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Furosemide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Furosemide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Furosemide in 10 mL of a solution of sodium hydroxide (1 in 50): the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 2.6 g of Furosemide in 90 mL of dilute sodium hydroxide TS, add 2 mL of nitric acid, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.020%).

(3) Sulfate <1.14>—To 20 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.35 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.030%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Furosemide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 25 mg of Furosemide in 25 mL of the dissolving solution, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the dissolving solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak appeared ahead of the peak of furosemide is not more than 2/5 times the peak area of furosemide from the standard solution, the area of each peak appeared behind the peak of furosemide is not more than 1/4 times the peak area of furosemide from the standard solution, and the total area of these peaks is not more than 2 times the peak area of furosemide from the standard solution.

Dissolving solution—To 22 mL of acetic acid (100) add a mixture of water and acetonitrile (1:1) to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 272 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetic acid (100) (70:30:1).

Flow rate: Adjust the flow rate so that the retention time of furosemide is about 18 minutes.

Time span of measurement: About 2.5 times as long as the retention time of furosemide beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the dissolving solution to make exactly 50 mL. Confirm that the peak area of furosemide obtained from 20 μ L of this solution is equivalent to 3.2 to 4.8% of that obtained from 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of furosemide is not less than 7000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of furosemide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Furosemide, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow to blue (indicator: 3 drops of bromothymol blue TS). Perform a blank determination with a mixture of 50 mL of *N,N*-dimethylformamide and 15 mL of water, and make any necessary correction

$$\begin{aligned} &\text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ &= 33.07 \text{ mg of } C_{12}H_{11}ClN_2O_5S \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Furosemide Tablets

フロセミド錠

Furosemide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$; 330.74).

Method of preparation Prepare as directed under Tablets,

with Furosemide.

Identification (1) Shake well a quantity of powdered Furosemide Tablets, equivalent to 0.2 g of Furosemide according to the labeled amount, with 40 mL of acetone, and filter. To 0.5 mL of the filtrate add 10 mL of 2 mol/L hydrochloric acid TS, and heat under a reflux condenser on a water bath for 15 minutes. After cooling, add 18 mL of sodium hydroxide TS to make the solution slightly acetic: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines, producing a red to red-purple color.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, between 269 nm and 273 nm, and between 330 nm and 336 nm.

Purity To a quantity of powdered Furosemide Tablets, equivalent to 40 mg of Furosemide according to the labeled amount, add about 30 mL of acetone, shake well, and add acetone to make exactly 50 mL. Centrifuge the solution, add 3.0 mL of water to 1.0 mL of the supernatant liquid, cool in ice, add 3.0 mL of dilute hydrochloric acid and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Add 1.0 mL of ammonium amidosulfate TS, shake well, allow to stand for 3 minutes, add 1.0 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS, shake well, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with 1.0 mL of acetone as the blank: the absorbance at 530 nm is not more than 0.10.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Furosemide Tablets add a suitable amount of 0.05 mol/L sodium hydroxide TS, shake to disintegrate, then add 0.05 mol/L sodium hydroxide TS to make exactly *V* mL so that each mL contains about 0.4 mg of furosemide ($C_{12}H_{11}ClN_2O_5S$). Filter the solution, discard the first 10 mL of the filtrate, pipet the subsequent 2 mL of the filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount (mg) of furosemide } (C_{12}H_{11}ClN_2O_5S) \\ &= W_S \times (A_T/A_S) \times (V/50) \end{aligned}$$

W_S : Amount (mg) of Furosemide Reference Standard

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Furosemide Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test for a 20-mg tablet or 30 minutes after for a 40-mg tablet, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly *V* mL so that each mL contains about 10 μ g of furosemide ($C_{12}H_{11}ClN_2O_5S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide Reference Standard, previously

dried at 105°C for 4 hours, and dissolve in 5 mL of methanol, and add 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 277 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the dissolution rates for a 20-mg tablet in 15 minutes and for a 40-mg tablet in 30 minutes are not less than 80%, respectively.

Dissolution rate (%) with respect to the labeled amount of furosemide ($C_{12}H_{11}ClN_2O_5S$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$$

W_S : Amount (mg) of Furosemide Reference Standard

C: Labeled amount (mg) of furosemide ($C_{12}H_{11}ClN_2O_5S$) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Furosemide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 40 mg of furosemide ($C_{12}H_{11}ClN_2O_5S$), add about 70 mL of 0.05 mol/L sodium hydroxide TS, shake well, and add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL. Filter, discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Furosemide Reference Standard, previously dried at 105°C for 4 hours, and dissolve in 0.05 mol/L sodium hydroxide TS to make exactly 50 mL. Pipet 2 mL of this solution, add 0.05 mol/L sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 271 nm as directed under the Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} &\text{Amount (mg) of furosemide (} C_{12}H_{11}ClN_2O_5S \text{)} \\ &= W_S \times (A_T/A_S) \times 2 \end{aligned}$$

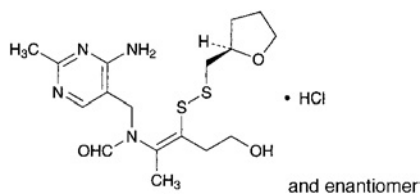
W_S : Amount (mg) of Furosemide Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Fursultiamine Hydrochloride

フルスルチアミン塩酸塩



$C_{17}H_{26}N_4O_3S_2 \cdot HCl$: 435.00

N-(4-Amino-2-methylpyrimidin-5-ylmethyl)-*N*-(1*Z*)-4-hydroxy-1-methyl-2-[(2*RS*)-tetrahydrofuran-2-ylmethyl-disulfanyl]but-1-en-1-yl]formamide monohydrochloride [804-30-8, Fursultiamine]

Fursultiamine Hydrochloride contains not less than

98.5% of $C_{17}H_{26}N_4O_3S_2 \cdot HCl$, calculated on the dried basis.

Description Fursultiamine Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a characteristic odor, and has a bitter taste.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 5 mg of Fursultiamine Hydrochloride in 6 mL of 0.1 mol/L hydrochloric acid TS, add 0.1 g of zinc powder, allow to stand for several minutes, and filter. To 3 mL of the filtrate, add 3 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS, then add 5 mL of 2-methyl-1-propanol, shake vigorously for 2 minutes, allow to stand to separate the 2-methyl-1-propanol layer, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. The fluorescence disappears by acidifying, and appears again by alkalinizing.

(2) Determine the infrared absorption spectrum of a solution of Fursultiamine Hydrochloride, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum, or with the spectrum of Fursultiamine Hydrochloride Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. If any differences appear, dissolve the Fursultiamine Hydrochloride in water, evaporate the water, and dry the residue in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and repeat the test.

(3) A solution of Fursultiamine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Clarity of solution—Dissolve 1.0 g of Fursultiamine Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Proceed with 1.5 g of Fursultiamine Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Fursultiamine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 0.10 g of Fursultiamine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following condition. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of fursultiamine from the sample solution is not larger than the peak area of fursultiamine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detector sensitivity: Adjust the detection sensitivity so that

the peak height of fursultiamine from 10 μ L of the standard solution is between 20 mm and 30 mm.

Time span of measurement: About 3 times as long as the retention time of fursultiamine.

Water <2.48> Not more than 5.0% (0.3 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 55 mg each of Fursultiamine Hydrochloride and Fursultiamine Hydrochloride Reference Standard (previously determined the water <2.48> in the same manner as Fursultiamine Hydrochloride) and dissolve each in 50 mL of water, and add exactly 10 mL each of the internal standard solution, then add water to make exactly 100 mL. To 8 mL each of the solution add water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of fursultiamine to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of } C_{17}H_{26}N_4O_3S_2 \cdot HCl \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Fursultiamine Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of isopropyl 4-aminobenzoate in ethanol (95) (3 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 1.01 g of sodium 1-heptane sulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 675 mL of this solution add 325 mL of a mixture of methanol and acetonitrile (3:2).

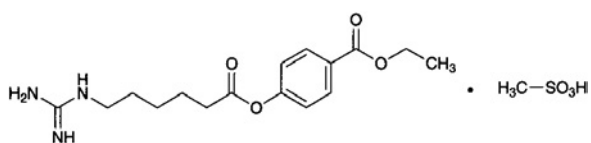
Flow rate: Adjust the flow rate so that the retention time of Fursultiamine is about 9 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions and calculate the resolution. Use a column giving elution of fursultiamine and the internal standard in this order with the resolution between these peaks being not less than 10.

Containers and storage Containers—Tight containers.

Gabexate Mesilate

ガベキサートメシル酸塩



$C_{16}H_{23}N_3O_4 \cdot CH_4O_3S$: 417.48

Ethyl 4-(6-guanidinohexanoyloxy)benzoate

monomethanesulfonate [56974-61-9]

Gabexate Mesilate, when dried, contains not less than 98.5% of $C_{16}H_{23}N_3O_4 \cdot CH_4O_3S$.

Description Gabexate Mesilate occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 4 mL of a solution of Gabexate Mesilate (1 in 2000) add 2 mL of 1-naphthol TS and 1 mL of diacetyl TS, and allow to stand for 10 minutes: a red color develops.

(2) Dissolve 1 g of Gabexate Mesilate in 5 mL of water, add 2 mL of sodium hydroxide TS, and heat in a water bath for 5 minutes. After cooling, add 2 mL of dilute nitric acid and 5 mL of ethanol (95), shake, add 5 drops of iron (III) chloride TS, and shake: a purple color develops.

(3) Determine the absorption spectrum of a solution of Gabexate Mesilate (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gabexate Mesilate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 0.1 g of Gabexate Mesilate add 0.2 g of sodium hydroxide, fuse by heating gently, and continue the heating for 20 to 30 seconds. After cooling, add 0.5 mL of water and 3 mL of dilute hydrochloric acid, and warm: the gas evolved changes a potassium iodate-starch paper to blue.

pH <2.54> Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the pH of the solution is between 4.5 and 5.5.

Melting point <2.60> 90 – 93°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Gabexate Mesilate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Gabexate Mesilate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Dissolve 2.0 g of Gabexate Mesilate in 20 mL of 1 mol/L hydrochloric acid TS by heating in a water bath, and continue the heating for 20 minutes. After cooling, centrifuge, and use 10 mL of the supernatant liquid as the test solution. Perform the test (not more than 2 ppm).

(4) Ethyl parahydroxybenzoate—Weigh 50 mg of Gabexate Mesilate, previously dried, and dissolve in dilute ethanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, dissolve 5.0 mg of ethyl parahydroxybenzoate in dilute ethanol to make exactly 100 mL. Pipet 1 mL of this solution, and add dilute ethanol to make exactly 20 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of ethyl parahydroxybenzoate to that of the internal standard: Q_T is not larger than Q_S .

Internal standard solution—A solution of butyl parahydroxybenzoate in dilute ethanol (1 in 5000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

(5) Related substances—Dissolve 0.20 g of Gabexate Mesilate in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate until it has no acetic odor. Spray evenly a solution of 8-quinolinol in acetone (1 in 1000) on the plate, and after air-drying, spray evenly bromine-sodium hydroxide TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Gabexate Mesilate and Gabexate Mesilate Reference Standard, previously dried, and dissolve each in dilute ethanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 3 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of gabexate to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } C_{16}H_{23}N_3O_4 \cdot CH_4O_3S \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Gabexate Mesilate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in dilute ethanol (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol, a solution of sodium lauryl sulfate (1 in 1000), a solution of sodium 1-heptane sulfonate (1 in 200) and acetic acid (100) (540:200:20:1).

Flow rate: Adjust the flow rate so that the retention time of gabexate is about 13 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating condi-

tions, the internal standard and gabexate are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of gabexate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

β -Galactosidase (*Aspergillus*)

β -ガラクトシダーゼ (アスペルギルス)

[9031-11-2]

β -Galactosidase (*Aspergillus*) contains an enzyme produced by *Aspergillus oryzae*. It is an enzyme drug having lactose decomposition activity.

It contains 8000 to 12000 units per g.

Usually, it is diluted with a mixture of Maltose Hydrate and Dextrin, Maltose Hydrate and D-Mannitol, or Maltose Hydrate, Dextrin and D-Mannitol.

Description β -Galactosidase (*Aspergillus*) occurs as a white to light yellow powder.

It is slightly soluble in water with a turbidity, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 25 mg of β -Galactosidase (*Aspergillus*) in 100 mL of water, then to 1 mL of this solution add 9 mL of lactose substrate TS, and stand at 30°C for 10 minutes. To 1 mL of this solution add 6 mL of glucose detection TS, and stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.1 g of β -Galactosidase (*Aspergillus*) in 100 mL of water, and filter the solution if necessary. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Odor— β -Galactosidase (*Aspergillus*) has no any rancid odor.

(2) Heavy metals <1.07>—Proceed with 1.0 g of β -Galactosidase (*Aspergillus*) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of β -Galactosidase (*Aspergillus*) according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 9.0% (0.5 g, in vacuum, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 3% (0.5 g).

Nitrogen content Weigh accurately about 70 mg of β -Galactosidase (*Aspergillus*), and perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is between 0.5% and 5.0%, calculated on the dried basis.

Assay (i) Substrate solution: Dissolve 0.172 g of 2-nitrophenyl- β -D-galactopyranoside in disodium hydrogenphosphate-citric acid buffer solution, pH 4.5 to make 100 mL.

(ii) Procedure: Weigh accurately about 25 mg of β -Galactosidase (*Aspergillus*), dissolve in water to make exactly 100 mL, then pipet 2 mL of this solution, add water to make exactly 50 mL, and use this solution as the sample solution. Take exactly 3.5 mL of the substrate solution, stand at $30 \pm 0.1^\circ\text{C}$ for 5 minutes, add exactly 0.5 mL of the sample solution, immediately mix, and stand at $30 \pm 0.1^\circ\text{C}$ for exactly 10 minutes, then add exactly 1 mL of sodium carbonate TS and mix immediately. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbance, A_1 , of this solution at 420 nm using water as the control. Separately, take exactly 3.5 mL of the substrate solution, add exactly 1 mL of sodium carbonate TS and mix, then add exactly 0.5 mL of the sample solution and mix. Determine the absorbance, A_2 , of this solution in the same manner as above.

$$\begin{aligned} &\text{Units per g of } \beta\text{-Galactosidase (Aspergillus)} \\ &= (1/W) \times \{(A_1 - A_2)/0.917\} \times (1/0.5) \times (1/10) \end{aligned}$$

0.917: Absorbance of 1 $\mu\text{mol}/5$ mL of *o*-nitrophenol

W : Amount (g) of the sample in the sample solution per mL

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl- β -D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Storage—In a cold place.

β -Galactosidase (Penicillium)

β -ガラクトシダーゼ (ペニシリウム)

[9031-11-2]

β -Galactosidase (*Penicillium*) contains an enzyme, having lactose decomposition activity, produced by *Penicillium multicolor*.

It contains not less than 8500 units and not more than 11,500 units in each g.

Usually, it is diluted with D-mannitol.

Description β -Galactosidase (*Penicillium*) occurs as a white to pale yellowish white, crystalline powder or powder.

It is soluble in water with a turbidity, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 0.05 g of β -Galactosidase (*Penicillium*) in 100 mL of water, then to 0.2 mL of this solution add 0.2 mL of lactose substrate TS, and allow to stand at 30°C for 10 minutes. To this solution add 3 mL of glucose detection TS, and allow to stand at 30°C for 10 minutes: a red to red-purple color develops.

(2) Dissolve 0.15 g of β -Galactosidase (*Penicillium*) in 100 mL of water, filter if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maxi-

mum between 278 nm and 282 nm.

Purity (1) Odor— β -Galactosidase (*Penicillium*) has no any rancid odor.

(2) Heavy metals <1.07>—Proceed with 1.0 g of β -Galactosidase (*Penicillium*) according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of β -Galactosidase (*Penicillium*) according to Method 3, and perform the test (not more than 2 ppm).

(4) Nitrogen—Weigh accurately about 0.1 g of β -Galactosidase (*Penicillium*), and perform the test as directed under Nitrogen Determination <1.08>: not more than 3 mg of nitrogen (N: 14.01) is found for each labeled 1000 Units.

(5) Protein contaminants—Dissolve 0.15 g of β -Galactosidase (*Penicillium*) in 4 mL of water, and use this solution as the sample solution. Perform the test with 15 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak having retention time of about 19 minutes is not more than 75% of the total area of all peaks, and the areas of peaks other than the peaks having retention times of about 3, 16 and 19 minutes are not more than 15% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7.5 mm in inside diameter and about 75 mm in length, packed with strongly acidic ion-exchange resin for liquid chromatography of sulfopropyl group-binding hydrophilic polymer (10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C .

Mobile phase: A solution obtained by dissolving 2.83 g of sodium acetate in 1000 mL of water, and adjusting to pH 4.5 with acetic acid (100) (mobile phase A), and a solution obtained by dissolving 29.2 g of sodium chloride in 1000 mL of mobile phase A (mobile phase B).

Flow system: Adjust a linear concentration gradient from the mobile phase A to the mobile phase B immediately after injection of the sample so that the retention times of non-retaining protein and the enzyme protein are about 3 minutes and 19 minutes, respectively, when the flow runs 0.8 mL per minute, and then continue the running of the mobile phase B.

Selection of column: Dissolve 15 mg of β -lactoglobulin in 4.5 mL of water, add 0.5 mL of a solution of cytosine (1 in 5000), and use this solution as the column-selecting solution. Proceed with 15 μL of the column-selecting solution under the above operating conditions, and calculate the resolution. Use a column giving elution of cytosine and β -lactoglobulin in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of β -lactoglobulin from 15 μL of the column-selecting solution is between 5 cm and 14 cm.

Time span of measurement: About 1.4 times as long as the retention time of β -lactoglobulin.

Loss on drying <2.41> Not more than 5.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 2% (1 g).

Assay (i) Substrate solution—Dissolve 0.603 g of 2-nitrophenyl-β-D-galactopyranoside in disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 to make 100 mL.

(ii) Procedure—Weigh accurately about 0.15 g of β-Galactosidase (Penicillium), dissolve in water with thorough shaking to make exactly 100 mL, and allow to stand at room temperature for an hour. Pipet 2 mL of this solution, add disodium hydrogen phosphate-citric acid buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the sample solution. Transfer exactly 0.5 mL of the sample solution to a test tube, stand at 30 ± 0.1°C for 10 minutes, add exactly 0.5 mL of the substrate solution previously kept at 30 ± 0.1°C, then mix immediately, and stand at 30 ± 0.1°C for exactly 10 minutes. Then add exactly 1 mL of sodium carbonate TS, mix immediately to stop the reaction. To this solution add exactly 8 mL of water, mix, and use as the colored sample solution. Separately, pipet 0.5 mL of disodium hydrogen phosphate-citric acid buffer solution, pH 4.5, then proceed in the same manner as the sample solution, and use the solution so obtained as the colored blank solution. Perform the test with the colored sample solution and the colored blank solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_B , at 420 nm.

$$\begin{aligned} &\text{Units per g of } \beta\text{-Galactosidase (Penicillium)} \\ &= (1/W) \times \{(A_T - A_B)/0.459\} \times (1/10) \end{aligned}$$

0.459: Absorbance of 1 μmol/10 mL of *o*-nitrophenol

W : Amount (g) of the sample in 0.5 mL of the sample solution

Unit: One unit indicates an amount of the enzyme which decomposes 1 μmol of 2-nitrophenyl-β-D-galactopyranoside in 1 minute under the above conditions.

Containers and storage Containers—Tight containers.

Gallium (⁶⁷Ga) Citrate Injection

クエン酸ガリウム (⁶⁷Ga) 注射液

Gallium (⁶⁷Ga) Citrate Injection is an aqueous solution for injection containing gallium-67 (⁶⁷Ga) in the form of gallium citrate.

It conforms to the requirements of Gallium (⁶⁷Ga) Citrate Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Gallium (⁶⁷Ga) Citrate Injection is a clear, colorless or light red liquid.

Gas Gangrene Antitoxin, Equine

ガスエソウマ抗毒素

Gas Gangrene Antitoxin, Equine, is a liquid for in-

jection containing *Clostridium perfringens* (*C. welchii*) Type A antitoxin, *Clostridium septicum* (*Vibrion septique*) antitoxin and *Clostridium oedematiens* (*C. novyi*) antitoxin in immunoglobulin of horse origin.

It may contain also *Clostridium histolyticum* antitoxin.

It conforms to the requirements of Gas Gangrene Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Gas Gangrene Antitoxin, Equine, is a colorless to light yellow-brown, clear liquid or a slightly whitish turbid liquid.

Gelatin

ゼラチン

Gelatin is a product prepared from aqueous extract of raw collagen by heating. The raw collagen is obtained by acid or alkali treatment of the bone, skin, ligament or tendon of animals.

Description Gelatin occurs as colorless or white to light yellow-brown sheets, shreds, granules or powder. It is odorless and tasteless.

Gelatin is very soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

Gelatin does not dissolve in water, but slowly swells and softens when immersed in it, gradually absorbing water 5 to 10 times its own mass.

Gelatin derived from an acid-treated collagen exhibits an isoelectric point between pH 7.0 and 9.0, and Gelatin derived from an alkali-treated collagen exhibits an isoelectric point between pH 4.5 and 5.0.

Identification (1) To 5 mL of a solution of Gelatin (1 in 100) add chromium (VI) oxide TS or 2,4,6-trinitrophenol TS dropwise: a precipitate is formed.

(2) To 5 mL of a solution of Gelatin (1 in 5000) add tannic acid TS dropwise: the solution becomes turbid.

Purity (1) Foreign odor and water-insoluble substances—Dissolve 1.0 g of Gelatin in 40 mL of water by heating: the solution has no disagreeable odor. It is clear, or only slightly opalescent. The solution has no more color than Matching Fluid A.

(2) Sulfite—Take 20.0 g of Gelatin in a round-bottomed flask, dissolve in 150 mL of hot water, and add 3 to 5 drops of silicone resin, 5 mL of phosphoric acid and 1 g of sodium hydrogen carbonate. Attach a condenser, immediately distill the solution, immersing the end of the condenser into a receiver containing 50 mL of iodine TS, and continue the distillation until 50 mL of distillate is obtained. Acidify the distillate with 2 to 3 drops of hydrochloric acid, add 2 mL of barium chloride TS, and heat on a water bath until the color of iodine TS is discharged. Collect the precipitates, wash with water, and ignite: the mass of the residue is not more than 4.5 mg, but the mass of the residue obtained from Gelatin for use in the preparation of capsules and tablets is not more than 75 mg. Perform a blank determination, and make any necessary correction.

(3) Heavy metals <1.07>—Proceed with 0.5 g of Gelatin

according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(4) Arsenic <1.11>—Take 15.0 g of Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and heat until solution is effected. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following standard stain.

Standard stain: Proceed with 15 mL of Standard Arsenic Solution, instead of Gelatin, in the same manner (not more than 1 ppm).

(5) Mercury—Place 2.0 g of Gelatin in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), heat gently under a reflux condenser, and boil for 2 hours. If the solution becomes clear during boiling, reduce the temperature of the solution to about 60°C, add further 5 mL of a solution of potassium permanganate (3 in 50), boil again, and repeat the above-mentioned procedure until the precipitate of manganese dioxide remains for about 20 minutes. Cool, add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 150 mL, and use the solution as the sample solution. Perform the test as directed under Atomic Absorption Spectrophotometry <2.23> (Cold vapor type) using the sample solution. Place the sample solution in a sample water bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Determine the absorbance A_T of the sample solution at 253.7 nm when the indication of the recorder has risen rapidly and become constant. On the other hand, place 2.0 mL of Standard Mercury Solution in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), and proceed in the same manner as for the sample solution. Determine the absorbance A_S of the standard solution: A_T is not more than A_S (not more than 0.1 ppm).

Loss on drying Not more than 15.0%. Take about 1 g of Gelatin, accurately weighed, in a tared 200-mL beaker containing 10 g of sea sand (No. 1) previously dried at 110°C for 3 hours. Add 20 mL of water, allow to stand for 30 minutes with occasional shaking, evaporate to dryness on a water bath with occasional shaking, and dry the residue at 110°C for 3 hours.

Residue on ignition <2.44> Not more than 2.0% (0.5 g).

Containers and storage Containers—Tight containers.

Purified Gelatin

精製ゼラチン

Purified Gelatin is a product prepared from aqueous

extract of raw collagen by heating. The raw collagen is obtained by acid or alkali treatment of the bone, skin, ligament, or tendon of animals.

Description Purified Gelatin occurs as colorless to light yellow sheets, shreds, pellets or powder. It is odorless and tasteless.

It is very soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

Purified Gelatin does not dissolve in water. It slowly swells and softens when immersed in water, and absorbs water 5 to 10 times its own mass.

Purified Gelatin derived from an acid-treated collagen has an isoelectric point at pH 7.0 to 9.0, and Purified Gelatin derived from an alkali-treated collagen has an isoelectric point at pH 4.5 to 5.0.

Identification (1) To 5 mL of a solution of Purified Gelatin (1 in 100) add chromium (VI) oxide TS or 2,4,6-trinitrophenol TS dropwise: a precipitate is formed.

(2) To 5 mL of a solution of Purified Gelatin (1 in 5000) add tannic acid TS dropwise: the solution becomes turbid.

Purity (1) Foreign odor and water-insoluble substances—Dissolve 1.0 g of Purified Gelatin in 40 mL of water by heating: the solution is clear, colorless and free from any disagreeable odor when the layer of the solution is 20 mm in depth.

(2) Sulfite—Take 20.0 g of Purified Gelatin in a round-bottomed flask, dissolve in 150 mL of hot water, and add 3 to 5 drops of silicone resin, 5 mL of phosphoric acid and 1 g of sodium hydrogen carbonate. Attach a condenser, immediately distill the solution, immersing the end of the condenser into a receiver containing 50 mL of iodine TS, and continue the distillation until 50 mL of distillate is obtained. Acidify the distillate by dropwise addition of hydrochloric acid, add 2 mL of barium chloride TS, and heat on a water bath until the color of iodine TS is discharged. Collect the precipitates, wash with water, and ignite: the mass of the residue is not more than 1.5 mg. Perform a blank determination, and make any necessary correction.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Purified Gelatin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Place 15.0 g of Purified Gelatin in a flask, add 60 mL of diluted hydrochloric acid (1 in 5), and heat until solution is effected. Add 15 mL of bromine TS, heat until the excess of bromine is expelled, neutralize with ammonia TS, add 1.5 g of disodium hydrogen phosphate dodecahydrate, and allow to cool. To this solution add 30 mL of magnesia TS, allow to stand for 1 hour, and collect the precipitates. Wash the precipitates with five 10-mL portions of diluted ammonia TS (1 in 4), and dissolve in diluted hydrochloric acid (1 in 4) to make exactly 50 mL. Perform the test with 5 mL of this solution: the solution has no more color than the following standard solution.

Standard solution: Proceed with 15 mL of Standard Arsenic Solution, instead of Purified Gelatin, in the same manner (not more than 1 ppm).

(5) Mercury—Place 2.0 g of Purified Gelatin in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), heat gently under a reflux condenser, and boil for 2 hours. If

the solution becomes clear during boiling, reduce the temperature of the solution to about 60°C, add further 5 mL of a solution of potassium permanganate (3 in 50), boil again, and repeat the above-mentioned procedure until the precipitate of manganese dioxide remains for about 20 minutes. Cool, add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 150 mL, and use the solution as the sample solution. Perform the test as directed under Atomic Absorption Spectrophotometry <2.23> (Cold vapor type) using the sample solution. Place the sample solution in a sample water bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Determine the absorbance A_T of the sample solution at 253.7 nm when the indication of the recorder has risen rapidly and become constant. On the other hand, place 2.0 mL of Standard Mercury Solution in a decomposition flask, add 20 mL of diluted sulfuric acid (1 in 2) and 100 mL of a solution of potassium permanganate (3 in 50), and proceed in the same manner as for the sample solution. Determine the absorbance A_S of the standard solution: A_T is not more than A_S (not more than 0.1 ppm).

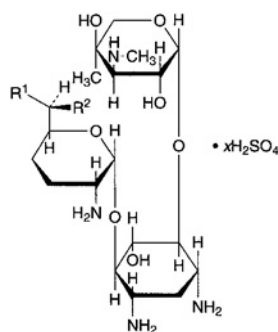
Loss on drying Not more than 15.0%. Take about 1 g of Purified Gelatin, accurately weighed, in a tared 200-mL beaker containing 10 g of sea sand (No. 1), previously dried at 110°C for 3 hours. Add 20 mL of water, allow to stand for 30 minutes with occasional shaking, evaporate on a water bath to dryness with occasional shaking, and dry the residue at 110°C for 3 hours.

Residue on ignition <2.44> Not more than 2.0% (0.5 g).

Containers and storage Containers—Tight containers.

Gentamicin Sulfate

ゲンタマイシン硫酸塩



Gentamicin Sulfate C_1 : $R^1 = CH_3$ $R^2 = NHCH_3$

Gentamicin Sulfate C_2 : $R^1 = CH_3$ $R^2 = NH_2$

Gentamicin Sulfate C_{1a} : $R^1 = H$ $R^2 = NH_2$

Gentamicin Sulfate C_1 :

(6*R*)-2-Amino-2,3,4,6-tetradeoxy-6-methylamino-6-methyl- α -D-erythro-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino- β -L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate

Gentamicin Sulfate C_2 :

(6*R*)-2,6-Diamino-2,3,4,6-tetradeoxy-6-methyl- α -D-erythro-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino- β -L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate

Gentamicin Sulfate C_{1a} :

2,6-Diamino-2,3,4,6-tetradeoxy- α -D-erythro-hexopyranosyl-(1→4)-[3-deoxy-4-*C*-methyl-3-methylamino- β -L-arabinopyranosyl-(1→6)]-2-deoxy-D-streptamine sulfate

[1405-41-0, Gentamicin Sulfate]

Gentamicin Sulfate is the sulfate of a mixture of aminoglycoside substances having antibacterial activity produced by the growth of *Micromonospora purpurea* or *Micromonospora echinospora*.

It contains not less than 590 μ g (potency) and not more than 775 μ g (potency) per mg, calculated on the dried basis. The potency of Gentamicin Sulfate is expressed as mass (potency) of gentamicin C_1 ($C_{21}H_{43}N_5O_7$: 477.60).

Description Gentamicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 50 mg of Gentamicin Sulfate in 1 mL of water, and add 2 drops of a solution of 1-naphthol in ethanol (95) (1 in 500). Gently superimpose this solution on 1 mL of sulfuric acid: a blue-purple color develops at the zone of contact.

(2) Dissolve 50 mg each of Gentamicin Sulfate and Gentamicin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², and without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapors: three principal spots from the sample solution are the same with the corresponding spots from the standard solution in color tone and the R_f value, respectively.

(3) Dissolve 50 mg of Gentamicin Sulfate in 5 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{25}$: +107 – +121° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.20 g of Gentamicin Sulfate in 5 mL of water is between 3.5 and 5.5.

Content ratio of the active principle Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 20

μL of the sample solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor. Determine the integral absorbances, A_a , A_b and A_c , of the colored spots of gentamicin C_1 (R_f value: about 0.3), gentamicin C_2 (R_f value: about 0.2) and gentamicin C_{1a} (R_f value: about 0.1), respectively, by a densitometer (wavelength: 450 nm) while covering the plate with a glass plate, and calculate these amounts by the following formulae: gentamicin C_1 is between 25% and 55%, gentamicin C_2 is between 25% and 50%, and gentamicin C_{1a} is between 5% and 30%.

$$\begin{aligned} \text{Amount (\%)} \text{ of gentamicin } C_1 \\ = \{A_a / (A_a + 1.35A_b + A_c)\} \times 100 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} \text{ of gentamicin } C_2 \\ = \{1.35A_b / (A_a + 1.35A_b + A_c)\} \times 100 \end{aligned}$$

$$\begin{aligned} \text{Amount (\%)} \text{ of gentamicin } C_{1a} \\ = \{A_c / (A_a + 1.35A_b + A_c)\} \times 100 \end{aligned}$$

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Gentamicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Gentamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Gentamicin Sulfate in water to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Separately, shake a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) in a separator, and allow the mixture to stand for more than 1 hour. To 20 mL of the lower layer so obtained add 0.5 mL of methanol, and use this as the developing solvent. Develop the plate with the developing solvent to a distance of about 17 cm in a developing container with a cover, having an opening of about 20 mm², without putting a filter paper in the container, and air-dry the plate. Allow the plate to stand in iodine vapor, and compare the colored spots while covering with a glass plate: the spots other than the spots of gentamicin C_1 (R_f value: about 0.3), gentamicin C_2 (R_f value: about 0.2) and gentamicin C_{1a} (R_f value: about 0.1) obtained from the sample solution are not more intense than the spot of gentamicin C_2 from the standard solution.

Loss on drying <2.41> Not more than 18.0% (0.15 g, reduced pressure not exceeding 0.67 kPa, 110°C, 3 hours). Handle the sample avoiding absorption of moisture.

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate

method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus epidermidis* ATCC 12228

(ii) Agar media for seed and base layer—	
Glucose	1.0 g
Peptone	6.0 g
Meat extract	1.5 g
Yeast extract	3.0 g
Sodium chloride	10.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 7.8 to 8.0 after sterilization.

(iii) Agar medium for transferring test organisms—Use the medium ii in 2) Medium for other organisms under (2) Agar media for transferring test organisms.

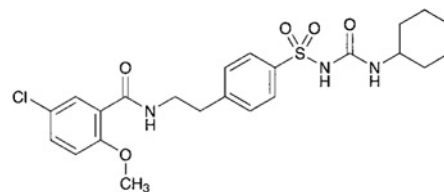
(iv) Standard solutions—Weigh accurately an amount of Gentamicin Sulfate Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 15°C or lower, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(v) Sample solutions—Weigh accurately an amount of Gentamicin Sulfate, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μg (potency) and 1 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Glibenclamide

グリベンクラミド



$C_{23}H_{28}ClN_3O_5S$: 494.00

4-[2-(5-Chloro-2-methoxybenzoylamino)ethyl]-*N*-(cyclohexylcarbamoyl)benzenesulfonamide [10238-21-8]

Glibenclamide, when dried, contains not less than 98.5% of $C_{23}H_{28}ClN_3O_5S$.

Description Glibenclamide occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in dimethylformamide, sparingly soluble

in chloroform, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Glibenclamide in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Glibenclamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Glibenclamide as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 169 – 174°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Glibenclamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Glibenclamide in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, chloroform and diluted ammonia TS (4 in 5) (11:7:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

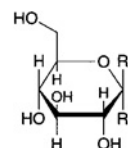
Assay Weigh accurately about 0.9 g of Glibenclamide, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination with a solution prepared by adding 18 mL of water to 50 mL of *N,N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 49.40 mg of $C_{23}H_{28}ClN_3O_5S$

Containers and storage Containers—Tight containers.

Glucose

ブドウ糖



α -D-Glucopyranose : R¹=H, R²=OH
 β -D-Glucopyranose : R¹=OH, R²=H

$C_6H_{12}O_6$: 180.16

D-Glucopyranose [50-99-7]

Glucose is α -D-glucopyranose, β -D-glucopyranose, or a mixture of them, and when dried, it contains not less than 99.5% of $C_6H_{12}O_6$.

Description Glucose occurs as white crystals or crystalline powder. It is odorless, and has a sweet taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification Add 2 to 3 drops of a solution of Glucose (1 in 20) to 5 mL of boiling Fehling's TS: a red precipitate is produced.

Purity (1) Clarity and color of solution—Add 25 g of Glucose to 30 mL of water in a Nessler tube, warm at 60°C in a water bath until solution is effected, cool, and add water to make 50 mL: the solution is clear and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 3.0 mL of Ferric Chloride stock CS, and 2.0 mL of Cupric Sulfate Stock CS, add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Acidity—Dissolve 5.0 g of Glucose in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS and 0.60 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(4) Sulfate <1.14>—Perform the test with 2.0 g of Glucose. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Heavy metals <1.07>—Proceed with 5.0 g of Glucose according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(6) Arsenic <1.11>—Dissolve 1.5 g of Glucose in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, and concentrate to 5 mL. After cooling, perform the test with this solution as the test solution (not more than 1.3 ppm).

(7) Dextrin—To 1.0 g of Glucose add 20 mL of ethanol (95), and boil under a reflux condenser: the solution is clear.

(8) Soluble starch and sulfite—Dissolve 1.0 g of Glucose in 10 mL of water, and add 1 drop of iodine TS: a yellow color develops.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C,

6 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 10 g of Glucose, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, allow to stand for 30 minutes, and determine the optical rotation, α_D , of this solution at $20 \pm 1^\circ\text{C}$ in a 100-mm cell as directed under Optical Rotation Determination <2.49>.

$$\text{Amount (mg) of } \text{C}_6\text{H}_{12}\text{O}_6 = \alpha_D \times 1895.4$$

Containers and storage Containers—Tight containers.

Glucose Injection

ブドウ糖注射液

Glucose Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of glucose ($\text{C}_6\text{H}_{12}\text{O}_6$; 180.16).

Method of preparation Prepare as directed under Injections, with Glucose. No preservative is added.

Description Glucose Injection is a clear, colorless liquid. It has a sweet taste. It occurs as a colorless to pale yellow, clear liquid when its labeled concentration exceeds 40%.

Identification Measure a volume of Glucose Injection, equivalent to 0.1 g of Glucose according to the labeled amount, and, if necessary, add water or evaporate on a water bath to a volume of 2 mL. Add 2 to 3 drops of the solution to 5 mL of boiling Fehling's TS: a red precipitate is produced.

pH <2.54> 3.5 – 6.5 In the case where the labeled concentration of the injection exceeds 5%, dilute to 5% with water before the test.

Purity 5-Hydroxymethylfurfural and related substances—Measure exactly a volume of Glucose Injection, equivalent to 2.5 g of Glucose according to the labeled amount, and add water to make exactly 100 mL. Determine the absorbance of this solution at 284 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.80.

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

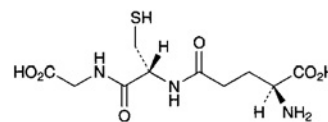
Assay Measure accurately a volume of Glucose Injection, equivalent to about 4 g of glucose ($\text{C}_6\text{H}_{12}\text{O}_6$), and add 0.2 mL of ammonia TS and water to make exactly 100 mL. Shake the solution well, allow to stand for 30 minutes, and determine the optical rotation, α_D , at $20 \pm 1^\circ\text{C}$ in a 100-mm cell as directed under Optical Rotation Determination <2.49>.

$$\text{Amount (mg) of glucose (C}_6\text{H}_{12}\text{O}_6) = \alpha_D \times 1895.4$$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Glutathione

グルタチオン



$\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6\text{S}$; 307.32

(2S)-2-Amino-4-[1-(carboxymethyl)carbamoyl-(2R)-2-sulfanylethylcarbamoyl]butanoic acid [70-18-8]

Glutathione, when dried, contains not less than 98.0% and not more than 101.0% of $\text{C}_{10}\text{H}_{17}\text{N}_3\text{O}_6\text{S}$.

Description Glutathione occurs as a white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Melting point: about 185°C (with decomposition).

Identification Determine the infrared absorption spectrum of Glutathione, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-15.5 - -17.5^\circ$ (after drying, 2 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Glutathione in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Glutathione according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Glutathione according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 50 mg of Glutathione in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10 \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak having the relative retention time of about 4 with respect to glutathione is not more than 3/4 times the peak area of glutathione from the standard solution, and the total area of the peaks other than the peak of glutathione is not more than the peak area of glutathione from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5 \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen phosphate and 2.02 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 970 mL of this solution add 30 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of glutathione is about 5 minutes.

Time span of measurement: About 6 times as long as the retention time of glutathione beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of glutathione obtained from 10 μ L of this solution is equivalent to 8 to 12% of that obtained from 10 μ L of the standard solution.

System performance: Dissolve 50 mg of glutathione, 10 mg of D-phenylglycine and 50 mg of ascorbic acid in 100 mL of water. When the procedure is run with 10 μ L of this solution under the above operating conditions, ascorbic acid, glutathione and D-phenylglycine are eluted in this order, and the resolutions between the peaks of ascorbic acid and glutathione and between the peaks of glutathione and D-phenylglycine are not less than 5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glutathione is not more than 1.5%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Glutathione, previously dried, dissolve in 50 mL of a solution of metaphosphoric acid (1 in 50), and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS
= 30.73 mg of C₁₀H₁₇N₃O₆S

Containers and storage Containers—Tight containers.

Glycerin

Glycerol

グリセリン

C₃H₈O₃: 92.09

Glycerin contains not less than 84.0% and not more than 87.0% of C₃H₈O₃.

Description Glycerin is a clear, colorless, viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum

of Glycerin as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.449 – 1.454

Specific gravity <2.56> d_{20}^{20} : 1.221 – 1.230

Purity (1) Color—Place 50 mL of Glycerin in a Nessler tube, and observe downward: the solution has no more color than the following control solution. Control solution: Place 0.40 mL of Ferric Chloride Colorimetric Stock Solution in a Nessler tube, and add water to make 50 mL.

(2) Acidity or alkalinity—To 2 mL of Glycerin add 8 mL of water and mix: the solution is neutral.

(3) Chloride <1.03>—Take 10.0 g of Glycerin, and perform the test: Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(4) Sulfate <1.14>—Take 10.0 g of Glycerin, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(5) Ammonium—To 5 mL of Glycerin add 5 mL of a solution of sodium hydroxide (1 in 10), and boil: the gas evolved does not change moistened red litmus paper to blue.

(6) Heavy metals <1.07>—Proceed with 5.0 g of Glycerin according to Method 1, and perform the test: Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(7) Calcium—To 5 mL of the solution obtained in (2) add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) Arsenic <1.11>—Prepare the test solution with 1.0 g of Glycerin according to Method 1, and perform the test (not more than 2 ppm).

(9) Acrolein, glucose, and other reducing substances—To 1.0 g of Glycerin add 1 mL of ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes: no yellow color is produced. Take the solution out of the water bath, add 3 drops of silver nitrate TS immediately, and allow to stand in a dark place for 5 minutes: the color of the solution does not change, and no turbidity is produced.

(10) Fatty acids and esters—Mix 50 g of Glycerin with 50 mL of freshly boiled and cooled water, add exactly 10 mL of 0.1 mol/L sodium hydroxide VS, boil the mixture for 15 minutes, cool, and titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: 0.1 mol/L sodium hydroxide VS consumed is not more than 3.0 mL (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

(11) Readily carbonizable substances—To 5 mL of Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has not more color than Matching Fluid H.

Water <2.48> 13 – 17% (0.1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Weigh accurately about 10 g of Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. After cooling, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

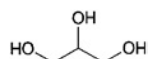
$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 9.209 \text{ mg of } C_3H_8O_3 \end{aligned}$$

Containers and storage Containers—Tight containers.

Concentrated Glycerin

Concentrated Glycerol

濃グリセリン



$C_3H_8O_3$: 92.09
Propane-1,2,3-triol [56-81-5]

Concentrated Glycerin contains not less than 98.0% and not more than 101.0% of glycerin ($C_3H_8O_3$), calculated of the anhydrous basis.

Description Concentrated Glycerin is a clear, colorless and viscous liquid. It has a sweet taste.

It is miscible with water and with ethanol (99.5).

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Concentrated Glycerin as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : Not less than 1.470.

Specific gravity <2.56> d_{20}^{20} : Not less than 1.258.

Purity (1) Color—Place 50 mL of Concentrated Glycerin in a Nessler tube, and observe downward: the solution has no more color than the following control solution.

Control solution: Pipet 0.40 mL of Ferric Chloride Colorimetric Stock Solution into a Nessler tube, and add water to make 50 mL.

(2) Acidity or alkalinity—To 2 mL of Concentrated Glycerin add 8 mL of water and mix: the solution is neutral.

(3) Chloride <1.03>—Take 10.0 g of Concentrated Glycerin, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.001%).

(4) Sulfate <1.14>—Take 10.0 g of Concentrated Glycerin, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(5) Ammonium—To 5 mL of Concentrated Glycerin add 5 mL of a solution of sodium hydroxide (1 in 10), and boil:

the gas evolved does not change moistened red litmus paper to blue.

(6) Heavy metals <1.07>—Proceed with 5.0 g of Concentrated Glycerin according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(7) Calcium—To 5 mL of the solution obtained in (2) add 3 drops of ammonium oxalate TS: the solution remains unchanged.

(8) Arsenic <1.11>—Prepare the test solution with 1.0 g of Concentrated Glycerin according to Method 1, and perform the test (not more than 2 ppm).

(9) Acrolein, glucose, or other reducing substances—To 1.0 g of Concentrated Glycerin add 1 mL of ammonia TS, mix, and warm in a water bath at 60°C for 5 minutes: no yellow color is produced. Take the solution out of the water bath, add 3 drops of silver nitrate TS immediately, and allow to stand in a dark place for 5 minutes: the color of the solution does not change, and no turbidity is produced.

(10) Fatty acids and esters—Mix 50 g of Concentrated Glycerin with 50 mL of freshly boiled and cooled water, add 10 mL of 0.1 mol/L sodium hydroxide VS, accurately measured, boil the mixture for 15 minutes, cool, and titrate the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS: not more than 3.0 mL of 0.1 mol/L sodium hydroxide VS is consumed (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

(11) Readily carbonizable substances—To 5 mL of Concentrated Glycerin add carefully 5 mL of sulfuric acid for readily carbonizable substances, mix gently at a temperature between 18°C and 20°C, and allow to stand for 1 hour between 15°C and 25°C: the solution has no more color than Matching Fluid H.

Water <2.48> Not more than 2.0% (6 g, volumetric titration, direct titration).

Residue on ignition <2.44> Weigh accurately about 10 g of Concentrated Glycerin in a tared crucible, heat to boiling, and fire to burn immediately. Cool, moisten the residue with 1 to 2 drops of sulfuric acid, and ignite cautiously to constant mass: the mass of the residue is not more than 0.01%.

Assay Weigh accurately about 0.2 g of Concentrated Glycerin, transfer into a glass-stoppered flask, add 50 mL of water, mix, add exactly 50 mL of sodium periodate TS, shake, and allow to stand in a dark place at a room temperature for about 30 minutes. Add 10 mL of a mixture of water and ethylene glycol (1:1), allow to stand for about 20 minutes, add 100 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make the necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 9.209 \text{ mg of } C_3H_8O_3 \end{aligned}$$

Containers and storage Containers—Tight containers.

Glycerin and Potash Solution

グリセリンカリ液

Method of preparation

Potassium Hydroxide	3 g
Glycerin	200 mL
Ethanol	250 mL
Aromatic substance	a suitable quantity
Water or Purified Water	a sufficient quantity

To make 1000 mL

Dissolve Potassium Hydroxide in a portion of Water or Purified Water, add Glycerin, Ethanol, a suitable quantity of aromatic substance and another portion of Water or Purified Water to volume, and filter. Concentrated Glycerin may be used in place of Glycerin.

Description Glycerin and Potash Solution is a clear, colorless liquid, having an aromatic odor.

The pH of a solution of Glycerin and Potash Solution (1 in 5) is about 12.

Specific gravity d_{20}^{20} : about 1.02

Identification (1) A solution of Glycerin and Potash Solution (1 in 2) is alkaline (potassium hydroxide).

(2) Place 10 mL of a solution of Glycerin and Potash Solution (1 in 10) in a glass-stoppered test tube, add 2 mL of sodium hydroxide TS and 1 mL of copper (II) sulfate TS, and shake: a blue color is produced (glycerin).

(3) Glycerin and Potash Solution responds to the Qualitative Tests <1.09> for potassium salt.

Containers and storage Containers—Tight containers.

Glyceryl Monostearate

モノステアリン酸グリセリン

Glyceryl Monostearate is a mixture of α - and β -glyceryl monostearate and other fatty acid esters of glycerin.

Description Glyceryl Monostearate occurs as white to light yellow, waxy masses, thin flakes, or granules. It has a characteristic odor and taste.

It is very soluble in hot ethanol (95), soluble in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water and in ethanol (95).

It is slowly affected by light.

Identification (1) Heat 0.2 g of Glyceryl Monostearate with 0.5 g of potassium hydrogen sulfate until thoroughly charred: the irritative odor of acrolein is perceptible.

(2) Dissolve 0.1 g of Glyceryl Monostearate in 2 mL of ethanol (95) by warming, heat with 5 mL of dilute sulfuric acid in a water bath for 30 minutes, and cool: a white to yellow solid is produced. This separated solid dissolves when shaken with 3 mL of diethyl ether.

Melting point <1.13> Not below 55°C.

Acid value <1.13> Not more than 15.

Saponification value <1.13> 157 – 170

Iodine value <1.13> Not more than 3.0. Use chloroform instead of cyclohexane.

Purity <1.13> Acidity or alkalinity—To 1.0 g of Glyceryl Monostearate add 20 mL of boiling water, and cool with swirling: the solution is neutral.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Glycine

Aminoacetic Acid

グリシン



$\text{C}_2\text{H}_5\text{NO}_2$: 75.07

Aminoacetic acid [56-40-6]

Glycine, when dried, contains not less than 98.5% of $\text{C}_2\text{H}_5\text{NO}_2$.

Description Glycine occurs as white crystals or crystalline powder. It is odorless. It has a sweet taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of Glycine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Glycine in water, evaporate the water to dryness, and repeat the test with the residue.

pH <2.54> Dissolve 1.0 g of Glycine in 20 mL of water: the pH of the solution is between 5.6 and 6.6.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Glycine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of Glycine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of Glycine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test using 0.25 g of Glycine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Glycine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Glycine according to Method 1, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of Glycine in 25

mL of water and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

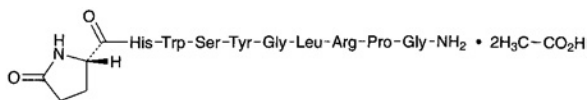
Assay Weigh accurately about 80 mg of Glycine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 7.507 mg of C₂H₅NO₂

Containers and storage Containers—Well-closed containers.

Gonadorelin Acetate

ゴナドレリン酢酸塩



C₅₅H₇₅N₁₇O₁₃·2C₂H₄O₂: 1302.39

5-Oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-glycyl-L-leucyl-L-arginyl-L-prolyl-glycinamide diacetate [34973-08-5]

Gonadorelin Acetate contains not less than 96.0% and not more than 102.0% of C₅₅H₇₅N₁₇O₁₃·2C₂H₄O₂, calculated on the anhydrous basis.

Description Gonadorelin Acetate occurs as a white to pale yellow powder. It is odorless or has a slight, acetic odor.

It is freely soluble in water, in methanol and in acetic acid (100), and sparingly soluble in ethanol (95).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Gonadorelin Acetate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Gonadorelin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the

spectrum of Gonadorelin Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Gonadorelin Acetate in 0.5 mL of ethanol (99.5), add 1 mL of sulfuric acid, and heat: the odor of ethyl acetate is perceptible.

Optical rotation <2.49>: $[\alpha]_D^{20}$: -53.0 - -57.0° (0.10 g calculated on the anhydrous basis, diluted acetic acid (100) (1 in 100), 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Gonadorelin Acetate in 10 mL of water: the pH of this solution is between 4.8 and 5.8.

Constituent amino acids Put 10 mg of Gonadorelin Acetate in a test tube for hydrolysis, add 0.5 mL of hydrochloric acid and 0.5 mL of a solution of mercaptoacetic acid (2 in 25), seal the tube under reduced pressure, and heat at 110°C for 5 hours. After cooling, open the tube, transfer the hydrolyzate into a beaker, and evaporate to dryness on a water bath. Add exactly 100 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh exactly 0.105 g of L-serine, 0.147 g of L-glutamic acid, 0.115 g of L-proline, 75 mg of glycine, 0.131 g of L-leucine, 0.181 g of L-tyrosine, 0.210 g of L-histidine hydrochloride monohydrate, 0.204 g of L-tryptophan and 0.211 g of L-arginine hydrochloride, which are all previously dried at 105°C for 3 hours, add 50 mL of 1 mol/L hydrochloric acid TS to dissolve them, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the peaks of nine constituent amino acids are observed on the chromatogram, and their respective molar ratios with respect to arginine are 0.7 - 1.0 for serine and tryptophan, 0.8 - 1.2 for proline, 0.9 - 1.1 for glutamic acid, leucine, tyrosine and histidine, respectively, and 1.8 - 2.2 for glycine.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm for proline and 570 nm for others).

Column: A stainless steel column 4 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography composed with a sulfonated polystyrene copolymer (5 μ m in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Mobile phase: Prepare the mobile phases A, B, C and D according to the following table.

Mobile phase	A	B	C	D
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g	—
Sodium hydroxide	—	—	—	8.00 g
Sodium chloride	5.66 g	7.07 g	54.35 g	—
Citric acid monohydrate	19.80 g	22.00 g	6.10 g	—
Ethanol (99.5)	130 mL	20 mL	—	100 mL
Benzyl alcohol	—	—	5 mL	—
Thiodiglycol	5 mL	5 mL	—	—
Lauromacrogol solution in diethyl ether (1 in 4)	4 mL	4 mL	4 mL	4 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount	a sufficient amount
Total volume	1000 mL	1000 mL	1000 mL	1000 mL

Flowing of mobile phase: Control the gradient by mixing the mobile phases A, B, C and D as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)	Mobile phase D (vol%)
0~9	100	0	0	0
9~25	0	100	0	0
25~61	0	100→0	0→100	0
61~76	0	0	100	0
76~96	0	0	0	100

Reaction reagent: Dissolve 204 g of lithium acetate dihydrate in 336 mL of water, add 123 mL of acetic acid (100) and 401 mL of 1-methoxy-2-propanol, and use as Solution A. Separately, dissolve 39 g of ninhydrin and 81 mg of sodium borohydride in 979 mL of 1-methoxy-2-propanol, and use as Solution B. Mix the same volume of Solution A and Solution B before use.

Flow rate of mobile phase: 0.25 mL per minute.

Flow rate of reaction reagent: 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, serine, glutamic acid, proline, glycine, leucine, tyrosine, histidine, tryptophan and arginine are eluted in this order with enough separation between these peaks.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 0.10 g of Gonadorelin Acetate in 10 mL of water is clear, and the absorbance of this solution at 350 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.10.

(2) Related substances—Dissolve 50 mg of Gonadorelin Acetate in 100 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area other than gonadorelin from the sample solution is not more than 1/5 times the peak area of gonadorelin from the standard solution, and the total area of the peaks other than the peak of gonadorelin is not more than 3/5 of the peak area of

gonadorelin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of gonadorelin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 100 mL. Confirm that the peak area of gonadorelin obtained from 10 μ L of this solution is equivalent to 1 to 3% of that obtained from 10 μ L of the standard solution.

System performance: Dissolve 4 mg of Gonadorelin Acetate in a suitable amount of the mobile phase, add 5 mL of a solution of phenacetin in acetonitrile (1 in 1000) and the mobile phase to make 50 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, gonadorelin and phenacetin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of gonadorelin is not more than 5%.

Water <2.48> Not more than 8.0% (0.15 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (0.1 g).

Assay Weigh accurately about 40 mg of Gonadorelin Acetate and Gonadorelin Acetate Reference Standard (separately determine the water <2.48> in the same manner as Gonadorelin Acetate) and dissolve in diluted acetic acid (100) (1 in 1000) to make exactly 25 mL each. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution and water to make 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of gonadorelin to that of the internal standard.

$$\text{Amount (mg) of } C_{55}H_{75}N_{17}O_{13} \cdot 2C_2H_4O_2 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Gonadorelin Acetate Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of phenacetin in a mixture of water and acetonitrile (3:2) (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile (90:17).

Flow rate: Adjust the flow rate so that the retention time of gonadorelin is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, gonadorelin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of gonadorelin to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Human Chorionic Gonadotrophin

Chorionic Gonadotrophin

ヒト絨毛性性腺刺激ホルモン

Human Chorionic Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained from the urine of healthy pregnant women after the manufacturing process to remove or inactivate the virus.

It contains not less than 2500 human chorionic gonadotrophin Units per mg, and contains not less than 3000 chorionic gonadotrophin Units per mg protein.

It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

Description Human Chorionic Gonadotrophin occurs as a white to light yellow-brown powder.

It is freely soluble in water.

Identification Calculate b by the following equation, using Y_3 and Y_4 obtained in the Assay: b is not less than 120.

$$b = (E/I)$$

$$E = (Y_3 - Y_4)/f$$

f : Number of test animals per group.

$$I = \log (T_H/T_L)$$

Purity (1) Clarity and color of solution—Dissolve 0.05 g of Human Chorionic Gonadotrophin in 5 mL of isotonic sodium chloride solution: the solution is clear and colorless or light yellow.

(2) Estrogen—Inject subcutaneously into each of three female albino rats or albino mice ovariectomized at least two weeks before the test, single dose of 100 units according to the labeled Units dissolved in 0.5 mL of isotonic sodium chloride solution. Take vaginal smear twice daily, on the third, fourth and fifth day. Place the smear thinly on a slide glass, dry, stain with Giemsa's TS, wash with water, and again dry: no estrus figure is shown microscopically <5.01>.

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins <4.01> Less than 0.03 EU/unit.

Abnormal toxicity Dilute Human Chorionic Gonadotrophin with isotonic sodium chloride solution so that each mL of the solution contains 120 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution

into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

Specific activity When calculate from the results obtained by the Assay and the following test, the specific activity is not less than 3000 human chorionic gonadotrophin Units per mg protein.

(i) Sample solution—To an exactly amount of Chorionic Gonadotrophin add water to make a solution so that each mL contains about 500 Units of human chorionic gonadotrophin according to the labeled amount.

(ii) Standard solution—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μg of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 mL each of the sample solution and the standard solutions, put them in glass test tubes about 18 mm in inside diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin's TS (1 in 2), mix, and warm in a water bath at 30°C for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained in the same manner with 0.5 mL of water as the blank.

Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and determine the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of the protein in the sample.

Assay (i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solution—Dissolve a quantity of Human Chorionic Gonadotrophin Reference Standard in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, having 7.5, 15, 30 and 60 Units per 2.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and weigh their ovaries, as directed in procedure of (iv). Inject bovine serum albumin-isotonic sodium chloride solution to another group, and use this group as the control group. According to the result of this test, designate the concentration of the reference standard which will increase the masses of the ovaries about 2.5 times the mass of the ovaries of the control group as a low-dose concentration of the standard solution, and the concentration 1.5 to 2.0 times the low-dose concentration as a high-dose concentration. Dissolve a quantity of Human Chorionic Gonadotrophin Reference Standard, in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L whose concentrations are equal to those determined by the above test.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable quantity of Human Chorionic Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose sample solution T_H and a low-dose sample solution T_L having Units equal to the standard solutions in equal volumes, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.5 mL of S_H , S_L , T_H and T_L in each group for 5 days. On the sixth day, excise the ovaries, remove the fat and other unwanted tissues attached to the ovaries, and remove the adhering water by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H , S_L , T_H and T_L as y_1 , y_2 , y_3 and y_4 , respectively. Sum up y_1 , y_2 , y_3 and y_4 on each set to obtain Y_1 , Y_2 , Y_3 and Y_4 .

Units per mg of Human Chorionic Gonadotrophin
= antilog $M \times (\text{units per mL of } S_H) \times (b/a)$

$$M = Y_a / Y_b$$

$$I = \log (S_H/S_L) = \log (T_H/T_L)$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a : Mass (mg) of sample.

b : Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution.

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L ($P = 0.95$) by the following equation: L should be not more than 0.3. If F' exceeds F_1 , or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2 / 4fs^2$$

f : Number of test animals per group.

$$s^2 = \{\Sigma y^2 - (Y/f)\} / n$$

Σy^2 : The sum of the squares of each y_1 , y_2 , y_3 and y_4 .

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f - 1)$$

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = Y_b^2 / (Y_b^2 - 4fs^2I^2)$$

I^2 : Value shown in the following table against n used to calculate s^2 .

n	$I^2 = F_1$	n	$I^2 = F_1$	n	$I^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Human Chorionic Gonadotrophin for Injection

Chorionic Gonadotrophin for Injection

注射用ヒト絨毛性性腺刺激ホルモン

Human Chorionic Gonadotrophin for Injection is a preparation for injection which is dissolved before use.

It contains not less than 80% and not more than 125% of the labeled human chorionic gonadotrophin Units.

Method of preparation Prepare as directed under Injections with Human Chorionic Gonadotrophin.

Description Human Chorionic Gonadotrophin for Injection occurs as a white to light yellow-brown powder or masses.

Identification Proceed as directed in the Identification under Human Chorionic Gonadotrophin.

pH <2.54> Prepare a solution so that each mL of isotonic sodium chloride solution contains 2 mg of Human Chorionic Gonadotrophin for Injection: the pH of this solution is between 5.0 and 7.0.

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins <4.01> Less than 0.03 EU/unit.

Uniformity of dosage units <6.02> When calculate the acceptance value using the mean of estimated contents of the units tested as M, it meets the requirements of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Proceed as directed in the Assay under Human Chorionic Gonadotrophin. The ratio of the assayed Units to the labeled Units should be calculated by the following equation.

$$\begin{aligned} & \text{The ratio of the assayed Units to the labeled Units} \\ & = \text{antilog } M \end{aligned}$$

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, and in a cold place.

Human Menopausal Gonadotrophin

ヒト下垂体性性腺刺激ホルモン

Human Menopausal Gonadotrophin is a dried preparation of gonad-stimulating hormone obtained

from the urine of postmenopausal healthy women, after processing for virus removal or inactivation. It has follicle-stimulating hormonal action and luteinizing hormonal action.

It contains not less than 40 follicle-stimulating hormone Units per mg.

Description Human Menopausal Gonadotrophin occurs as a white to pale yellow powder.

It is soluble in water.

Purity Interstitial cell-stimulating hormone—Perform the test according to the following method: the ratio of the unit of interstitial cell-stimulating hormone (luteinizing hormone) to that of follicle-stimulating hormone is not more than 1.

(i) Test animals—Select healthy male albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin Reference Standard, and dissolve in bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare three kinds of solutions, containing 10, 20 and 40 interstitial cell-stimulating hormone (luteinizing hormone) units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their seminal vesicles as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the seminal vesicle 20 to 35 mg, as the high-dose standard solution, S_H . Dilute the S_H to 1.5 to 2.0 times the initial volume with the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, and designate this solution as the low-dose standard solution, S_L . Store these solutions at 2 – 8°C.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, and dissolve in the bovine serum albumin-sodium chloride-phosphate buffer solution, pH 7.2, to prepare the high-dose sample solution, T_H and the low-dose sample solution, T_L , so that their concentrations are similar to those of the corresponding standard solutions, respectively. Store these solutions at 2 – 8°C.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously once every day 0.2 mL each of S_H , S_L , T_H and T_L into the animals in each group. On the sixth day, excise the seminal vesicles, remove extraneous tissue, remove fluid adhering to the vesicles and the contents of the vesicles by lightly pressing between filter papers, and weigh the vesicles.

(v) Calculation—Proceed as directed in (v) in the Assay.

Bacterial endotoxins <4.01> Dissolve Human Menopausal Gonadotrophin in water for bacterial endotoxins test to prepare a solution containing 75 follicle-stimulating hormone Units per mL, and perform the test: less than 0.66 EU/ follicle-stimulating hormone Unit.

Water <2.48> Not more than 5.0% (0.2 g, volumetric titration, direct titration).

Specific activity Perform the test with Human Menopausal Gonadotrophin according to the following method, and calculate the specific activity using the amount (Unit) obtained in the Assay: it is not less than 50 follicle-stimulating hormone Units per 1 mg of protein.

(i) Sample solution—Weigh accurately about 10 mg of Human Menopausal Gonadotrophin, dissolve in water so that each mL contains exactly 200 μ g, and use this solution as the sample solution.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin, and dissolve in water to make exactly 20 mL. To this solution add water to make four solutions containing exactly 300 μ g, 200 μ g, 100 μ g and 50 μ g of the albumin per mL, respectively, and use these solutions as the standard solutions.

(iii) Procedure—To glass test tubes, about 18 mm in inside diameter and about 130 mm in height, add separately exactly 0.5 mL each of the sample solution and the standard solutions. To these tubes add exactly 5 mL of alkaline copper TS, warm in a water bath at 30°C for 10 minutes, then add exactly 0.5 mL of diluted Folin's TS (1 in 2), and warm in a water bath at 30°C for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrometry <2.24>, and determine the absorbances at 750 nm, using a liquid obtained with 0.5 mL of water in the same manner as above as a blank. Prepare a calibration curve from the absorbances of the standard solutions, with absorbance on the vertical axis and concentration on the horizontal axis. Determine the amount of protein in the sample solution from the absorbance of the sample solution using the curve, and calculate the protein content of the sample.

Assay

(i) Test animals—Select healthy female albino rats weighing about 45 to 65 g.

(ii) Standard solutions—Weigh accurately a suitable amount of Human Menopausal Gonadotrophin Reference Standard, dissolve in human chorionic gonadotrophin TS to make three solutions which contain 0.75, 1.5 and 3.0 follicle-stimulating hormone Units per 1.0 mL, respectively. Inject these solutions into three groups consisting of five test animals each, and weigh their ovaries, as directed in (iv). According to the result of the test, designate the concentration of the reference standard, which will make the mass of the ovary about 120 to 160 mg, as the high-dose standard solution, S_H . Dilute the S_H to 1.5 to 2.0 times the initial volume with the human chorionic gonadotrophin TS, and designate the solution as the low-dose standard solution, S_L .

(iii) Sample solutions—According to the labeled units, weigh accurately a suitable amount of Human Menopausal Gonadotrophin, dissolve in human chorionic gonadotrophin TS, and prepare the high-dose sample solution, T_H , and the low-dose sample solution, T_L , which have similar numbers of units to those of corresponding standard solutions in equal volume, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject subcutaneously 0.2 mL each of S_H , S_L , T_H and T_L into the animals in each group, once in the afternoon on the first day, three times in the morning, noon and afternoon on the second day, and two times in the morning and afternoon on the third day. On the fifth day, excise the ovaries, remove the fat and extraneous tissue, remove fluid adhering to the ovaries by lightly pressing between filter papers, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H , S_L , T_H and T_L as y_1 , y_2 , y_3 and y_4 , respectively. Sum up y_1 , y_2 , y_3 and y_4 on each set to obtain Y_1 , Y_2 , Y_3 and Y_4 .

Units per mg of Human Menopausal Gonadotrophin
= antilog $M \times (\text{units per mL of } S_H) \times (b/a)$

$M = IY_a/Y_b$

$$I = \log (S_H/S_L) = \log (T_H/T_L)$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a : Mass (mg) of sample.

b : Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution.

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L ($P = 0.95$) by the following equation: L should be not more than 0.3. If F' exceeds F_1 , or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = (Y_1 - Y_2 - Y_3 - Y_4)^2 / (4fs^2)$$

f : Number of test animals per group.

$$s^2 = \{ \sum y^2 - (Y/f) \} / n$$

$\sum y^2$: The sum of the squares of each y_1, y_2, y_3 and y_4 .

$$Y = -Y_{12} - Y_{22} + Y_{32} + Y_{42}$$

$$n = 4(f - 1)$$

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = Y_b^2 / (Y_a^2 - 4fs^2t^2)$$

t^2 : Value shown in the following table against n used to calculate s^2 .

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Serum Gonadotrophin

血清性腺刺激ホルモン

Serum Gonadotrophin is a dried preparation of gonad-stimulating hormone, obtained from pregnant mares' serum which has adequately inspected viruses, and subjected to a suitable process for removal or inac-

tivation of viruses.

It contains not less than 2000 serum gonadotrophin Units per mg.

It contains not less than 80% and not more than 125% of the labeled serum gonadotrophin Units.

Description Serum Gonadotrophin occurs as a white powder.

It is freely soluble in water.

Identification Calculate b by the following equation, using Y_3 and Y_4 obtained in the Assay: b is not less than 120.

$$b = E/I$$

$$E = (Y_3 - Y_4)/f$$

f : Number of test animals per group.

$$I = \log (T_H/T_L)$$

Purity Clarity and color of solution—Dissolve Serum Gonadotrophin in isotonic sodium chloride solution to prepare a solution containing 9000 units per mL according to the labeled Units: the solution is clear and colorless.

Loss on drying <2.4I> Not more than 8.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins <4.0I> Less than 0.1 EU/unit.

Specific activity When calculated from the results obtained by the Assay and the following test, Serum Gonadotrophin contains not less than 3000 serum gonadotrophin Units per mg of protein.

(i) Standard solutions—Dissolve about 3 mg of bovine serum albumin in water to make a solution containing 500 μ g of the albumin in each mL. To this solution add water to make four standard solutions so that each mL contains exactly 200 μ g, 150 μ g, 100 μ g and 50 μ g of the albumin, respectively.

(ii) Sample solution—Dissolve about 1 mg of Serum Gonadotrophin in water to make a solution containing exactly 180 μ g in each mL.

(iii) Sodium carbonate solution—Dissolve 2 g of sodium carbonate (standard reagent) in 0.1 mol/L sodium hydroxide TS to make 100 mL.

(iv) Sodium tartrate solution—Dissolve about 1 g of sodium tartrate dihydrate in water to make 100 mL.

(v) Copper (II) sulfate solution—Dissolve 0.5 g of copper (II) sulfate pentahydrate in the sodium tartrate solution to make 100 mL.

(vi) Alkaline copper solution—Mix 50 mL of the sodium carbonate solution and 1 mL of the copper (II) sulfate solution. Prepare before use. Use within the day of preparation.

(vii) Procedure—Pipet 0.5 mL each of the standard solutions and the sample solution in small test tubes, add 3 mL of the alkaline copper solution to them, and mix. Allow them to stand at the room temperature for not less than 10 minutes, add 0.3 mL of diluted Folin's TS (1 in 2), mix immediately, and allow to stand for not less than 30 minutes. Determine the absorbances of these solutions so obtained at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner with 0.5 mL of water, as the blank. Plot the calibration curve from the absorbances obtained with the standard solutions, and determine the amount of protein in the sample solution from

this curve.

$$\begin{aligned} &\text{Specific activity (unit/mg protein)} \\ &= [(\text{units per mg, obtained in the Assay}) / \\ &\quad (\text{amount (\%) of protein in the sample})] \times 100 \end{aligned}$$

Abnormal toxicity Dissolve Serum Gonadotrophin in isotonic sodium chloride solution so that each 5 mL of the solution contains 4000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and inject 0.5 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy mice aged about 5 weeks. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

Assay

(i) Test animals—Select healthy female albino rats weighing about 45 g.

(ii) Standard solution—Dissolve a quantity of Serum Gonadotrophin Reference Standard in bovine serum albumin-isotonic sodium chloride solution to prepare four kinds of solutions, containing 10, 20, 40 and 80 Units per 0.5 mL, respectively. Inject these solutions into four groups consisting of five test animals each, and weigh their ovaries, as directed in procedure of (iv). Inject bovine serum albumin-isotonic sodium chloride solution to another group, and use this group as the control group. According to the result of this test, designate the concentration of the reference standard which will increase the masses of the ovaries about 3 times the mass of the ovaries of the control group as a low-dose concentration of the standard solution, and the concentration 1.5 to 2.0 times the low-dose concentration as a high-dose concentration. Weigh accurately a suitable quantity of Serum Gonadotrophin Reference Standard, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose standard solution S_H and a low-dose standard solution S_L whose concentrations are equal to those determined by the above test.

(iii) Sample solution—According to the labeled units, weigh accurately a suitable quantity of Serum Gonadotrophin, dissolve in bovine serum albumin-isotonic sodium chloride solution, and prepare a high-dose sample solution T_H and a low-dose sample solution T_L having Units equal to the standard solutions in equal volumes, respectively.

(iv) Procedure—Divide the test animals at random into 4 groups, A, B, C and D, with not less than 10 animals and equal numbers in each group. Inject once subcutaneously 0.5 mL of S_H , S_L , T_H and T_L in each group. On the sixth day, excise the ovaries, remove the fat and other unwanted tissues attached to the ovaries, and remove the adhering water by lightly pressing between filter paper, and immediately weigh the ovaries.

(v) Calculation—Designate the mass of ovaries by S_H , S_L , T_H and T_L as y_1 , y_2 , y_3 and y_4 , respectively. Sum up y_1 , y_2 , y_3 and y_4 on each set to obtain Y_1 , Y_2 , Y_3 and Y_4 .

$$\begin{aligned} &\text{Units per mg of Serum Gonadotrophin} \\ &= \text{antilog } M \times (\text{units per mL of SH}) \times (b/a) \end{aligned}$$

$$M = IY_a/Y_b$$

$$I = \log (S_H/S_L) = \log (T_H/T_L)$$

$$Y_a = -Y_1 - Y_2 + Y_3 + Y_4$$

$$Y_b = Y_1 - Y_2 + Y_3 - Y_4$$

a : Mass (mg) of sample.

b : Total volume (mL) of the high dose of the test solution prepared by diluting with bovine serum albumin-isotonic sodium chloride solution.

F' computed by the following equation should be smaller than F_1 against n when s^2 is calculated. And compute L ($P = 0.95$) by the following equation: L should be not more than 0.3. If F' exceeds F_1 , or if L exceeds 0.3, repeat the test increasing the number of the test animals or arranging the assay method in a better way until F' is smaller than F_1 or L is not more than 0.3.

$$F' = (Y_1 - Y_2 - Y_3 + Y_4)^2 / 4fs^2$$

f : Number of test animals per group.

$$s^2 = \{\Sigma y^2 - (Y/f)\} / n$$

Σy^2 : The sum of the squares of each y_1 , y_2 , y_3 and y_4 .

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f - 1)$$

$$L = 2\sqrt{(C-1)(CM^2 + I^2)}$$

$$C = Y_b^2 / (Y_b^2 - 4fs^2t^2)$$

t^2 : Value shown in the following table against n used to calculate s^2 .

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Serum Gonadotrophin for Injection

注射用血清性腺刺激ホルモン

Serum Gonadotrophin for Injection is a preparation for injection which is dissolved before use.

It contains not less than 80% and not more than 125% of the labeled Serum Gonadotrophin Units.

Method of preparation Prepare as directed under Injections with Serum Gonadotrophin.

Description Serum Gonadotrophin for Injection occurs as white powder or masses.

Identification Proceed as directed in the Identification under Serum Gonadotrophin.

pH <2.54> Dissolve 30 mg of Serum Gonadotrophin for In-

jection in 20 mL of isotonic sodium chloride solution: the pH of this solution is between 5.0 and 7.0.

Loss on drying <2.41> Not more than 5.0% (0.1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Bacterial endotoxins <4.01> Less than 0.1 EU/unit.

Assay Proceed as directed in the Assay under Serum Gonadotrophin. The ratio of the Units assayed to the labeled Units should be calculated by the following equation.

$$\frac{\text{The ratio of the assayed Units to the labeled Units}}{= \text{antilog } M}$$

Containers and storage Containers—Hermetic containers. Storage—Light-resistant, and in a cold place.

Gramicidin

グラミシジン

[1405-97-6]

Gramicidin is a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus brevis* Dubos.

It contains not less than 900 µg (potency) per mg, calculated on the dried basis. The potency of Gramicidin is expressed as mass (potency) of gramicidin.

Description Gramicidin occurs as a white to light yellowish white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) To 10 mg of Gramicidin add 2 mL of 6 mol/L hydrochloric acid TS, and heat in a water bath for 30 minutes with occasional stirring. After cooling, neutralize with 6 mol/L sodium hydroxide TS, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 2 minutes: a blue-purple to red-purple color develops.

(2) Determine the absorption spectrum of a solution of Gramicidin in ethanol (95) (1 in 20,000), as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Gramicidin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Loss on drying <2.41> Not more than 3.0% (0.1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Turbidimetric method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- (i) Test organism—*Enterococcus hirae* ATCC 10541
- (ii) Agar medium for transferring test organism

Glucose	10.0 g
Casein peptone	5.0 g
Yeast extract	20.0 g
Potassium dihydrogen phosphate	2.0 g
Polysorbate 80	0.1 g

Agar	15.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH of the solution so that it will be 6.7 to 6.8 after sterilization.

(iii) Liquid medium for suspending test organism—Use the culture medium (2).

(iv) Preparation of the test organism suspension—Puncture the test organism in the medium, prepared by dispensing 10 mL of the agar medium for transferring test organism in a test tube about 16 mm in inside diameter, incubate at 36.5 to 37.5°C for 20 to 24 hours. After sub-culturing at least three times, keep between 1 to 5°C. Transfer the organism so obtained in 10 mL of the liquid medium for suspending test organism, incubate at 36.5 to 37.5°C for 20 to 24 hours, and use this medium as the test organism stock suspension. Before use, add the test organism stock suspension to the liquid medium for suspending test organism so that the transmittance at 580 nm is 50 to 60%. Mix one volume of this suspension and 200 volume of the liquid medium for suspending test organism, and use this as the test organism suspension.

(v) Standard solution—Weigh accurately an amount of Gramicidin Reference Standard, previously dried under reduced pressure not exceeding 0.67 kPa at 60°C for 3 hours, equivalent to about 10 mg (potency), dissolve in ethanol (99.5) to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add the following diluting solution to make a solution so that each mL contains 0.02 µg (potency), and use this solution as the standard solution.

Diluting solution: To 390 mL of propylene glycol add 210 mL of a mixture of ethanol (99.5) and acetone (9:1) and Sterile Purified Water to make 1000 mL.

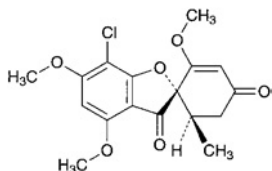
(vi) Sample solution—Weigh accurately an amount of Gramicidin, equivalent to about 10 mg (potency), and dissolve in ethanol (99.5) to make exactly 100 mL. Take exactly a suitable amount of this solution, add the diluting solution obtained in (v) to make a solution so that each mL contains 0.02 µg (potency), and use this solution as the sample solution.

(vii) Procedure—Transfer 0.155 mL, 0.125 mL, 0.100 mL, 0.080 mL and 0.065 mL each of the standard solution, 0.100 mL of the sample solution and 0.100 mL of the diluting solution, separately, in test tubes about 14 mm in inside diameter and about 15 cm in length, and make three sets for each. To each of the test tube add 10 mL of the test organism suspension, stopper the tube, incubate in a water bath at 36.5 to 37.5°C for 180 to 270 minutes, add 0.5 mL of a solution of formaldehyde (1 in 3), and determine their transmittances at 580 nm.

Containers and storage Containers—Tight containers.

Griseofulvin

グリセオフルビン



$C_{17}H_{17}ClO_6$: 352.77

(2*S*,6'*R*)-7-Chloro-2',4,6-trimethoxy-6'-methylspiro[benzo[*b*]furan-2(3*H*),1'-(cyclohex-2'-ene)]-3,4'-dione [126-07-8]

Griseofulvin is a substance having antifungal activity produced by the growth of *Penicillium griseofulvum* or *Penicillium janczewskii*.

It contains not less than 960 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Griseofulvin is expressed as mass (potency) of griseofulvin ($C_{17}H_{17}ClO_6$).

Description Griseofulvin occurs as white, crystals or crystalline powder.

It is soluble in *N,N*-dimethylformamide, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Griseofulvin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Griseofulvin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Griseofulvin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Griseofulvin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +350 – +364° (0.25 g calculated on the dried basis, *N,N*-dimethylformamide, 25 mL, 100 mm).

Melting point <2.60> 218 – 222°C

Purity (1) Acidity—Dissolve 0.25 g of Griseofulvin in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 1.0 mL of 0.02 mol/L sodium hydroxide VS: the color of the solution is red.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Griseofulvin according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 25 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Griseofulvin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—To 0.10 g of Griseofulvin add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the sample solution. Separately, to 5.0 mg of Griseofulvin Reference Standard add exactly 1 mL of the internal standard solution and acetone to make 10 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, determine each peak area by the automatic integration method, and calculate the ratio, Q_1 , of the peak area of dechlorogriseofulvin, having the relative retention time of about 0.6 with respect to griseofulvin, to that of the internal standard obtained from the sample solution, the ratio, Q_2 , of the peak area of dehydrogriseofulvin, having the relative retention time of about 1.2 with respect to griseofulvin, to that of the internal standard obtained from the sample solution and the ratio, Q_S , of the peak area of griseofulvin to that of the internal standard obtained from the standard solution: Q_1/Q_S is not more than 0.6, and Q_2/Q_S is not more than 0.15. **Internal standard solution**—A solution of 9,10-diphenylanthracene in acetone (1 in 500).

Operating conditions—

Detector: An hydrogen flame-ionization detector.

Column: A glass column 4 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography in the ratio of 1% (150 – 180 μm in particle diameter).

Column temperature: A constant temperature of about 250°C.

Temperature of injection port: A constant temperature of about 270°C.

Temperature of detector: A constant temperature of about 300°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of griseofulvin is about 10 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the internal standard solution diluted with acetone (1 in 10) to make exactly 10 mL. Confirm that the ratio of the peak area of griseofulvin to that of the internal standard obtained from 2 μL of this solution is equivalent to 7 to 13% of that obtained from 2 μL of the standard solution.

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and griseofulvin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of griseofulvin to that of the internal standard is not more than 5.0%.

(5) Petroleum ether soluble substances—To 1.0 g of Griseofulvin add 20 mL of petroleum ether, shake, and boil for 10 minutes under a reflux condenser. After cooling, filter through a dried filter paper, wash the filter paper with two 15-mL portions of petroleum ether, combine the washings to the filtrate, evaporate the petroleum ether on a water bath, and dry the residue at 105°C for 1 hour: the amount of the

residue is not more than 0.2%.

Loss on drying <2.41> Not more than 1.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Griseofulvin and Griseofulvin Reference Standard, equivalent to about 50 mg (potency), dissolve each in 50 mL of *N,N*-dimethylformamide, add exactly 20 mL of the internal standard solution and water to make 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of griseofulvin to that of the internal standard.

$$\begin{aligned} & \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{17}\text{H}_{17}\text{ClO}_6 \\ & = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Griseofulvin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of griseofulvin is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, griseofulvin and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

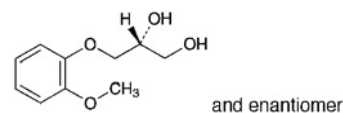
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of griseofulvin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Guaifenesin

Guaiacol Glyceryl Ether

グアイフェネシン



$\text{C}_{10}\text{H}_{14}\text{O}_4$: 198.22

(2*RS*)-3-(2-Methoxyphenoxy)propane-1,2-diol [93-14-1]

Guaifenesin, when dried, contains not less than 98.0% and not more than 102.0% of $\text{C}_{10}\text{H}_{14}\text{O}_4$.

Description Guaifenesin occurs as a white crystals or crystalline powder.

It is freely soluble in ethanol (95), and sparingly soluble in water.

A solution of ethanol (95) (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Guaifenesin (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Guaifenesin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Guaifenesin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Guaifenesin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 80 – 83°C

pH <2.54> Dissolve 1.0 g of Guaifenesin in 100 mL of water: the pH of the solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Guaifenesin in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.7 g of Guaifenesin in 25 mL of water by warming. Cool, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.020%).

(3) Heavy metals <1.07>—Dissolve 2.0 g of Guaifenesin in 25 mL of water by warming. Cool, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Guaifenesin according to Method 3, and perform the test (not more than 2 ppm).

(5) Free guaiacol—To 1.0 g of Guaifenesin add exactly 25 mL of water, dissolve by warming, cool, and use this solu-

tion as the sample solution. Separately, dissolve 0.100 g of guaiacol in water to make exactly 1000 mL. Pipet 3 mL of this solution, add exactly 22 mL of water, and use this solution as the standard solution. To each of the sample solution and the standard solution add 1.0 mL of potassium hexacyanoferrate (III) TS and 5.0 mL of a solution of 4-aminoantipyrine (1 in 200), and immediately after shaking for exactly 5 seconds add a solution of sodium hydrogen carbonate (1 in 1200) to make exactly 100 mL. Determine the absorbances of these solutions at 500 nm exactly 15 minutes after the addition of the 4-aminoantipyrine solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner with 25 mL of water, as the blank: the absorbance of the solution obtained from the sample solution is not greater than that from the standard solution.

(6) Related substances—Dissolve 1.0 g of Guaifenesin in 100 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, ethanol (95), and ammonia solution (28) (40:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 60 mg of Guaifenesin and Guaifenesin Reference Standard, previously dried, and dissolve each then in water to make exactly 100 mL. Pipet 5 mL of these solutions, and add water to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 273 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

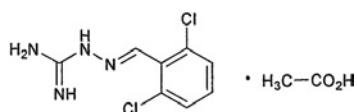
$$\begin{aligned} & \text{Amount (mg) of } C_{10}H_{14}O_4 \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Guaifenesin Reference Standard

Containers and storage Containers—Tight containers.

Guanabenz Acetate

グアナベンズ酢酸塩



$C_8H_8Cl_2N_4 \cdot C_2H_4O_2$: 291.13
(*E*)-1-(2,6-Dichlorobenzylideneamino)guanidine monoacetate [23256-50-0]

Guanabenz Acetate, when dried, contains not less than 98.5% of $C_8H_8Cl_2N_4 \cdot C_2H_4O_2$.

Description Guanabenz Acetate occurs as white crystals or crystalline powder.

It is freely soluble in acetic acid (100), soluble in methanol and in ethanol (95), slightly soluble in water, and practically insoluble in diethyl ether.

It is gradually affected by light.

Melting point: about 190°C (with decomposition).

Identification (1) To 5 mL of a solution of Guanabenz Acetate (1 in 1000) add 0.5 mL of a diluted ethanol (95) (5 in 6) which contains 16 g of urea and 0.2 g of 1-naphthol in 100 mL, and add 1 mL of *N*-bromosuccinimide TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of Guanabenz Acetate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Guanabenz Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 0.1 g of Guanabenz Acetate add 5 mL of water and 1 mL of ammonia TS, shake, filter, and neutralize the filtrate with dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> (3) for acetate.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Guanabenz Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.05 g of Guanabenz Acetate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 10 mL, then pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (80:20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Place the plate in a chamber filled with iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 50°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.25 g of Guanabenz Acetate, previously dried, dissolve in 50 mL of acetic acid

(100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

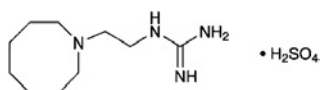
Each mL of 0.1 mol/L perchloric acid VS
= 29.11 mg of $C_8H_8Cl_2N_4 \cdot C_2H_4O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Guanethidine Sulfate

グアネチジン硫酸塩



$C_{10}H_{22}N_4 \cdot H_2SO_4$: 296.39

1-[2-(Hexahydroazocin-1(2H)-yl)ethyl]guanidine
monosulfate [645-43-2]

Guanethidine Sulfate, when dried, contains not less than 98.5% of $C_{10}H_{22}N_4 \cdot H_2SO_4$.

Description Guanethidine Sulfate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a bitter taste.

It is very soluble in formic acid, freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: 251 – 256°C (an evacuated sealed capillary tube, with decomposition).

Identification (1) To 4 mL of a solution of Guanethidine Sulfate (1 in 4000) add 2 mL of 1-naphthol TS, 1 mL of diacetyl TS and 15 mL of water, and allow to stand for 30 minutes: a red color develops.

(2) Determine the infrared absorption spectrum of Guanethidine Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Guanethidine Sulfate (1 in 10) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the pH of the solution is between 4.7 and 5.7.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Guanethidine Sulfate in 50 mL of water: the solution is clear and colorless.

(2) Methylisothiurea sulfate—Dissolve 2.0 g of Guanethidine Sulfate in 80 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Add 60 mL of hydrochloric acid, 2 g of sodium bromide and water to make 200 mL. Then, to this solution add 0.70 mL of 1/60 mol/L potassium bromate VS and 2 mL of zinc iodide-starch paste TS: a blue color develops.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Guanethidine Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C,

4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Guanethidine Sulfate, previously dried, dissolve in 2 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.64 mg of $C_{10}H_{22}N_4 \cdot H_2SO_4$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Adsorbed Habu-venom Toxoid

沈降はぶトキソイド

Adsorbed Habu-venom Toxoid is a liquid for injection containing habu toxoid prepared by treating toxic substances produced by habu (*Trimeresurus flavoviridis*) with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Habu-venom Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Habu-venom Toxoid becomes a uniform whitish turbid liquid on shaking.

Freeze-dried Habu Antivenom, Equine

乾燥はぶウマ抗毒素

Freeze-dried Habu Antivenom, Equine, is a preparation for injection which is dissolved before use.

It contains *Trimeresurus flavoviridis* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Habu Anti-venom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Habu Antivenom, Equine, becomes colorless or light yellow-brown, clear liquid or a slightly whitish turbid liquid on addition of solvent.

Adsorbed Hepatitis B Vaccine

沈降 B 型肝炎ワクチン

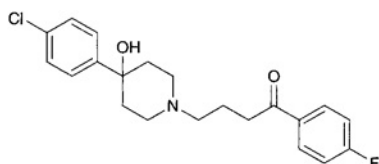
Adsorbed Hepatitis B Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing a surface antigen of hepatitis B virus to make the HBs antigen insoluble.

It conforms to the requirements of Adsorbed Hepatitis B Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Hepatitis B Vaccine becomes a homogeneous, whitish turbid liquid on shaking.

Haloperidol

ハロペリドール



$C_{21}H_{23}ClFNO_2$: 375.86

4-[4-(4-Chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one [52-86-8]

Haloperidol, when dried, contains not less than 99.0% and not more than 101.0% of $C_{21}H_{23}ClFNO_2$.

Description Haloperidol occurs as white to pale yellow crystals or powder.

It is freely soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in 2-propanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 30 mg of Haloperidol in 100 mL of 2-propanol. To 5 mL of the solution add 10 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Haloperidol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 149 – 153°C

Purity (1) Sulfate <1.14>—To 1.0 g of Haloperidol add 50 mL of water, shake, and filter. To 25 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Haloperidol according to method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 25 mg of Haloperidol in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the fol-

lowing conditions, and determine each peak area by the automatic integration method: the area of the peak other than haloperidol is not larger than the peak area of haloperidol from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of haloperidol from the standard solution. For this calculation, use the peak areas for the related substances, having the relative retention time of about 0.5, about 1.2 and about 2.6 with respect to haloperidol, after multiplying by their relative response factors, 0.75, 1.47 and 0.76, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 300 mL of this solution add 700 mL of methanol and 1.0 g of sodium lauryl sulfate.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of haloperidol beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of haloperidol obtained with 10 μ L of this solution is equivalent to 15 to 25% of that with 10 μ L of the standard solution.

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of haloperidol are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloperidol is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, phosphorus (V) oxide, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of haloperidol, previously dried, and dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 37.59 mg of $C_{21}H_{23}ClFNO_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Haloperidol Tablets

ハロペリドール錠

Haloperidol Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of haloperidol ($C_{21}H_{23}ClFNO_2$; 375.86).

Method of preparation Prepare as directed under Tablets, with Haloperidol.

Identification To pulverized Haloperidol Tablets, equivalent to 6 mg of Haloperidol according to the labeled amount, add 70 mL of 2-propanol, and heat on a water bath until to boiling while shaking. After cooling, add 2-propanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add 2 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 20 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 219 nm and 223 nm and between 243 nm and 247 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Haloperidol Tablets add 5 mL of the mobile phase, disperse the particle with the aid of ultrasonic waves, add 30 mL of the mobile phase, and extract for 30 minutes with the aid of ultrasonic waves with occasional shaking. Shake for more 30 minutes, and add the mobile phase to make exactly 50 mL. Centrifuge the solution, pipet V mL of the supernatant liquid, equivalent to about 0.3 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add exactly 2 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in the mobile phase to make exactly 100 mL. Pipet 15 mL of this solution, and add the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 2 mL of the internal standard solution and the mobile phase to make 25 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of haloperidol to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of haloperidol (C}_{21}\text{H}_{23}\text{ClFNO}_2\text{)} \\ & = W_S \times (Q_T/Q_S) \times (1/V) \times (3/4) \end{aligned}$$

W_S : Amount (mg) of haloperidol for assay

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 6700).

Operating conditions—

Proceed as detected in the operating condition in the Assay.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Dissolution Being specified separately.

Assay Weigh accurately, and powder not less than 20 Haloperidol Tablets. Weigh accurately a portion of the powder, equivalent to about 10 mg of haloperidol ($C_{21}H_{23}ClFNO_2$), add 10 mL of water, disperse the particle with the aid of ultrasonic waves, add exactly 20 mL of the internal standard solution, extract for 30 minutes with the aid of ultrasonic waves with occasional shaking, and add the mobile phase to make 100 mL. Centrifuge after shaking for more 30 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 25 mg of haloperidol for assay, previously dried in vacuum at 60°C for 3 hours on phosphorus (V) oxide, and dissolve in methanol to make exactly 25 mL. Pipet 10 mL of this solution, add exactly 20 mL of the internal standard solution and the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of haloperidol to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of haloperidol (C}_{21}\text{H}_{23}\text{ClFNO}_2\text{)} \\ & = W_S \times (Q_T/Q_S) \times (2/5) \end{aligned}$$

W_S : Amount (mg) of haloperidol for assay

Internal standard solution—A solution of diphenyl in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.95 g of trisodium citrate dihydrate in 900 mL of water, adjust to pH 3.5 with dilute hydrochloric acid, and add water to make 1000 mL. To 250 mL of this solution add 750 mL of methanol and 1.0 g of sodium lauryl sulfate, and mix to dissolve.

Flow rate: Adjust the flow rate so that the retention time of haloperidol is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, haloperidol and diphenyl are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of haloperidol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant for the tablets without coating.

Halothane

ハロタン

C₂HBrClF₃: 197.38(2*RS*)-2-Bromo-2-chloro-1,1,1-trifluoroethane [151-67-7]

Halothane contains not less than 0.008% and not more than 0.012% of Thymol as a stabilizer.

Description Halothane is a clear, colorless, and mobile liquid.

It is miscible with ethanol (95), with diethyl ether and with isooctane.

It is slightly soluble in water.

It is a volatile, nonflammable liquid, and setting fire to its heated vapor does not support combustion.

It is affected by light.

Refractive index n_D^{20} : 1.369 – 1.371

Identification Transfer about 3 μ L of Halothane to a gas cell having light path 10 cm in length, and determine the infrared absorption spectrum as directed in the gas sampling method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{20}^{20} : 1.872 – 1.877

Purity (1) Acidity or alkalinity—Shake 60 mL of Halothane with 60 mL of freshly boiled and cooled water vigorously for 3 minutes. Separate the water layer, and use this as the sample solution. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.10 mL of 0.01 mol/L sodium hydroxide VS: a red-purple color develops. To 20 mL of the sample solution add 1 drop of bromocresol purple TS and 0.6 mL of 0.01 mol/L hydrochloric acid VS: a yellow color is produced.

(2) Halide and halogen—To 5 mL of the sample solution obtained in (1) add 1 drop of nitric acid and 0.20 mL of silver nitrate TS: no turbidity is produced. To 10 mL of the sample solution obtained in (1) add 1 mL of potassium iodide TS and 2 drops of starch TS, and allow to stand for 5 minutes: no blue color develops.

(3) Phosgene—Transfer 50 mL of Halothane to a dried 300-mL conical flask, suspend a strip of phosgene test paper vertically inside the flask with the lower end about 10 mm above the surface of the liquid, insert the stopper, and allow to stand at a dark place for 20 to 24 hours: the test paper shows no yellow color.

(4) Residue on evaporation—Pipet 50 mL of Halothane, evaporate on a water bath, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1.0 mg.

(5) Volatile related substances—To 100 mL of Halothane add exactly 5.0 μ L of the internal standard, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Gas Chromatography <2.02>, and determine each peak area by the automatic integration method: the total area of the peaks other than those

of halothane and the internal standard is not larger than the peak area of the internal standard.

Internal standard—1,1,2-Trichloro-1,2,2-trifluoroethane.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and 3 m in length, at the first 2 m from the injection port, having macrogol 400 coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 μ m in particle diameter), and at the remaining 1 m, having dinonyl phthalate coated in the ratio of 30% on siliceous earth for gas chromatography (180 to 250 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is 2 to 3 minutes.

Selection of column: Mix 3 mL of Halothane and 1 mL of the internal standard. Proceed with 1 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and halothane in this order with the resolution between these peaks being not less than 10.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of the internal standard obtained from 5 μ L of the sample solution composes 30 to 70% of the full scale.

Time span of measurement: About 3 times as long as the retention time of halothane.

Distilling range <2.57> Not less than 95 vol distills within a 1°C range between 49°C and 51°C.

Thymol To 0.50 mL of Halothane add 5.0 mL of isooctane and 5.0 mL of titanium (IV) oxide TS, shake vigorously for 30 seconds, and allow to stand: the separated upper layer has more color than the following control solution A, and has no more color than the following control solution B.

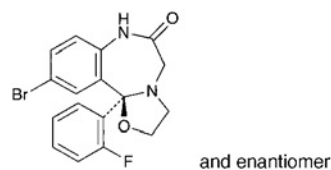
Control solution: Dissolve 0.225 g of thymol for assay in isooctane to make exactly 100 mL. To 10 mL each of this solution, accurately measured, add isooctane to make exactly 150 mL and 100 mL, respectively. Proceed with 0.50 mL each of these solutions in the same manner as Halothane, and use the separated upper layers so obtained as the control solution A and B, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

Haloxazolam

ハロキサゾラム

C₁₇H₁₄BrFN₂O₂: 377.21(11*bRS*)-10-Bromo-11*b*-(2-fluorophenyl)-2,3,7,11*b*-tetrahydro[1,3]oxazolo[3,2-*d*][1,4]benzodiazepin-6(5*H*)-one [59128-97-1]

Haloxazolam, when dried, contains not less than 99.0% of $C_{17}H_{14}BrFN_2O_2$.

Description Haloxazolam occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), sparingly soluble in acetonitrile, in methanol and in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 183°C (with decomposition).

Identification (1) Dissolve 0.01 g of Haloxazolam in 10 mL of methanol, add 1 drop of hydrochloric acid: the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). To this solution add 1 mL of sodium hydroxide TS: the fluorescence disappears immediately.

(2) Prepare the test solution with 0.05 g of Haloxazolam as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 20 mL of dilute sodium hydroxide TS and 1 mL of hydrogen peroxide (30) as an absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for bromide and for fluoride.

(3) Determine the absorption spectrum of a solution of Haloxazolam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Haloxazolam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1cm}^{1\%}$ (247 nm): 390 – 410 (10 mg, methanol, 1000 mL).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Haloxazolam in 20 mL of ethanol (99.5): the solution is clear and colorless.

(2) Soluble halides—To 1.0 g of Haloxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test <1.03>. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Haloxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—To 1.0 g of Haloxazolam in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, place a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, heat, repeat this procedure twice, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution: the solution has no more color than the following control solution (not more than 2 ppm).

Control solution: Proceed in the same manner as above

without using Haloxazolam, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and proceed in the same manner as the test solution.

(5) Related substances—Dissolve 0.10 g of Haloxazolam in 100 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the haloxazolam from the sample solution is not larger than the peak area of the haloxazolam from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.2 g of boric acid and 7.5 g of potassium chloride in 900 mL of water, adjust the pH with triethylamine to 8.5, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of haloxazolam is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of haloxazolam beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of haloxazolam obtained from 10 μ L of this solution is equivalent to 8 to 12% of that of haloxazolam obtained from 10 μ L of the standard solution.

System performance: Dissolve 10 mg each of Haloxazolam and cloxazolam in 200 mL of acetonitrile. When the procedure is run with 10 μ L of this solution under the above operating conditions, haloxazolam and cloxazolam are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of haloxazolam is not more than 1.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Haloxazolam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 37.72 mg of $C_{17}H_{14}BrFN_2O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Heparin Sodium

ヘパリンナトリウム

Heparin Sodium is obtained from the livers, the lungs and the intestinal mucosa of healthy edible animals, and prolongs the clotting time of blood. Heparin Sodium obtained from the livers and the lungs contains not less than 110 Heparin Units per mg, and that obtained from the intestinal mucosa contains not less than 130 Heparin Units per mg.

Heparin Sodium, calculated on the dried basis, contains not less than 90% and not more than 110% of the labeled Units.

Label the name of the organ used as the starting material.

Description Heparin Sodium occurs as a white to grayish brown powder or grains. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

pH <2.54> The pH of a solution of 1.0 g of Heparin Sodium in 100 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Heparin Sodium in 20 mL of water: the solution is clear and colorless to light yellow.

(2) Barium—Dissolve 0.03 g of Heparin Sodium in 3.0 mL of water, and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(3) Total nitrogen—Weigh accurately about 0.1 g of Heparin Sodium, previously dried at 60°C for 3 hours under reduced pressure, and perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not more than 3.0%.

(4) Protein—To 1.0 mL of the sample solution obtained in (2) add 5 drops of a solution of trichloroacetic acid (1 in 5): neither a precipitate nor turbidity is produced.

Loss on drying <2.41> Not more than 10% (20 mg, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 40% (after drying, 20 mg).

Pyrogen <4.04> Dissolve Heparin Sodium in isotonic sodium chloride solution so as to contain 1000 Units per mL according to the labeled Units. Inject into rabbits 2 mL of this solution per kg: it meets the requirement.

Assay (i) Substrate solution: Dissolve 15 mg of *N*-benzoyl-L-isoleucyl-L-glutamyl(γ -OR)-glycyl-L-arginyl-*p*-nitroanilide hydrochloride in 20 mL of water.

(ii) Antithrombin III solution: Dissolve human antithrombin III in water to make a solution containing 1 Unit per mL.

(iii) Activated blood coagulation factor X solution: Dissolve bovine activated blood coagulation factor X in water to

make a solution containing 0.426 Units per mL.

(iv) Buffer solution: Dissolve 6.06 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 750 mL of water, adjust the pH to 8.4 with 1 mol/L hydrochloric acid TS, and add water to make 1000 mL.

(v) Reaction stop solution: To 20 mL of acetic acid (100) add water to make 40 mL.

(vi) Heparin standard solutions: Dissolve Heparin Sodium Reference Standard in isotonic sodium chloride solution to make a solution containing 10 Units per mL, and use as the standard stock solution. To 100 μ L of the standard stock solution add the buffer solution to make exactly 5 mL, and use this solution as the standard solution. Prepare the heparin standard solutions (1), (2), (3), (4) and (5) by addition of antithrombin III solution, human normal plasma and the buffer solution to the standard solution as directed in the following table.

Heparin standard solution		Buffer solution (μ L)	Antithrombin III solution (μ L)	Human normal plasma (μ L)	Standard solution (μ L)
No.	Heparin concentration (Unit/mL)				
(1)	0	800	100	100	0
(2)	0.02	700	100	100	100
(3)	0.04	600	100	100	200
(4)	0.06	500	100	100	300
(5)	0.08	400	100	100	400

(vii) Sample solution: Weigh accurately an adequate amount of Heparin Sodium, dissolve in isotonic sodium chloride solution so that each mL contains about 0.5 Units according to the labeled amount. To 100 μ L of this solution add 100 μ L of antithrombin III solution, 100 μ L of human normal plasma and 700 μ L of the buffer solution, and use this solution as the sample solution.

(viii) Procedure: Transfer 400 μ L of the sample solution to a test tube, and warm at 37°C for 4 minutes. Add 200 μ L of the activated blood coagulation factor X solution, mix well, warm at 37°C for exactly 30 seconds, add 400 μ L of the substrate solution, previously warmed at 37°C, and mix well. Allow the tube to stand at 37°C for exactly 3 minutes, add 600 μ L of the reaction stop solution, mix immediately, and determine the absorbance at 405 nm, using the blank solution prepared by addition of 600 μ L of the reaction stop solution and 600 μ L of water to 400 μ L of the sample solution. Proceed the same way with the heparin standard solution (1), the heparin standard solution (2), the heparin standard solution (3), the heparin standard solution (4) and the heparin standard solution (5), and determine their absorbances.

(ix) Calculation: Plot the absorbances of the standard solutions on the vertical axis and their heparin concentrations on the horizontal axis to prepare a calibration curve. Determine the heparin concentration, *C*, of the sample solution from its absorbance by using the curve, and calculate heparin Units per mg of Heparin Sodium from the following formula.

$$\text{Units per mg of Heparin Sodium} = C \times 10 \times (b/a)$$

- a*: Amount of sample (mg)
b: Total volume (mL) of isotonic sodium chloride solution used to dissolve the sample to make the solution containing about 0.5 Units per mL

Containers and storage Containers—Tight containers.

Heparin Sodium Injection

ヘパリンナトリウム注射液

Heparin Sodium Injection is an aqueous solution for injection. It contains not less than 90% and not more than 110% of the labeled heparin Units.

Label the name of organ used as the starting material of Heparin Sodium supplied for preparing Heparin Sodium Injection.

Method of preparation Dissolve Heparin Sodium in Isotonic Sodium Chloride Solution and prepare as directed under Injections.

Description Heparin Sodium Injection is a clear, colorless to light yellow liquid.

pH <2.54> 5.5 – 8.0

Purity (1) Barium—Measure exactly a portion of Heparin Sodium Injection, equivalent to 3000 Units of Heparin Sodium according to the labeled Unit. Add water to make 3.0 mL and use this solution as the sample solution. To 1.0 mL of the sample solution add 3 drops of dilute sulfuric acid, and allow to stand for 10 minutes: no turbidity is produced.

(2) Protein—Proceed as directed in the Purity (4) under Heparin Sodium.

Bacterial endotoxins <4.01> Less than 0.0030 EU/unit.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter Perform the test according to Method 1: it meets the requirement.

Sterility Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Proceed as directed in the Assay under Heparin Sodium, replacing the sample solution indicated in (vii) and the calculation in (ix) with the following.

Sample solution: Measure exactly an adequate portion of Heparin Sodium Injection according to the labeled Units, dilute it with isotonic sodium chloride solution so that each mL contains about 0.5 Units. To 100 μ L of this solution add 100 μ L of antithrombin III solution, 100 μ L of human normal plasma and 700 μ L of the buffer solution, and use this solution as the sample solution.

Calculation: Plot the absorbances of the standard solutions on the vertical axis and their heparin concentrations on the horizontal axis to prepare a calibration curve. Determine the heparin concentration, *C*, of the sample solution from its absorbance by using the curve, and calculate heparin Units per mL of Heparin Sodium Injection from the following formula.

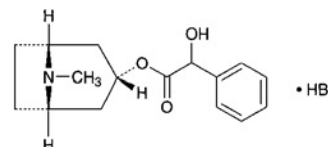
$$\begin{aligned} &\text{Units per mL of Heparin Sodium Injection} \\ &= C \times 10 \times (b/a) \end{aligned}$$

- a*: Amount of sample (mL)
b: Total volume (mL) of isotonic sodium chloride solution used to dilute the sample to make the solution containing about 0.5 Units per mL

Containers and storage Containers—Hermetic containers.
 Storage—Light-resistant.

Homatropine Hydrobromide

ホマトロピン臭化水素酸塩



$C_{16}H_{21}NO_3 \cdot HBr$: 356.25

(1*R*,3*r*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-3-yl [(2*RS*)-2-hydroxy-2-phenyl]acetate monohydrobromide [51-56-9]

Homatropine Hydrobromide contains not less than 99.0% of $C_{16}H_{21}NO_3 \cdot HBr$, calculated on the dried basis.

Description Homatropine Hydrobromide occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether. It is affected by light.

Melting point: about 214°C (with decomposition).

Identification (1) To 5 mL of a solution of Homatropine Hydrobromide (1 in 20) add 2 to 3 drops of iodine TS: a brown precipitate is produced.

(2) Dissolve 0.05 g of Homatropine Hydrobromide in 5 mL of water, and add 3 mL of 2,4,6-trinitrophenol TS: a yellow precipitate is produced. Filter the precipitate, wash with five 10-mL portions of water, and dry at 105°C for 2 hours: it melts <2.60> between 184°C and 187°C.

(3) A solution of Homatropine Hydrobromide (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

Purity (1) Acidity—Dissolve 1.0 g of Homatropine Hydrobromide in 20 mL of water, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red-methylene blue TS: a green color develops.

(2) Atropine, hyoscyamine and scopolamine—To 10 mg of Homatropine Hydrobromide add 5 drops of nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: no red-purple color is produced.

(3) Related substances—Dissolve 0.15 g of Homatropine Hydrobromide in 3 mL of water, and use this solution as the sample solution.

- (i) To 1 mL of the sample solution add 2 to 3 drops of tannic acid TS: no precipitate is produced.
 (ii) To 1 mL of the sample solution add 2 to 3 drops each

of dilute hydrochloric acid and platinic chloride TS: no precipitate is produced.

Loss on drying <2.41> Not more than 1.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (0.2 g).

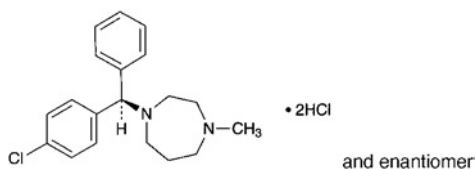
Assay Dissolve by warming about 0.4 g of Homatropine Hydrobromide in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3). Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 35.63 mg of C₁₉H₂₁NO₃·HBr

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Homochlorcyclizine Hydrochloride

ホモクロルシクリジン塩酸塩



C₁₉H₂₃ClN₂·2HCl: 387.77

1-[(*RS*)-(4-Chlorophenyl)(phenyl)methyl]-
4-methylhexahydro-1*H*-1,4-diazepine dihydrochloride
[1982-36-1]

Homochlorcyclizine Hydrochloride, when dried, contains not less than 98.0% of C₁₉H₂₃ClN₂·2HCl.

Description Homochlorcyclizine Hydrochloride occurs as white to pale brown, crystals or powder.

It is very soluble in water, freely soluble in acetic acid (100), slightly soluble in ethanol (99.5), and very slightly soluble in acetonitrile and in acetic anhydride.

It dissolves in 0.1 mol/L hydrochloric acid TS.

It is hygroscopic.

It is colored slightly by light.

A solution of Homochlorcyclizine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 227°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Homochlorcyclizine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Homochlorcyclizine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Homochlorcyclizine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of

Homochlorcyclizine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Homochlorcyclizine Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Measure exactly 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method: the areas of the peaks other than homochlorcyclizine obtained from the sample solution are not more than 1/2 times the peak area of homochlorcyclizine from the standard solution, and the total area of the peaks other than homochlorcyclizine from the sample solution is not more than the peak area of homochlorcyclizine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 223 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and perchloric acid (134:66:1).

Flow rate: Adjust the flow rate so that the retention time of homochlorcyclizine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of homochlorcyclizine.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 50 mL. Confirm that the peak area of homochlorcyclizine obtained from 10 μL of this solution is equivalent to 7 to 13% of that of homochlorcyclizine obtained from 10 μL of the standard solution.

System performance: Dissolve 5 mg each of Homochlorcyclizine Hydrochloride and methyl parahydroxybenzoic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, methyl parahydroxybenzoic acid and homochlorcyclizine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of homochlorcyclizine is not more than 1.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, 110°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.3 g of Homochlorcyclizine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS

= 19.39 mg of $C_{19}H_{23}ClN_2 \cdot 2HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Human Normal Immunoglobulin

人免疫グロブリン

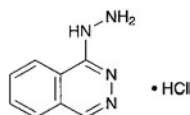
Human Normal Immunoglobulin is a liquid for injection containing immunoglobulin G in serum globulins of humans.

It conforms to the requirements of Human Normal Immunoglobulin in the Minimum Requirements for Biological Products.

Description Human Normal Immunoglobulin is a clear, colorless or yellow-brown liquid.

Hydralazine Hydrochloride

ヒドララジン塩酸塩



$C_8H_8N_4 \cdot HCl$: 196.64

Phthalazin-1-ylhydrazine monohydrochloride [304-20-1]

Hydralazine Hydrochloride, when dried, contains not less than 98.0% of $C_8H_8N_4 \cdot HCl$.

Description Hydralazine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 275°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Hydralazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Hydralazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Hydralazine Hydrochloride (1 in 4000) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydralazine Hydrochloride in 50 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Hydrala-

zine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 8 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Hydralazine Hydrochloride, previously dried, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, add 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking until the purple color of the chloroform layer disappears. The end point is reached when the red-purple color no more reappears in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4 \cdot HCl$

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride for Injection

注射用ヒドララジン塩酸塩

Hydralazine Hydrochloride for Injection is a preparation for injection which is dissolved before use. It contains not less than 99% and not more than 113% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4 \cdot HCl$: 196.64).

Method of preparation Prepare as directed under Injections, with Hydralazine Hydrochloride.

Description Hydralazine Hydrochloride for Injection occurs as a white to pale yellow powder or mass. It is odorless, and has a bitter taste.

Identification Determine the absorption spectrum of a solution of Hydralazine Hydrochloride for Injection (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

pH <2.54> Dissolve 1.0 g of Hydralazine Hydrochloride for Injection in 50 mL of water: the pH of this solution is between 3.5 and 4.5.

Assay Weigh accurately the contents of not less than 10 samples of Hydralazine Hydrochloride for Injection. Weigh accurately about 0.15 g of the contents, transfer it to a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, cool to room temperature, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4 \cdot HCl$

Containers and storage Containers—Hermetic containers.

Hydralazine Hydrochloride Powder

ヒドララジン塩酸塩散

Hydralazine Hydrochloride Powder contains not less than 95% and not more than 105% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4.HCl$: 196.64).

Method of preparation Prepare as directed under Powder, with Hydralazine Hydrochloride.

Identification Weigh a portion of Hydralazine Hydrochloride Powder, equivalent to 25 mg of Hydralazine Hydrochloride according to the labeled amount, add 100 mL of water, shake well, and filter, if necessary. Add water to 2 mL of the filtrate to make 50 mL and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm, and between 313 nm and 317 nm.

Assay Weigh accurately a portion of Hydralazine Hydrochloride Powder, equivalent to about 0.15 g of Hydralazine Hydrochloride, transfer it to a glass-stoppered flask, add 25 mL of water, shake well, add 25 mL of hydrochloric acid, cool to room temperature, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4.HCl$

Containers and storage Containers—Tight containers.

Hydralazine Hydrochloride Tablets

ヒドララジン塩酸塩錠

Hydralazine Hydrochloride Tablets contain not less than 95% and not more than 105% of the labeled amount of hydralazine hydrochloride ($C_8H_8N_4.HCl$: 196.64).

Method of preparation Prepare as directed under Tablets, with Hydralazine Hydrochloride.

Identification Weigh a quantity of powdered Hydralazine Hydrochloride Tablets, equivalent to 25 mg of Hydralazine Hydrochloride according to the labeled amount, add 100 mL of water, mix well, and filter if necessary. To 2 mL of this solution add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 238 nm and 242 nm, between 258 nm and 262 nm, between 301 nm and 305 nm and between 313 nm and 317 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Hydralazine Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 30 mL or more of the dissolved solution 45 minutes af-

ter start of the dissolution test, and filter through a membrane filter with pore size of not more than 0.8 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 11 μg of hydralazine hydrochloride ($C_8H_8N_4.HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of hydralazine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Hydralazine Hydrochloride Tablets in 45 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of hydralazine hydrochloride ($C_8H_8N_4.HCl$)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S : Amount (mg) of hydralazine hydrochloride for assay.

C : Labeled amount (mg) of hydralazine hydrochloride ($C_8H_8N_4.HCl$) in 1 tablet.

Assay Weigh accurately not less than 20 Hydralazine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of hydralazine hydrochloride ($C_8H_8N_4.HCl$), transfer it to a glass-stoppered flask, and proceed as directed in the Assay under Hydralazine Hydrochloride.

Each mL of 0.05 mol/L potassium iodate VS
= 9.832 mg of $C_8H_8N_4.HCl$

Containers and storage Containers—Tight containers.

Hydrochloric Acid

塩酸

Hydrochloric Acid contains not less than 35.0% and not more than 38.0% of hydrogen chloride (HCl: 36.46).

Description Hydrochloric Acid is a colorless liquid having a pungent odor.

It is fuming but ceases to fume when it is diluted with 2 volumes of water.

Specific gravity d_{20}^{20} , about 1.18

Identification (1) Allow a glass stick wet with ammonia TS to come near the surface of Hydrochloric Acid: a remarkable white smoke evolves.

(2) A solution of Hydrochloric Acid (1 in 100) changes blue litmus paper to red, and responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Sulfate <1.14>—To 15 mL of Hydrochloric Acid add water to make 50 mL, and use this solution as the sample solution. To 3.0 mL of the sample solution add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of the sample solution obtained in (1) add 5 mL of water and 1 drop of iodine TS: the color of io-

dine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of the sample solution obtained in (1) in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals <1.07>—Evaporate 5 mL of Hydrochloric Acid on a water bath to dryness, and add 2 mL of dilute acetic acid and water to the residue to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 5 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.7 mL of Hydrochloric Acid according to Method 1, and perform the test (not more than 1 ppm).

(7) Mercury—Dilute 20 mL of Hydrochloric Acid with water to make exactly 100 mL, and use the solution as the sample solution. Perform the test with this sample solution as directed under Atomic Absorption Spectrophotometry <2.23> (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air, and determine the absorbance A_T of the sample solution after the recorder reading has risen rapidly, and becomes constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and determine the absorbance A_S of the solution obtained by the same procedure as used for the sample solution: A_T is smaller than A_S (not more than 0.04 ppm).

Residue on ignition <2.44> Pipet 10 mL of Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness, and ignite: not more than 1.0 mg of residue remains.

Assay Weigh accurately a glass-stoppered flask containing 20 mL of water, add about 3 mL of Hydrochloric Acid, and weigh accurately again. Dilute with 25 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 36.46 \text{ mg of HCl} \end{aligned}$$

Containers and storage Containers—Tight containers.

Dilute Hydrochloric Acid

希塩酸

Dilute Hydrochloric Acid contains not less than 9.5 w/v% and not more than 10.5 w/v% of hydrogen chloride (HCl: 36.46).

Description Dilute Hydrochloric Acid is a colorless liquid. It is odorless and has a strong acid taste.

Specific gravity d_{20}^{20} : about 1.05

Identification A solution of Dilute Hydrochloric Acid (1 in 30) changes blue litmus paper to red and responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Sulfate—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 5 drops of barium chloride TS, and allow to stand for 1 hour: no turbidity is produced.

(2) Sulfite—To 3.0 mL of Dilute Hydrochloric Acid add 5 mL of water and 1 drop of iodine TS: the color of iodine TS does not disappear.

(3) Bromide or iodide—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 1 mL of chloroform and 1 drop of 0.002 mol/L potassium permanganate VS, and shake well: the chloroform layer remains colorless.

(4) Bromine or chlorine—Place 10 mL of Dilute Hydrochloric Acid in a glass-stoppered test tube, add 5 drops of potassium iodide TS and 1 mL of chloroform, and shake for 1 minute: the chloroform layer remains free from a purple color.

(5) Heavy metals <1.07>—Evaporate 9.5 mL of Dilute Hydrochloric Acid on a water bath to dryness, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 3 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 4.0 mL of Dilute Hydrochloric Acid according to Method 1, and perform the test (not more than 0.5 ppm).

(7) Mercury—Dilute 80 mL of Dilute Hydrochloric Acid with water to make exactly 100 mL, and use this solution as the sample solution. Perform the test with this solution according to the Atomic Absorption Spectrophotometry <2.23> (cold vapor type). Place the sample solution in a sample bottle of the atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the spectrophotometer, circulate air, and read the absorbance A_T of the sample solution after the recorder reading has risen rapidly and become constant at a wavelength of 253.7 nm. On the other hand, to 8 mL of Standard Mercury Solution add water to make exactly 100 mL, and read the absorbance A_S of the solution obtained by the same procedure as used for the sample solution: A_T is smaller than A_S (not more than 0.01 ppm).

Residue on ignition <2.44> Pipet 10 mL of Dilute Hydrochloric Acid, add 2 drops of sulfuric acid, evaporate to dryness, and ignite: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 10 mL of Dilute Hydrochloric Acid, and dilute with 20 mL of water. Titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 to 3 drops of methyl red TS).

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 36.46 \text{ mg of HCl} \end{aligned}$$

Containers and storage Containers—Tight containers.

Hydrochloric Acid Lemonade

塩酸リモナーデ

Method of preparation

Dilute Hydrochloric Acid	5 mL
Simple Syrup	80 mL
Purified Water	a sufficient quantity
To make 1000 mL	

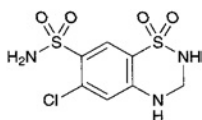
Prepare before use as directed under Lemonades, with the above ingredients.

Description Hydrochloric Acid Lemonade is a clear, colorless liquid. It has a sweet, cool, acid taste.

Containers and storage Containers—Tight containers.

Hydrochlorothiazide

ヒドロクロロチアジド



$C_7H_8ClN_3O_4S_2$: 297.74

6-Chloro-3,4-dihydro-2*H*-1,2,4-benzothiazine-7-sulfonamide 1,1-dioxide [58-93-5]

Hydrochlorothiazide, when dried, contains not less than 99.0% of $C_7H_8ClN_3O_4S_2$.

Description Hydrochlorothiazide occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetone, sparingly soluble in acetonitrile, very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 267°C (with decomposition).

Identification (1) To 5 mg of Hydrochlorothiazide add 5 mL of disodium chlomotropate TS, and allow to stand for 5 minutes: a purple color develops.

(2) Fuse a mixture of 0.1 g of Hydrochlorothiazide and 0.5 g of sodium carbonate decahydrate cautiously: the gas evolved changes moistened red litmus paper to blue. After cooling, crush with a glass rod, add 10 mL of water, stir, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is produced.

(3) To 4 mL of the filtrate obtained in (2) add 5 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white precipitate is produced.

(4) Dissolve 12 mg of Hydrochlorothiazide in 100 mL of sodium hydroxide TS. Dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spec-

trophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Hydrochlorothiazide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Hydrochlorothiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Hydrochlorothiazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Primary aromatic amines—Dissolve 80 mg of Hydrochlorothiazide in acetone to make exactly 100 mL. Measure exactly 1 mL of the solution, add 3.0 mL of dilute hydrochloric acid, 3.0 mL of water and 0.15 mL of sodium nitrite TS, shake, and allow to stand for 1 minute. Shake this solution with 1.0 mL of ammonium amidosulfate TS, allow to stand for 3 minutes, then add 1.0 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate TS, shake, and allow to stand for 5 minutes. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 1.0 mL of acetone in the same manner as the blank: the absorbance at 525 nm is not more than 0.10.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 30 mg each of Hydrochlorothiazide and Hydrochlorothiazide Reference Standard, previously dried, and dissolve in 150 mL of the mobile phase, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrochlorothiazide to that of the internal standard.

$$\text{Amount (mg) of } C_7H_8ClN_3O_4S_2 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Hydrochlorothiazide Reference Standard

Internal standard solution—A solution of 4-aminoacetophenone in acetonitrile (9 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L sodium dihydrogen phosphate TS, pH 3.0 and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of hydrochlorothiazide is about 10 minutes.

System suitability—

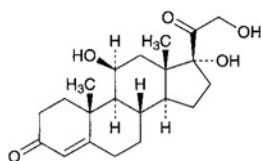
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrochlorothiazide and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrochlorothiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Hydrocortisone

ヒドロコルチゾン



$\text{C}_{21}\text{H}_{30}\text{O}_5$: 362.46
11 β ,17,21-Trihydroxypregn-4-ene-3,20-dione [50-23-7]

Hydrocortisone, when dried, contains not less than 97.0% and not more than 102.0% of $\text{C}_{21}\text{H}_{30}\text{O}_5$.

Description Hydrocortisone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol, in ethanol (95) and in 1,4-dioxane, slightly soluble in chloroform, and very slightly soluble in diethyl ether and in water.

Melting point: 212 – 220°C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone: the solution shows a yellow-green fluorescence immediately, and the color of the solution changes gradually from orange to dark red. Dilute carefully the solution with 10 mL of water: the color changes through yellow to orange-yellow with green fluorescence, and a small amount of a flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is formed.

(3) Determine the infrared absorption spectrum of Hydrocortisone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Reference Standard: both spectra exhibit similar intensities

of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone and Hydrocortisone Reference Standard in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +150 – +156° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 20 mg of Hydrocortisone in 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone and Hydrocortisone Reference Standard, previously dried and accurately weighed, in 20 mL each of a mixture of chloroform and methanol (9:1), add 10 mL each of the internal standard solution, then add a mixture of chloroform and methanol (9:1) to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μL each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrocortisone to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } \text{C}_{21}\text{H}_{30}\text{O}_5 \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Hydrocortisone Reference Standard

Internal standard solution—A solution of prednisone in a mixture of chloroform and methanol (9:1) (9 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of chloroform, methanol and acetic acid (100) (1000:20:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone is about 15 minutes.

System suitability—

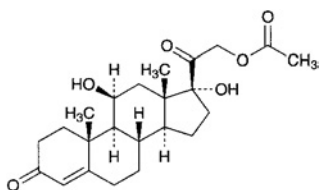
System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, the internal standard and hydrocortisone are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone Acetate

ヒドロコルチゾン酢酸エステル



$\text{C}_{23}\text{H}_{32}\text{O}_6$: 404.50

11 β ,17,21-Trihydroxypregn-4-ene-3,20-dione 21-acetate
[50-03-3]

Hydrocortisone Acetate, when dried, contains not less than 97.0% and not more than 102.0% of $\text{C}_{23}\text{H}_{32}\text{O}_6$.

Description Hydrocortisone Acetate occurs as white crystals or crystalline powder. It is odorless.

It is sparingly soluble in 1,4-dioxane, slightly soluble in methanol, in ethanol (95) and in chloroform, very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 220°C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Acetate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone Acetate in 1 mL of methanol by warming, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.05 g of Hydrocortisone Acetate add 2 mL of potassium hydroxide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(4) Determine the infrared absorption spectra of Hydrocortisone Acetate and Hydrocortisone Acetate Reference Standard, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: both the sample and the Reference Standard exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and Reference Standard in ethanol (95), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +158 – +165° (after drying, 50 mg, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 40 mg of Hydrocortisone Acetate in 25 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, add a mixture of chloroform and methanol (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (160:30:8:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 20 mg each of Hydrocortisone Acetate and Hydrocortisone Acetate Reference Standard, previously dried and accurately weighed, in methanol, add exactly 10 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrocortisone acetate to that of the internal standard, respectively.

$$\text{Amount (mg) of } \text{C}_{23}\text{H}_{32}\text{O}_6 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Hydrocortisone Acetate Reference Standard

Internal standard solution—A solution of benzyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone acetate is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, hydrocortisone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone and Diphenhydramine Ointment

ヒドロコルチゾン・ジフェンヒドラミン軟膏

Method of preparation

Hydrocortisone Acetate	5 g
Diphenhydramine	5 g
White Petrolatum	a sufficient quantity

To make 1000 g

Prepare as directed under Ointments, with the above ingredients.

Description Hydrocortisone and Diphenhydramine Ointment is white to pale yellow in color.

Identification (1) To 1 g of Hydrocortisone and Diphenhydramine Ointment add 10 mL of ethanol (95), heat on a water bath for 5 minutes with occasional shaking, cool, and filter. Take 5 mL of the filtrate, distill off the ethanol, and to the residue add 2 mL of sulfuric acid: the solution shows a yellow-green fluorescence immediately and the color of the solution gradually changes through yellow to yellow-brown. Add carefully 10 mL of water to this solution: the color changes to yellow with green fluorescence, and a light yellow, flocculent precipitate is formed (hydrocortisone acetate).

(2) To 1 mL of the filtrate obtained in (1) add 5 mL of potassium hydrogen phthalate buffer solution, pH 4.6, and 2 mL of bromophenol blue TS, and add further 5 mL of chloroform. Shake well, and allow to stand: a yellow color develops in the chloroform layer (diphenhydramine).

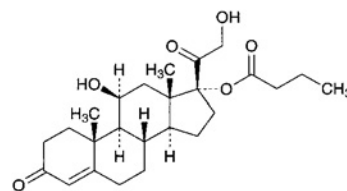
(3) To 1 g of Hydrocortisone and Diphenhydramine Ointment add 5 mL of methanol, warm, and shake. After cooling, separate the methanol layer, and use this layer as the sample solution. Dissolve 0.01 g each of hydrocortisone acetate and diphenhydramine in 10 mL each of methanol, and use these solutions as standard solutions (1) and (2). Perform the test with the sample solution and standard solutions (1) and (2) as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of these solutions on a plate of silica gel with a complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethyl ether (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): two spots from the sample solution show the same R_f value as the corresponding spots from standard solutions (1) and (2).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Hydrocortisone Butyrate

ヒドロコルチゾン酪酸エステル



$C_{25}H_{36}O_6$: 432.55

11 β ,17,21-Trihydroxypregn-4-ene-3,20-dione 17-butyrate
[13609-67-1]

Hydrocortisone Butyrate, when dried, contains not less than 96.0% and not more than 104.0% of $C_{25}H_{36}O_6$.

Description Hydrocortisone Butyrate occurs as a white powder. It is odorless.

It is freely soluble in tetrahydrofuran, in chloroform and in 1,2-dichloroethane, soluble in methanol, sparingly soluble in ethanol (99.5), slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Hydrocortisone Butyrate: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light (main wavelength: 254 nm). Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Hydrocortisone Butyrate in 1 mL of methanol by warming, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.05 g of Hydrocortisone Butyrate add 2 mL of potassium hydroxide-ethanol TS, and heat on a water bath for 5 minutes. Cool, add 2 mL of diluted sulfuric acid (2 in 7), and boil gently for 1 minute: the odor of ethyl butyrate is perceptible.

(4) Determine the infrared absorption spectrum of Hydrocortisone Butyrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: +48 – +52° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Hydrocortisone Butyrate according to method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 25 mg of Hydrocortisone Butyrate in 5 mL of tetrahydrofuran, and use this solution as the sample solution. Pipet 2 mL of this solution, and add tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of

this solution, add tetrahydrofuran to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (470:30:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more than two in number, and not more intense than those from the standard solution in color.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

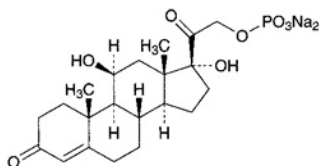
Assay Weigh accurately about 50 mg of Hydrocortisone Butyrate, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. Pipet 2 mL of this solution, and add ethanol (99.5) to make exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_{25}H_{36}O_6 = (A/375) \times 25,000$$

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Phosphate

ヒドロコルチゾンリン酸エステルナトリウム



$C_{21}H_{29}Na_2O_8P$: 486.40

Disodium 11 β ,17,21-trihydroxypregn-4-ene-3,20-dione 21-phosphate [6000-74-4]

Hydrocortisone Sodium Phosphate contains not less than 96.0% and not more than 102.0% of $C_{21}H_{29}Na_2O_8P$, calculated on the dried basis.

Description Hydrocortisone Sodium Phosphate occurs as a white to light yellow powder. It is odorless.

It is freely soluble in water, sparingly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To 2 mg of Hydrocortisone Sodium Phosphate add 2 mL of sulfuric acid: a yellowish green fluorescence is exhibited initially, then gradually changes through orange-yellow to dark red. Examine the solution under ultraviolet light (main wavelength: 254 nm): an intense, light green fluorescence is exhibited. To this solution add carefully 10 mL of water: the color changes from yellow to orange-yellow with a light green fluorescence and a yellow-brown, flocculent floating substance is formed.

(2) Determine the infrared absorption spectrum of

Hydrocortisone Sodium Phosphate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Hydrocortisone Sodium Phosphate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(3) Moisten 1.0 g of Hydrocortisone Sodium Phosphate with a small quantity of sulfuric acid, and incinerate by gradual heating. After cooling, dissolve the residue in 10 mL of dilute nitric acid, and heat in a water bath for 30 minutes. After cooling, filter if necessary. This solution responds to the Qualitative Tests <1.09> for sodium salt and for phosphate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +121 – +129° (1 g, calculated on the dried basis, phosphate buffer solution, pH 7.0, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 100 mL of water: the pH of this solution is between 7.5 and 9.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydrocortisone Sodium Phosphate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—Dissolve 0.30 g of Hydrocortisone Sodium Phosphate in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 100 mL. To 5 mL of this solution add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.600%).

(3) Heavy metals <1.07>—Proceed with 0.5 g of Hydrocortisone Sodium Phosphate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 40 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Hydrocortisone Sodium Phosphate according to Method 3, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.25 g of Hydrocortisone Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution into separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at $20 \pm 1^\circ\text{C}$ for 30 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S , of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the amount of free phosphoric acid is not more than 1.0%.

Content (%) of free phosphoric acid (H_3PO_4)

$$= (A_T/A_S) \times (1/W) \times 257.8$$

W : Amount (mg) of Hydrocortisone Sodium Phosphate, calculated on the dried basis.

(6) Free hydrocortisone—Dissolve 25 mg of Hydrocorti-

sone Sodium Phosphate in the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh 25 mg of Hydrocortisone Reference Standard, previously dried at 105°C for 3 hours, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of hydrocortisone from each solution: A_T is not larger than A_S .

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hydrocortisone is not more than 1.0%.

Loss on drying <2.41> Not more than 5.0% (1 g, in vacuum, 80°C, 5 hours).

Assay Weigh accurately about 20 mg each of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Phosphate Reference Standard (previously determine the loss on drying <2.41> in the same manner as Hydrocortisone Sodium Phosphate), dissolve each in 50 mL of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 200 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrocortisone phosphate to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{29}Na_2O_8P = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Hydrocortisone Sodium Phosphate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of isopropyl parahydroxybenzoate in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS, pH 2.6 and methanol (1:1).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone phosphate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, hydrocortisone phosphate and isopropyl para-

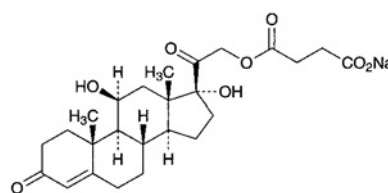
hydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone phosphate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Hydrocortisone Sodium Succinate

ヒドロコルチゾンコハク酸エステルナトリウム



$C_{25}H_{33}NaO_8$: 484.51

Monosodium 11 β ,17,21-trihydroxypregn-4-ene-3,20-dione 21-succinate [125-04-2]

Hydrocortisone Sodium Succinate, calculated on the dried basis, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{33}NaO_8$.

Description Hydrocortisone Sodium Succinate occurs as white powder or masses. It is odorless.

It is freely soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

It is gradually colored by light.

Identification (1) Dissolve 0.2 g of Hydrocortisone Sodium Succinate in 20 mL of water, and add 0.5 mL of dilute hydrochloric acid with stirring: a white precipitate is formed. Collect the precipitate, wash it with two 10-mL portions of water, and dry at 105°C for 3 hours. To 3 mg of this dried matter add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Dissolve 0.01 g of the dried matter obtained in (1) in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) To 0.1 g of the dried matter obtained in (1) add 2 mL of sodium hydroxide TS, and allow to stand for 10 minutes. Filter the solution to remove the precipitate formed, mix the filtrate with 1 mL of dilute hydrochloric acid, filter if necessary, then adjust the solution to a pH of about 6 with diluted ammonia TS (1 in 10), and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Determine the infrared absorption spectrum of the dried matter obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spec-

trum or the spectrum of previously dried Hydrocortisone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Sodium Succinate and Hydrocortisone Succinate Reference Standard in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

(5) Hydrocortisone Sodium Succinate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +135 – +145° (0.1 g, calculated on the dried basis, ethanol (95), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Hydrocortisone Sodium Succinate in 10 mL of water: the solution is clear and colorless.

(2) Other steroids—Dissolve 25 mg of Hydrocortisone Sodium Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 0.025 g of hydrocortisone in methanol to make exactly 10 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution (1). Pipet 6 mL of the standard solution (1), add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution (1) is not more intense than the spot from the standard solution (1). Any spot other than the principal spot and the above spot obtained from the sample solution is not more than one, and is not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 2.0% (0.5 g, 105°C, 3 hours).

Assay Weigh accurately about 10 mg of Hydrocortisone Sodium Succinate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.01 g of Hydrocortisone Succinate Reference Standard, previously dried at 105°C for 3 hours, proceed in the same manner as directed for the sample solution, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

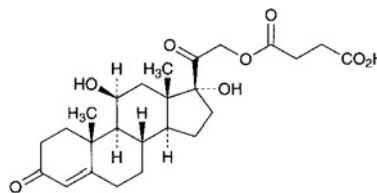
$$\begin{aligned} \text{Amount (mg) of } C_{25}H_{33}NaO_8 \\ = W_S \times (A_T/A_S) \times 1.0475 \end{aligned}$$

W_S : Amount (mg) of Hydrocortisone Succinate Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Hydrocortisone Succinate

ヒドロコルチゾンコハク酸エステル



$C_{25}H_{34}O_8$: 462.53

11 β ,17,21-Trihydroxypregn-4-ene-3,20-dione
21-(hydrogen succinate) [2203-97-6]

Hydrocortisone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{34}O_8$.

Description Hydrocortisone Succinate occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (99.5), sparingly soluble in ethanol (95), and practically insoluble in water.

Identification (1) To 3 mg of Hydrocortisone Succinate add 2 mL of sulfuric acid: the solution shows a yellowish green fluorescence immediately, and the color of the solution gradually changes through orange-yellow to dark red. This solution shows a strong light green fluorescence under ultraviolet light. Add carefully 10 mL of water to this solution: the color changes from yellow to orange-yellow with a light green fluorescence, and a yellow-brown flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Hydrocortisone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Hydrocortisone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Hydrocortisone Succinate and Hydrocortisone Succinate Reference Standard in methanol, respectively, then evaporate the methanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +147 – +153° (after drying, 0.1 g, ethanol (99.5), 10 mL, 100 mm).

Purity Related substances—Dissolve 25 mg of Hydrocortisone Succinate in exactly 10 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.025 g of hydrocortisone in exactly 10 mL of methanol. Pipet 1 mL of this solution, dilute with methanol to exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (99.5) and formic acid (150:10:1) to a distance of about 10 cm, and air-dry the plate. Examine un-

der ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 50 mg each of Hydrocortisone Succinate and Hydrocortisone Succinate Reference Standard, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, then add methanol to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions, and calculate the ratios, Q_T and Q_S , of the peak area of hydrocortisone succinate to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{25}H_{34}O_8 \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Hydrocortisone Succinate Reference Standard

Internal standard solution—A solution of butyl parahydroxy benzoate in methanol (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetic acid-sodium acetate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of hydrocortisone succinate is about 5 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, hydrocortisone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

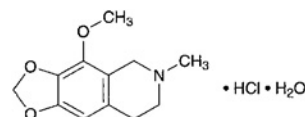
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of hydrocortisone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Hydrocotarnine Hydrochloride Hydrate

ヒドロコタルニン塩酸塩水和物



$C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$: 275.73

4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinoline monohydrochloride monohydrate [5985-55-7, anhydride]

Hydrocotarnine Hydrochloride Hydrate, when dried, contains not less than 98.0% of hydrocotarnine-hydrochloride ($C_{12}H_{15}NO_3HCl$: 257.72).

Description Hydrocotarnine Hydrochloride Hydrate occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and slightly soluble in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Hydrocotarnine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Hydrocotarnine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Hydrocotarnine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Hydrocotarnine Hydrochloride Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Hydrocotarnine Hydrochloride Hydrate in 10 mL of water: the solution is clear, and when perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, the absorbance at 400 nm is not more than 0.17.

(2) Heavy metals <1.07>—Proceeds with 1.0 g of Hydrocotarnine Hydrochloride Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Hydrocotarnine Hydrochloride Hydrate in 10 mL of diluted ethanol (99.5) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (99.5) (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these

solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Hydrocotarnine Hydrochloride Hydrate, previously dried. Dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 25.77 mg of $\text{C}_{12}\text{H}_{15}\text{NO}_3\text{HCl}$

Containers and storage Containers—Tight containers.

Hydrogenated Oil

硬化油

Hydrogenated Oil is the fat obtained by hydrogenation of fish oil or of other oils originating from animal or vegetable.

Description Hydrogenated Oil occurs as a white mass or powder and has a characteristic odor and a mild taste.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

The oil obtained by hydrogenation of castor oil is slightly soluble in diethyl ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Acid value <1.13> Not more than 2.0.

Purity (1) Moisture and coloration—Hydrogenated Oil (5.0 g), melted by heating on a water bath, forms a clear liquid, from which no water separates. In a 10-mm thick layer of the liquid, it is colorless or slightly yellow.

(2) Alkalinity—To 2.0 g of Hydrogenated Oil add 10 mL of water, melt by heating on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: no color develops.

(3) Chloride—To 1.5 g of Hydrogenated Oil add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the turbidity of the solution does not exceed that of the following control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, then add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

(4) Heavy metals—Heat 2.0 g of Hydrogenated Oil with 5 mL of dilute hydrochloric acid and 10 mL of water on a

water bath for 5 minutes with occasional shaking. After cooling, filter, and make 5 mL of the filtrate weakly alkaline with ammonia TS, then add 3 drops of sodium sulfide TS: the solution remains unchanged.

(5) Nickel—Place 5.0 g of Hydrogenated Oil in a quartz or porcelain crucible, heat slightly with caution at the beginning, and, after carbonization, incinerate by strong heating ($500 \pm 20^\circ\text{C}$). Cool, add 1 mL of hydrochloric acid, evaporate on a water bath to dryness, dissolve the residue in 3 mL of dilute hydrochloric acid, and add 7 mL of water. Then add 1 mL of bromine TS and 1 mL of a solution of citric acid monohydrate (1 in 5), make alkaline with 5 mL of ammonia TS, and cool in running water. To this solution add 1 mL of dimethylglyoxime TS, add water to make 20 mL, and use this solution as the test solution. Allow to stand for 5 minutes: the solution has no more color than the following control solution.

Control solution: Evaporate 1 mL of hydrochloric acid on a water bath to dryness, add 1 mL of Standard Nickel Solution and 3 mL of dilute hydrochloric acid, and add 6 mL of water. Then proceed as directed in the test solution, add water to make 20 mL, and allow to stand for 5 minutes.

Residue on ignition <2.44> Not more than 0.1% (5 g).

Containers and storage Containers—Well-closed containers.

Hydrophilic Ointment

親水軟膏

Method of preparation

White Petrolatum	250 g
Stearyl Alcohol	200 g
Propylene Glycol	120 g
Polyoxyethylene hydrogenated castor oil 60	40 g
Glycerin Monostearate	10 g
Methyl Parahydroxybenzoate	1 g
Propyl Parahydroxybenzoate	1 g
Purified Water	a sufficient quantity

To make 1000 g

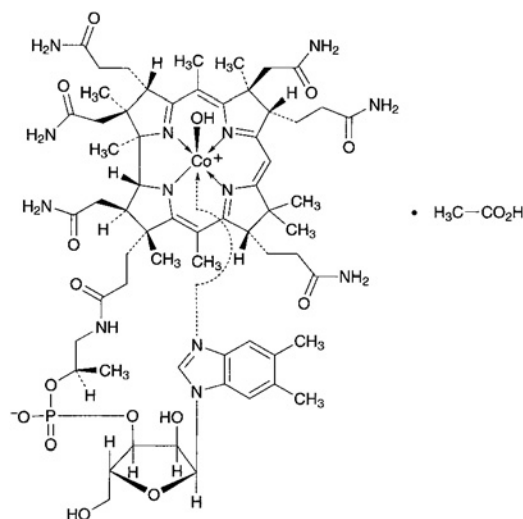
Melt White Petrolatum, Stearyl Alcohol, polyoxyethylene hydrogenated castor oil 60 and Glycerin Monostearate by heating on a water bath, stir, and keep temperature of the mixture at about 75°C. To Propylene Glycol add Methyl Parahydroxybenzoate and Propyl Parahydroxybenzoate, melt by warming if necessary, dissolve in Purified Water, and warm to about 75°C. Add this solution to the above mixture, stir to form emulsion, cool, and stir thoroughly until it congeals.

Description Hydrophilic Ointment is white in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Hydroxocobalamin Acetate

ヒドロキソコバラミン酢酸塩



$C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2$: 1406.41
 $Co\alpha$ -[α -(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]- $Co\beta$ -hydroxocobamide monoacetate
 [13422-51-0, Hydroxocobalamin]

Hydroxocobalamin Acetate contains not less than 95.0% of $C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2$, calculated on the dried basis.

Description Hydroxocobalamin Acetate occurs as dark red crystals or powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Hydroxocobalamin Acetate in acetic acid-sodium acetate buffer solution, pH 4.5 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Hydroxocobalamin Acetate with 0.05 g of potassium hydrogen sulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until the solution develops a light red. Then add 0.5 g of sodium acetate trihydrate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color develops immediately. Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

(3) Add 0.5 mL of ethanol (99.5) and 1 mL of sulfuric acid to 0.02 g of Hydroxocobalamin Acetate, and heat the mixture: the odor of ethyl acetate is perceptible.

Purity Cyanocobalamin and colored impurities—Dissolve 50 mg of Hydroxocobalamin Acetate in exactly 5 mL each of

acetic acid-sodium acetate buffer solution, pH 5.0, in two tubes. To one tube add 0.15 mL of potassium thiocyanate TS, allow to stand for 30 minutes, and use this solution as the sample solution (1). To the other tube add 0.10 mL of potassium cyanide TS, allow to stand for 30 minutes, and use this solution as the sample solution (2). Separately, dissolve 3.0 mg of Cyanocobalamin Reference Standard in exactly 10 mL of acetic acid-sodium acetate buffer solution, pH 5.0, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Apply 20 μ L each of the sample solution and standard solution 25 mm in length along the starting line, 10 mm apart from each other, on a plate of silica gel for thin-layer chromatography. Develop the plate for 18 hours with 2-butanol saturated with water, while supporting the plate at an angle of about 15° to a horizontal plane, and air-dry the plate: the spot from the sample solution (1) corresponding to that from the standard solution is not more intense than the spot from the standard solution, and the spots other than the principal spot from the sample solution (2) are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 12% (50 mg, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 100°C, 6 hours).

Assay Weigh accurately about 20 mg of Hydroxocobalamin Acetate, and dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 2 mL of this solution into a 50-mL volumetric flask, add 1 mL of a solution of potassium cyanide (1 in 1000), and allow to stand for 30 minutes at ordinary temperature. Add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Cyanocobalamin Reference Standard after determining the loss on drying in the same manner as for Cyanocobalamin, and dissolve in water to make exactly 50 mL. To 2 mL of this solution, exactly measured, add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 361 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{62}H_{89}CoN_{13}O_{15}P \cdot C_2H_4O_2 \\ = W_S \times (A_T/A_S) \times 1.0377 \end{aligned}$$

W_S : Amount (mg) of Cyanocobalamin Reference Standard, calculated on the dried basis

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Hydroxypropylcellulose

ヒドロキシプロピルセルロース

Hydroxypropylcellulose is a hydroxypropyl ether of cellulose.

Hydroxypropylcellulose, when dried, contains not less than 53.4% and not more than 77.5% of hydroxypropoxy group ($-OC_3H_6OH$: 75.09).

Description Hydroxypropylcellulose occurs as a white to

yellowish white powder.

It is practically insoluble in diethyl ether.

It forms a viscous liquid upon addition of water or ethanol (95).

Identification (1) To 1 g of Hydroxypropylcellulose add 100 mL of water, heat in a water bath at 70°C for 5 minutes with stirring, and cool while shaking. Allow to stand at room temperature until it becomes more homogeneous and viscous, and use this solution as the sample solution. To 2 mL of the sample solution add 1 mL of anthrone TS gently: a blue to green color develops at the zone of contact.

(2) Heat the sample solution obtained in (1): a white turbidity or precipitate is produced, and the turbidity or precipitate disappears when cooled.

(3) To 1 g of Hydroxypropylcellulose add 100 mL of ethanol (95), and allow to stand after stirring: a homogeneous and viscous liquid is produced.

pH <2.54> Dissolve 1.0 g of Hydroxypropylcellulose in 50 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.5.

Purity (1) Clarity of solution—Use an outer glass cylinder, 250 mm in height, 25 mm in internal diameter, 2 mm in thickness, with a high-quality glass plate 2 mm thick at the bottom, and inner glass cylinder, 300 mm in height, 15 mm in internal diameter, 2 mm in thickness, with a high-quality glass plate 2 mm thick at the bottom. In the outer cylinder place a solution prepared by adding 1.0 g of Hydroxypropylcellulose to 100 mL of water, heat while stirring in a water bath at 70°C, and then cool to room temperature. Place this cylinder on a sheet of white paper on which 15 parallel, black, 1-mm width lines are drawn at 1-mm intervals. Place the inner cylinder, and move it up and down while viewing downward through the bottom of the inner cylinder, and measure the minimum height of the solution between the bottom of the outer cylinder and the lower end of the inner cylinder at the time when the lines on the paper cannot be differentiated. The average value obtained from three repeated procedures is greater than that obtained from the following control solution treated in the same manner.

Control solution: To 5.50 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid, 5 mL of ethanol (95) and water to make 50 mL. To this solution add 2 mL of barium chloride TS, mix, allow to stand for 10 minutes, and shake well before use.

(2) Chloride <1.03>—Add 1.0 g of Hydroxypropylcellulose to 30 mL of water, heat in a water bath with stirring for 30 minutes, and filter while being hot. Wash the residue with three 15-mL portions of hot water, combine the washings with the filtrate, and add water to make 100 mL after cooling. To 10 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(3) Sulfate <1.14>—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Hydroxypropylcellulose according to Method 2 and perform the test.

Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Hydroxypropylcellulose according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay (i) Apparatus—Reaction flask: A 5-mL screw-cap pressure-tight glass bottle, having an inverted conical bottom inside, 20 mm in outside diameter, 50 mm in height up to the neck, 2 mL in capacity up to a height of about 30 mm, equipped with a pressure-tight septum of heat-resisting resin and also with an inside stopper or sealer of fluoroplastic.

Heater: A square aluminum block 60 to 80 mm thick, having holes 20.6 mm in diameter and 32 mm in depth, capable of maintaining the inside temperature within $\pm 1^\circ\text{C}$.

(ii) Procedure—Weigh accurately about 65 mg of Hydroxypropylcellulose, previously dried, transfer to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the flask tightly, and weigh accurately. Shake the flask for 30 seconds, heat at 150°C on the heater for 30 minutes with repeated shaking at 5-minute intervals, and continue heating for an additional 30 minutes. Allow the flask to cool, and again weigh accurately. If the mass loss is less than 10 mg, use the upper layer of the mixture as the sample solution. Separately, take 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in another reaction flask, stopper tightly, and weigh accurately. Add 50 μL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction flask for 30 seconds, and use the upper layer of the content as the standard solution. Perform the test as directed under Gas Chromatography <2.02> with 1 μL each of the sample solution and standard solution according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of isopropyl iodide to that of the internal standard.

$$\begin{aligned} \text{Amount (\% of hydroxypropoxy group (C}_3\text{H}_7\text{O}_2\text{))} \\ = (W_S/W_T) \times (Q_T/Q_S) \times 44.17 \end{aligned}$$

W_S : Amount (mg) of isopropyl iodide in the standard solution.

W_T : Amount (mg) of the sample

Internal standard solution—A solution of *n*-octane in *o*-xylene (4 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium (for thermal-conductivity detector); helium or nitrogen (for hydrogen flame-ionization detector).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

Selection of column: Proceed with 1 μL of the standard so-

lution under the above operating conditions. Use a column giving well-resolved peaks of isopropyl iodide and the internal standard in this order.

Containers and storage Containers—Well-closed containers.

Low Substituted Hydroxypropylcellulose

低置換度ヒドロキシプロピルセルロース

Low Substituted Hydroxypropylcellulose is a low substituted hydroxypropyl ether of cellulose.

Low Substituted Hydroxypropylcellulose, when dried, contains not less than 5.0% and not more than 16.0% of hydroxypropoxy group ($-\text{OC}_3\text{H}_6\text{OH}$: 75.09).

Description Low Substituted Hydroxypropylcellulose occurs as a white to yellowish white powder or granules. It is odorless or has a slight, characteristic odor. It is tasteless.

It is practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in a solution of sodium hydroxide (1 in 10), and produces a viscous solution.

It swells in water, in sodium carbonate TS and in 2 mol/L hydrochloric acid TS.

Identification (1) To 0.02 g of Low Substituted Hydroxypropylcellulose add 2 mL of water, shake, and produce a turbid solution. Add 1 mL of anthrone TS gently: a blue to blue-green color develops at the zone of contact.

(2) To 0.1 g of Low Substituted Hydroxypropylcellulose add 10 mL of water, stir and produce a turbid solution. Add 1 g of sodium hydroxide, shake until it becomes homogeneous, and use this solution as the sample solution. To 0.1 mL of the sample solution add 9 mL of diluted sulfuric acid (9 in 10), shake well, heat in a water bath for exactly 3 minutes, immediately cool in an ice bath, add carefully 0.6 mL of ninhydrin TS, shake well, and allow to stand at 25°C: a red color develops at first, and it changes to purple within 100 minutes.

(3) To 5 mL of the sample solution obtained in (2) add 10 mL of a mixture of acetone and methanol (4:1), and shake: a white, flocculent precipitate is produced.

pH <2.54> To 1.0 g of Low Substituted Hydroxypropylcellulose add 100 mL of freshly boiled and cooled water, and shake: the pH of the solution is between 5.0 and 7.5.

Purity (1) Chloride <1.03>—To 0.5 g of Low Substituted Hydroxypropylcellulose add 30 mL of hot water, stir well, heat on a water bath for 10 minutes, and filter the supernatant liquid by decantation while being hot. Wash the residue thoroughly with 50 mL of hot water, combine the washings with the filtrate, and add water to make 100 mL after cooling. To 5 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.335%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Low Substituted Hydroxypropylcellulose according to Method 2, and perform the test. Prepare the control solution with 2.0

mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Low Substituted Hydroxypropylcellulose, according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay (i) Apparatus—Reaction flask: A 5-mL screw-cap pressure-tight glass bottle, having an inverted conical bottom inside, 20 mm in outside diameter, 50 mm in height up to the neck, 2 mL in capacity up to a height of about 30 mm, equipped with a pressure-tight septum of heat-resisting resin and also with an inside stopper or sealer of fluoroplastic.

Heater: A square-shaped aluminum block 60 to 80 mm thick, having holes 20.6 mm in diameter and 32 mm in depth, capable of maintaining the inside temperature within $\pm 1^\circ\text{C}$.

(ii) Procedure—Weigh accurately about 65 mg of Low Substituted Hydroxypropylcellulose, previously dried, transfer to the reaction flask, add 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the flask tightly, and weigh accurately. Shake the flask for 30 seconds, heat at 150°C on the heater for 30 minutes with repeated shaking at 5-minute intervals, and continue heating for an additional 30 minutes. Allow the flask to cool, and again weigh accurately. If the mass loss is less than 10 mg, use the upper layer of the mixture as the sample solution. Separately, take 65 mg of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in another reaction flask, stopper tightly, and weigh accurately. Add 15 μL of isopropyl iodide for assay, and again weigh accurately. Shake the reaction flask for 30 seconds, and use the upper layer of the content as the standard solution. Perform the test as directed under Gas Chromatography <2.02> with 2 μL each of the sample solution and standard solution according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of isopropyl iodide to that of the internal standard.

$$\begin{aligned} \text{Amount (\% of hydroxypropoxy group (C}_3\text{H}_7\text{O}_2)) \\ = (W_S/W_T) \times (Q_T/Q_S) \times 44.17 \end{aligned}$$

W_S : Amount (mg) of isopropyl iodide in the standard solution.

W_T : amount (mg) of the sample

Internal standard solution—A solution of *n*-octane in *o*-xylene (1 in 50).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 3 m in length, packed with siliceous earth for gas chromatography, 180 to 250 μm in particle diameter, coated with methyl silicone polymer for gas chromatography at the ratio of 20%.

Column temperature: A constant temperature of about 100°C.

Carrier gas: Helium (for thermal-conductivity detector); helium or nitrogen (for hydrogen flame-ionization detector).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

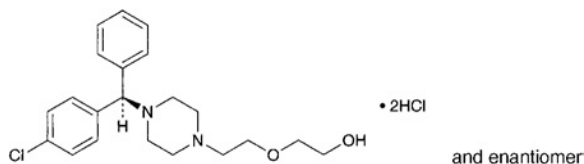
Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions. Use a column

giving well-resolved peaks of isopropyl iodide and the internal standard in this order.

Containers and storage Containers—Tight containers.

Hydroxyzine Hydrochloride

ヒドロキシジン塩酸塩



$C_{21}H_{27}ClN_2O_2 \cdot 2HCl$: 447.83

2-(2-[4-[(*RS*)-(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)ethanol dihydrochloride [2192-20-3]

Hydroxyzine Hydrochloride, when dried, contains not less than 98.5% of $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$.

Description Hydroxyzine Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in methanol, in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 200°C (with decomposition).

Identification (1) To 5 mL of a solution of Hydroxyzine Hydrochloride (1 in 100) add 2 to 3 drops of ammonium thiocyanate-cobaltous nitrate TS: a blue precipitate is formed.

(2) Determine the absorption spectrum of a solution of Hydroxyzine Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Hydroxyzine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Hydroxyzine Hydrochloride in 20 mL of water: the pH of this solution is between 1.3 and 2.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Hydroxyzine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Hydroxyzine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia solution (28) (150:95:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the

spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Hydroxyzine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

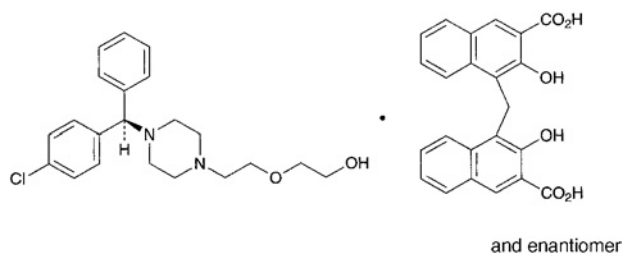
Each mL of 0.1 mol/L perchloric acid VS

= 22.39 mg of $C_{21}H_{27}ClN_2O_2 \cdot 2HCl$

Containers and storage Containers—Tight containers.

Hydroxyzine Pamoate

ヒドロキシジンパモ酸塩



$C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$: 763.27

2-(2-[4-[(*RS*)-(4-Chlorophenyl)phenylmethyl]piperazin-1-yl]ethoxy)ethanol mono[4,4'-methylenebis(3-hydroxy-2-naphthoate)] (1/1) [10246-75-0]

Hydroxyzine Pamoate contains not less than 98.0% of $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$, calculated on the anhydrous basis.

Description Hydroxyzine Pamoate occurs as a light yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in *N,N*-dimethylformamide, slightly soluble in acetone, and practically insoluble in water, in methanol, in ethanol (95) and in diethyl ether.

Identification (1) To 0.1 g of Hydroxyzine Pamoate add 25 mL of sodium hydroxide TS, and shake well. Extract with 20 mL of chloroform, and use the chloroform layer as the sample solution. Use the water layer for test (4). To 5 mL of the sample solution add 2 mL of ammonium thiocyanate-cobaltous nitrate TS, shake well, and allow to stand: a blue color is produced in the chloroform layer.

(2) Evaporate 2 mL of the sample solution obtained in (1) on a water bath to dryness, and dissolve the residue in 0.1 mol/L hydrochloric acid TS to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Hydroxyzine Pamoate as direct-

ed under Flame Coloration Test <1.04> (2): a green color appears.

(4) To 1 mL of the water layer obtained in (1), add 2 mL of 1 mol/L hydrochloric acid TS: a yellow precipitate is produced. Collect the precipitate, dissolve the precipitate in 5 mL of methanol, and add 1 drop of iron (III) chloride TS: a green color is produced.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Hydroxyzine Pamoate in 10 mL of *N,N*-dimethylformamide: the solution is clear, and shows a slightly greenish, light yellow-brown color.

(2) Chloride <1.03>—To 0.3 g of Hydroxyzine Pamoate add 6 mL of dilute nitric acid and 10 mL of water, shake for 5 minutes, and filter. Wash the residue with two 10-mL portions of water, combine the washings with the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.095%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Hydroxyzine Pamoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 2.0 g of Hydroxyzine Pamoate according to Method 3, and perform the test (not more than 1 ppm).

(5) Related substances—Dissolve 0.40 g of Hydroxyzine Pamoate in 10 mL of a mixture of sodium hydroxide TS and acetone (1:1), and use the solution as the sample solution. Pipet 1 mL of this solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 20 mL. Pipet 5 mL of this solution, add a mixture of sodium hydroxide TS and acetone (1:1) to make exactly 50 mL, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and ammonia TS (150:95:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots other than the spots from hydroxyzine and pamoic acid obtained from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 3.0% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

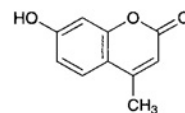
Assay Weigh accurately about 0.6 g of Hydroxyzine Pamoate, add 25 mL of sodium hydroxide TS, shake well, and extract with six 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, and evaporate the combined chloroform extracts on a water bath to about 30 mL. Add 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 38.16 mg of $C_{21}H_{27}ClN_2O_2 \cdot C_{23}H_{16}O_6$

Containers and storage Containers—Tight containers.

Hymecromone

ヒメクロモン



$C_{10}H_8O_3$: 176.17

7-Hydroxy-4-methylchromen-2-one [90-33-5]

Hymecromone, when dried, contains not less than 98.0% of $C_{10}H_8O_3$.

Description Hymecromone occurs as white crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in ethanol (95), in ethanol (99.5) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Hymecromone in 5 mL of ammonia-ammonium chloride buffer solution, pH 11.0: the solution shows an intense blue-purple fluorescence.

(2) Dissolve 0.025 g of Hymecromone in 5 mL of diluted ethanol (95) (1 in 2), and add 1 drop of iron (III) chloride TS: initially a blackish brown color develops, and when allowed to stand the color changes to yellow-brown.

(3) Determine the absorption spectrum of a solution of Hymecromone in ethanol (99.5) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Hymecromone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 187 – 191°C

Purity (1) Chloride <1.03>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.011%).

(2) Sulfate <1.14>—Dissolve 0.8 g of Hymecromone in 40 mL of a mixture of acetone and water (2:1), and add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and a mixture of acetone and water (2:1) to make 50 mL (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Hymecromone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead

Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Hymecromone according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 80 mg of Hymecromone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 50 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (10:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Hymecromone, previously dried, dissolve in 90 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 14 mL of water to 90 mL of *N,N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 17.62 mg of C₁₀H₈O₃

Containers and storage Containers—Tight containers.

Hypromellose

Hydroxypropylmethylcellulose

ヒプロメロース

Cellulose, 2-hydroxypropyl methyl ether
[9004-65-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbol (♦ ◆).

Hypromellose is a methyl and hydroxypropyl mixed ether of cellulose.

There are four substitution types of Hypromellose, 1828, 2208, 2910 and 2910. They contain methoxy (-OCH₃; 31.03) and hydroxypropoxy (-OC₃H₆OH; 75.09) groups conforming to the limits for the types of Hypromellose shown in the table below, calculated on the dried basis.

The viscosity is shown in millipascal second (mPa·s) on the label, together with the substitution type.

Substitution Type	Methoxy Group (%)		Hydroxypropoxy Group (%)	
	Min.	Max.	Min.	Max.
1828	16.5	20.0	23.0	32.0
2208	19.0	24.0	4.0	12.0
2906	27.0	30.0	4.0	7.5
2910	28.0	30.0	7.0	12.0

♦**Description** Hypromellose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

It swells with water and becomes a clear or slightly turbid, viscous solution.♦

Identification (1) Disperse evenly 1.0 g of Hypromellose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Hypromellose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 10°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of diluted sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color first, then changes to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

Viscosity <2.53> Method I: Apply to Hypromellose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Hypromellose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 10°C for 20 to 40 minutes while stirring. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

Method II: Apply to Hypromellose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Hypromellose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II (2)

under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)	Rotor No.	Rotation frequency /min	Conversion factor
Not less than 600 and less than 1400	3	60	20
" 1400 " 3500	3	12	100
" 3500 " 9500	4	60	100
" 9500 " 99500	4	6	1000
" 99500	4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

pH <2.54> Allow the sample solution obtained in the Viscosity to stand at $20 \pm 2^\circ\text{C}$ for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

Purity Heavy metals—Put 1.0 g of Hypromellose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C , 1 hour).

Residue on ignition <2.44> Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20 mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Hypromellose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is $130 \pm 2^\circ\text{C}$. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid in a reaction bottle, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay and 15 to 22 μL of isopropyl iodide for assay through the septum using micro-syringe with weighing accurately every time, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of iodomethane and isopropyl iodide to that of the internal standard obtained from the sample solution, and Q_{Sa} and Q_{Sb} , of the peak area of iodomethane and isopropyl iodide to that of the internal standard from the standard solution.

$$\begin{aligned} \text{Content (\%)} \text{ of methoxy group } (-\text{CH}_3\text{O}) \\ = (Q_{\text{Ta}}/Q_{\text{Sa}}) \times (W_{\text{Sa}}/W) \times 21.86 \end{aligned}$$

$$\begin{aligned} \text{Content (\%)} \text{ of hydroxypropoxy group } (-\text{C}_3\text{H}_7\text{O}_2) \\ = (Q_{\text{Tb}}/Q_{\text{Sb}}) \times (W_{\text{Sb}}/W) \times 44.17 \end{aligned}$$

W_{Sa} : Amount (mg) of iodomethane for assay.

W_{Sb} : Amount (mg) of isopropyl iodide for assay.

W: Amount (mg) of sample, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3–4 mm in inside diameter and 1.8–3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10–20%.

Column temperature: A constant temperature of about 100°C .

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order, with complete separation of these peaks.

◆**Containers and storage** Containers—Well-closed containers. ◆

Hypromellose Phthalate

ヒプロメロースフタル酸エステル

Hydroxypropyl methylcellulose benzene-1,2-dicarboxylate
[9050-31-1]

Hypromellose Phthalate is a monophthalic acid ester of hypromellose.

It contains methoxy group ($-\text{OCH}_3$: 31.03), hydroxypropoxy group ($-\text{OC}_3\text{H}_6\text{OH}$: 75.09), and carboxybenzoyl group ($-\text{COC}_6\text{H}_4\text{COOH}$: 149.12).

There are two substitution types, 200731 and 220824, and each type contains indicated amount of carboxybenzoyl group in the accompanying table, calculated on the anhydrous basis.

Substitution Type	Carboxybenzoyl group (%)	
	Min.	Max.
200731	27.0	35.0
220824	21.0	27.0

Its substitution type and its kinematic viscosity are shown in square mm per second (mm^2/s) on the label.

Description Hypromellose Phthalate occurs as white powder or granules. It is odorless and tasteless.

It is practically insoluble in acetonitrile, in ethanol (99.5), and in hexane.

It becomes a viscous liquid when a mixture of methanol and dichloromethane (1:1) or a mixture of ethanol (99.5) and acetone (1:1) is added.

It dissolves in sodium hydroxide TS.

Identification Determine the infrared absorption spectrum of Hypromellose Phthalate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of Hypromellose Phthalate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Viscosity <2.53> To 10 g of Hypromellose Phthalate, previously dried at 105°C for 1 hour, add 90 g of a mixture of methanol and dichloromethane in equal mass ratio, and stir to dissolve. Determine the viscosity at $20 \pm 0.1^\circ\text{C}$ as directed in Method I under Viscosity Determination: the viscosity is not less than 80% and not more than 120% of the labeled unit.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Hypromellose Phthalate in 40 mL of 0.2 mol/L sodium hydroxide VS,

add 1 drop of phenolphthalein TS, and add dilute nitric acid dropwise with vigorous stirring until the red color is discharged. Further add 20 mL of dilute nitric acid with stirring. Heat on a water bath with stirring until the gelatinous precipitate formed turns to granular particles. After cooling, centrifuge, and take off the supernatant liquid. Wash the precipitate with three 20-mL portions of water by centrifuging each time, combine the supernatant liquid and the washings, add water to make 200 mL, and filter. Perform the test with 50 mL of the filtrate. Control solution: To 0.50 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of 0.2 mol/L sodium hydroxide VS, 7 mL of dilute nitric acid and water to make 50 mL (not more than 0.07%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Hypromellose Phthalate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Phthalic acid—Weigh accurately about 0.2 g of Hypromellose Phthalate, add about 50 mL of acetonitrile to dissolve partially with the aid of ultrasonicator, add 10 mL of water, and dissolve further with the ultrasonicator. After cooling, add acetonitrile to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 12.5 mg of phthalic acid, dissolve in about 125 mL of acetonitrile by stirring, add 25 mL of water and acetonitrile to make exactly 250 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of phthalic acid, A_T and A_S , of both solutions: amount of phthalic acid ($\text{C}_8\text{H}_6\text{O}_4$: 166.13) is not more than 1.0%.

$$\begin{aligned} &\text{Amount (\%)} \text{ of phthalic acid} \\ &= (C/W) \times (A_T/A_S) \times 10 \end{aligned}$$

C : concentration of phthalic acid in the standard solution ($\mu\text{g}/\text{mL}$)

W : amount (mg) of the sample, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (3 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 20°C .

Mobile phase: A mixture of 0.1 mol/L cyanoacetic acid and acetonitrile (17:3).

Flow rate: About 2.0 mL per minute.

System repeatability: Repeat the test six times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of phthalic acid is not more than 1.0%.

Water <2.48> Not more than 5.0% (1 g, direct titration, using a mixture of ethanol (99.5) and dichloromethane (3:2) instead of methanol for Karl Fischer method).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 1 g of Hypromellose Phthalate, dissolve in 50 mL of a mixture of ethanol (95), acetone

and water (2:2:1), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

$$\text{Amount (\% of carboxybenzoyl group (C}_8\text{H}_5\text{O}_3\text{))} \\ = \{(0.01 \times 149.1 \times V)/W\} - \{(2 \times 149.1 \times P)/166.1\}$$

P: amount (%) of phthalic acid obtained in the Purity (3)

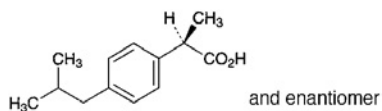
V: amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

W: amount (g) of the sample, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Ibuprofen

イブプロフェン



C₁₃H₁₈O₂: 206.28
(*2RS*)-2-[4-(2-Methylpropyl)phenyl]propanoic acid
[15687-27-1]

Ibuprofen, when dried, contains not less than 98.5% of C₁₃H₁₈O₂.

Description Ibuprofen occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

It dissolves in dilute sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Ibuprofen in dilute sodium hydroxide TS (3 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ibuprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 75 – 77°C

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Ibuprofen according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ibuprofen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.50 g of Ibuprofen in exactly 5 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica

gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, ethyl acetate and acetic acid (100) (15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ibuprofen, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

$$\text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 20.63 \text{ mg of C}_{13}\text{H}_{18}\text{O}_2$$

Containers and storage Containers—Well-closed containers.

Ichthammol

イクタモール

Ichthammol, calculated on the dried basis, contains not less than 2.5% of ammonia (NH₃: 17.030), not more than 8.0% of ammonium sulfate [(NH₄)₂SO₄: 132.14], and not less than 10.0% of total sulfur (as S: 32.07).

Description Ichthammol is a red-brown to blackish brown, viscous fluid. It has a characteristic odor.

It is miscible with water, and is partially soluble in ethanol (95) and in diethyl ether.

Identification (1) To 4 mL of a solution of Ichthammol (3 in 10) add 8 mL of hydrochloric acid: a yellow-brown to blackish brown, oily or resinous mass is produced. Cool the mass with ice to solidify, and discard the water layer. Wash the residue with diethyl ether: a part of the mass dissolves but it does not dissolve completely even when it is washed until almost no color develops in the washing. Perform the following tests with this residue.

(i) To 0.1 g of the residue add 1 mL of a mixture of diethyl ether and ethanol (95) (1:1): it dissolves.

(ii) To 0.1 g of the residue add 2 mL of water: it dissolves. To 1 mL of this solution add 0.4 mL of hydrochloric acid: a yellow-brown to blackish brown oily or resinous substance is produced.

(iii) To 1 mL of the solution obtained in (ii) add 0.3 g of sodium chloride: a yellow-brown or blackish brown oily or resinous substance is produced.

(2) Boil 2 mL of a solution of Ichthammol (1 in 10) with 2 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

Loss on drying <2.41> Not more than 50% (0.5 g, 105°C, 6 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay (1) Ammonia—Weigh accurately about 5 g of Ichthammol, transfer to a Kjeldahl flask, and add 60 mL of water, 1 mL of 1-octanol and 4.5 mL of a solution of sodium hydroxide (2 in 5). Connect the flask to a distilling tube with a spray trap and a condenser, and immerse the lower outlet of the condenser in the receiver containing exactly 30 mL of 0.25 mol/L sulfuric acid VS. Distil slowly, collect about 50 mL of the distillate, and titrate <2.50> the excess sulfuric acid with 0.5 mol/L sodium hydroxide VS (indicator: 3 drops of methyl red TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.25 mol/L sulfuric acid VS} \\ = 8.515 \text{ mg of NH}_3 \end{aligned}$$

(2) Ammonium sulfate—Weigh accurately about 1 g of Ichthammol, add 25 mL of ethanol (95), stir thoroughly, and filter. Wash with a mixture of diethyl ether and ethanol (95) (1:1) until the washings are clear and colorless. Dry the filter paper and the residue in air, dissolve the residue in 200 mL of hot water acidified slightly with hydrochloric acid, and filter. Boil the filtrate, add 30 mL of barium chloride TS slowly, heat for 30 minutes on a water bath, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate (BaSO_4 ; 233.39).

$$\begin{aligned} \text{Amount (mg) of ammonium sulfate } [(\text{NH}_4)_2\text{SO}_4] \\ = \text{amount (mg) of barium sulfate } (\text{BaSO}_4) \times 0.5662 \end{aligned}$$

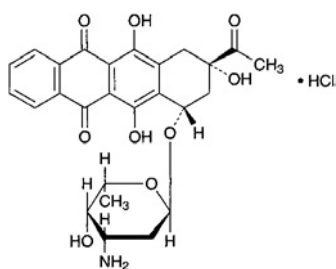
(3) Total sulfur—Weigh accurately about 0.6 g of Ichthammol, transfer to a 200-mL Kjeldahl flask, and add 30 mL of water and 5 g of potassium chlorate, then add slowly 30 mL of nitric acid, and evaporate the mixture to about 5 mL. Transfer the residue to a 300-mL beaker with the aid of 25 mL of hydrochloric acid, and evaporate again to 5 mL. Add 100 mL of water, boil, filter, and wash with water. Heat the combined filtrate and washings to boil, add gradually 30 mL of barium chloride TS, heat the mixture on a water bath for 30 minutes, and filter. Wash the precipitate with water, dry, and ignite to constant mass. Weigh the residue as barium sulfate (BaSO_4).

$$\begin{aligned} \text{Amount (mg) of total sulfur (S)} \\ = \text{amount (mg) of barium sulfate } (\text{BaSO}_4) \times 0.13739 \end{aligned}$$

Containers and storage Containers—Tight containers.

Idarubicin Hydrochloride

イダルビシン塩酸塩



$\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$: 533.95

(2*S*,4*S*)-2-Acetyl-4-(3-amino-2,3,6-trideoxy- α -L-lyxo-hexopyranosyloxy)-2,5,12-trihydroxy-

1,2,3,4-tetrahydrotetracene-6,11-dione monohydrochloride
[57852-57-0]

Idarubicin Hydrochloride contains not less than 960 μg (potency) and not more than 1030 μg (potency) per mg, calculated on the anhydrous basis. The potency of Idarubicin Hydrochloride is expressed as mass (potency) of idarubicin hydrochloride ($\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$).

Description Idarubicin Hydrochloride occurs as a yellow-red powder.

It is sparingly soluble in methanol, slightly soluble in water and in ethanol (95), and practically insoluble in acetonitrile and in diethyl ether.

Identification (1) Determine the absorption spectra of a solution of Idarubicin Hydrochloride in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idarubicin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectra of Idarubicin Hydrochloride and Idarubicin Hydrochloride Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 2 mg of Idarubicin Hydrochloride in 3 mL of water, and add 1 mL of dilute nitric acid and 3 drops of silver nitrate TS: a white turbidity is produced.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (482 nm): 204 – 210 (20 mg calculated on the anhydrous basis, methanol, 1000 mL).

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +191 – +197° (20 mg calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

pH <2.54> The pH of a solution of Idarubicin Hydrochloride (1 in 200) is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals—Being specified separately.

(3) Related substances—Being specified separately.

(4) Residual solvent—Being specified separately.

Water <2.48> Not more than 5.0% (0.1 g, coulometric titration).

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 8.9 EU/mg (potency).

Assay Weigh accurately an amount of Idarubicin Hydrochloride and Idarubicin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in the mobile phase containing no sodium lauryl sulfate to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine peak areas, A_T and A_S , of idarubicin of these solutions.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}$$

$$= W_S \times (A_T/A_S) \times 1000$$

W_S : Amount [mg (potency)] of Idarubicin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (4 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 10.2 g of potassium dihydrogenphosphate in a suitable amount of water, add 1 mL of phosphoric acid and water to make 750 mL, and add 250 mL of tetrahydrofuran. To 500 mL of this solution add 0.72 g of sodium lauryl sulfate and 0.5 mL of *N,N*-dimethyl-*n*-octylamine, and adjust to pH 4 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of idarubicin is about 15 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical steps of the peak of idarubicin is not less than 3000 steps.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of idarubicin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Idarubicin Hydrochloride for Injection

注射用イダルビシン塩酸塩

Idarubicin Hydrochloride for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than 110.0% of the labeled amount of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$; 533.95).

Method of preparation Prepare as directed under Injections, with Idarubicin Hydrochloride.

Description Idarubicin Hydrochloride for Injection occurs as yellow-red masses.

Identification (1) Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 2 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of sodium hydroxide TS: the solution shows a blue-purple color.

(2) Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 1 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 1 mL of water, and add methanol to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 285 nm and 289 nm,

between 480 nm and 484 nm, and between 510 nm and 520 nm.

pH <2.54> The pH of a solution prepared by dissolving an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of water is between 5.0 and 7.0.

Purity Clarity and color of solution—Dissolve an amount of Idarubicin Hydrochloride for Injection, equivalent to 5 mg (potency) of Idarubicin Hydrochloride according to the labeled amount, in 5 mL of water: the solution is clear and yellow-red.

Water <2.48> Weigh accurately the mass of one Idarubicin Hydrochloride for Injection, add 5 mL of methanol for Karl Fischer method using a syringe, dissolve with thorough shaking, and perform the test with 4 mL of this solution as directed in the Volumetric titration (direct titration). Use 4 mL of methanol for Karl Fischer method as the blank. Determine the mass of the content from the difference between the mass of one Idarubicin Hydrochloride for Injection obtained above and the mass of its bottle and rubber stopper, which are weighed accurately after washing with water then with ethanol (95), drying at 105°C for 1 hour and allowing to cool to room temperature in a desiccator (not more than 4.0%).

Bacterial endotoxins <4.01> Less than 8.9 EU/mg (potency).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To one Idarubicin Hydrochloride for Injection add the mobile phase prepared without addition of sodium lauryl sulfate to make exactly V mL so that each mL contains 0.2 mg (potency) of idarubicin hydrochloride ($C_{26}H_{27}NO_9 \cdot HCl$), and use this solution as the sample solution. Separately, weigh accurately an amount of Idarubicin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve in the mobile phase without containing sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

$$\text{Amount [mg (potency)] of idarubicin hydrochloride } (C_{26}H_{27}NO_9 \cdot HCl) = W_S \times (A_T/A_S) \times (V/50)$$

W_S : Amount [mg (potency)] of Idarubicin Hydrochloride Reference Standard

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Idarubicin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 5 mg (potency) according to the labeled amount, dissolve in the mobile phase prepared without addition of sodium lauryl sulfate to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of

Idarubicin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve in the mobile phase without containing sodium lauryl sulfate to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Idarubicin Hydrochloride.

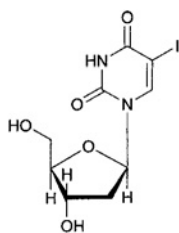
$$\begin{aligned} & \text{Amount [mg (potency)] of idarubicin hydrochloride} \\ & (\text{C}_{26}\text{H}_{27}\text{NO}_9 \cdot \text{HCl}) \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount [mg (potency)] of Idarubicin Hydrochloride Reference Standard

Containers and storage Containers—Hermetic containers.

Idoxuridine

イドクスウリジン



$\text{C}_9\text{H}_{11}\text{IN}_2\text{O}_5$; 354.10

5-Iodo-2'-deoxyuridine [54-42-2]

Idoxuridine, when dried, contains not less than 98.0% of $\text{C}_9\text{H}_{11}\text{IN}_2\text{O}_5$.

Description Idoxuridine occurs as colorless, crystals or a white, crystalline powder. It is odorless.

It is freely soluble in dimethylamide, slightly soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: about 176°C (with decomposition).

Identification (1) Dissolve 0.01 g of Idoxuridine in 5 mL of water by warming, add 5 mL of diphenylamine-acetic acid TS, and heat for 5 minutes: a blue color develops.

(2) Heat 0.1 g of Idoxuridine: a purple gas evolves.

(3) Dissolve 2 mg of Idoxuridine in 50 mL of 0.01 mol/L sodium hydroxide. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Idoxuridine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> $[\alpha]_D^{20}$: +28 – +31° (after drying, 0.2 g, sodium hydroxide TS, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Idoxuridine in 5 mL of a solution of sodium hydroxide (1 in 200): the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Idoxuridine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Idoxuridine in exactly 10 mL of a mixture of dilute ethanol and ammonia solution (28) (99:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 50 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diluted 2-propanol (2 in 3) (4:1) to a distance of about 10 cm, and air-dry the plate. Then develop two-dimensionally at right angles to the first, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

(4) Iodine and iodide—Dissolve 0.10 g of Idoxuridine in 20 mL of water and 5 mL of sodium hydroxide TS, and add immediately 5 mL of dilute sulfuric acid under ice-cooling. Allow to stand for 10 minutes with occasional shaking, and filter. Transfer the filtrate into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100), shake for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh accurately 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. To exactly 1 mL of this solution add 19 mL of water, 5 mL of sodium hydroxide TS and 5 mL of dilute sulfuric acid, mix, and filter. Transfer the filtrate to a Nessler tube, and proceed in the same manner.

Loss on drying <2.41> Not more than 0.5% (2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.7 g of Idoxuridine, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS until the color of the solution changes from yellow through yellow-green to blue (indicator: 5 drops of thymol blue-dimethylformamide TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L tetramethylammonium} \\ & \text{hydroxide VS} \\ & = 35.41 \text{ mg of } \text{C}_9\text{H}_{11}\text{IN}_2\text{O}_5 \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Idoxuridine Ophthalmic Solution

イドクスウリジン点眼液

Idoxuridine Ophthalmic Solution contains not less than 90% and not more than 110% of the labeled amount of idoxuridine ($\text{C}_9\text{H}_{11}\text{IN}_2\text{O}_5$; 354.10).

Method of preparation Prepare as directed under Ophthalmic Solutions, with Idoxuridine.

Description Idoxuridine Ophthalmic Solution is a clear, colorless liquid.

Identification (1) To a volume of Idoxuridine Ophthalmic Solution, equivalent to 5 mg of Idoxuridine according to the

labeled amount, add 5 mL of diphenylamine-acetic acid TS, and heat for 20 minutes: a light blue color develops.

(2) Place a volume of Idoxuridine Ophthalmic Solution, equivalent to 5 mg of Idoxuridine according to the labeled amount, in a porcelain crucible, add 0.1 g of anhydrous sodium carbonate, heat slowly, evaporate to dryness and ignite until the residue is incinerated. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and add 2 to 3 drops of sodium nitrite TS: a yellow-brown color develops. Then add 2 to 3 drops of starch TS: a deep blue color develops.

(3) To a volume of Idoxuridine Ophthalmic Solution, equivalent to 2 mg of Idoxuridine according to the labeled amount, add 0.01 mol/L sodium hydroxide TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 281 nm.

pH <2.54> 4.5 – 7.0

Purity 5-Iodouracil and 2'-deoxyuridine—To a volume of Idoxuridine Ophthalmic Solution, equivalent to 4.0 mg of Idoxuridine according to the labeled amount, add water to make exactly 5 mL, and use this solution as the sample solution. Separately, dissolve 12.0 mg of 5-iodouracil for liquid chromatography and 4.0 mg of 2'-deoxyuridine for liquid chromatography in water to make exactly 200 mL. Measure exactly 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of 5-iodouracil and 2'-deoxyuridine: the peak areas of 5-iodouracil and 2'-deoxyuridine of the sample solution are not more than the peak areas of 5-iodouracil and 2'-deoxyuridine of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (24:1).

Flow rate: Adjust the flow rate so that the retention time of 2'-deoxyuridine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, 2'-deoxyuridine and 5-iodouracil are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of 2'-deoxyuridine is not more than 1.0%.

Assay Measure exactly a volume of Idoxuridine Ophthalmic Solution, equivalent to 3 mg of idoxuridine ($C_9H_{11}IN_2O_5$) according to the labeled amount, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Separately weigh accurately about 10 mg of Idoxuridine

Reference Standard, previously dried at 60°C for 3 hours, dissolve in water to make exactly 10 mL. Measure exactly 3 mL of this solution, add exactly 2 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of idoxuridine to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of idoxuridine (C}_9\text{H}_{11}\text{IN}_2\text{O}_5\text{)} \\ & = W_S \times (Q_T/Q_S) \times (3/10) \end{aligned}$$

W_S : Amount (mg) of Idoxuridine Reference Standard

Internal standard solution—A solution of sulfathiazole in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (87:13).

Flow rate: Adjust the flow rate so that the retention time of idoxuridine is about 9 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, idoxuridine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

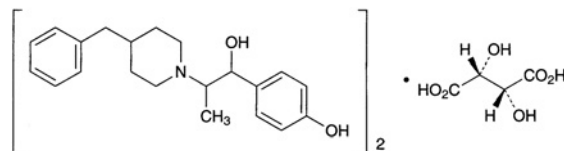
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of idoxuridine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, in a cold place, and avoid freezing.

Ifenprodil Tartrate

イフェンプロジル酒石酸塩



$(C_{21}H_{27}NO_2)_2 \cdot C_4H_6O_6$: 800.98
(1*RS*,2*SR*)-4-[2-(4-Benzylpiperidin-1-yl)-1-hydroxypropyl]phenol hemi-(2*R*,3*R*)-tartrate [23210-58-4]

Ifenprodil Tartrate contains not less than 98.5% of $(C_{21}H_{27}NO_2)_2 \cdot C_4H_6O_6$, calculated on the anhydrous basis.

Description Ifenprodil Tartrate occurs as a white crystalline

powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in ethanol (95), slightly soluble in water and in methanol, and practically insoluble in diethyl ether.

Optical rotation $[\alpha]_D^{20}$: +11 – +15° (1 g, calculated on the anhydrous basis, ethanol (95), 20 mL, 100 mm).

Melting point: about 148°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ifenprodil Tartrate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ifenprodil Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.4 g of Ifenprodil Tartrate in 40 mL of water by warming. After cooling, add 0.5 mL of ammonia TS to this solution, extract with two 40-mL portions of chloroform, and collect the water layer. Evaporate 30 mL of the water layer on a water bath to dryness, and after cooling, dissolve the residue in 6 mL of water: the solution responds to the Qualitative Tests <1.09> for tartrate.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ifenprodil Tartrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.30 g of Ifenprodil Tartrate in 10 mL of diluted ethanol (95) (3 in 4), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (3 in 4) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane, 1-butanol and ammonia solution (28) (140:40:20:1) to a distance of about 10 cm, and air-dry the plate. Spray hydrogen hexachloroplatinate (IV)-potassium iodide TS evenly on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 4.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Ifenprodil Tartrate, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

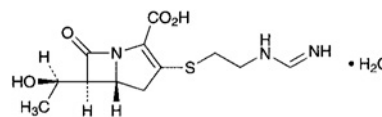
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 40.05 \text{ mg of } (\text{C}_{21}\text{H}_{27}\text{NO}_2)_2 \cdot \text{C}_4\text{H}_6\text{O}_6 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Imipenem Hydrate

イミペネム水和物



$\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S} \cdot \text{H}_2\text{O}$: 317.36

(5*R*,6*S*)-3-[2-(Formimidoylamino)ethylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate [74431-23-5]

Imipenem Hydrate contains not less than 980 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Imipenem Hydrate is expressed as mass (potency) of imipenem ($\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$: 299.35).

Description Imipenem Hydrate occurs as white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Imipenem Hydrate in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipenem Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Imipenem Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Imipenem Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +89 – +94° (50 mg calculated on the anhydrous basis, 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Imipenem Hydrate in 200 mL of water is between 4.5 and 7.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Imipenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Put 2.0 g of Imipenem Hydrate in a crucible, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat carefully until white fumes evolve. After cooling, add 2 mL of nitric acid, heat, and repeat this procedure once more. Then add 2 mL of hydrogen peroxide (30), heat, and repeat this procedure several times until the color of the solution changes to colorless to pale yellow. After cooling, heat again until white fumes evolve. After cooling, add water to make 5 mL, and perform the test with this solution as the test

solution (not more than 1 ppm).

(3) Related substances—Dissolve 50 mg of Imipenem Hydrate in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of thienamycin, having the relative retention time of about 0.8 with respect to imipenem, obtained from the sample solution is not more than 1.4 times the peak area of imipenem from the standard solution, the area of the peak other than imipenem and thienamycin from the sample solution is not more than 1/3 times the peak area of imipenem from the standard solution, and the total area of the peaks other than imipenem and thienamycin from the sample solution is not more than the peak area of imipenem from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of imipenem.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL. Confirm that the peak area of imipenem from 10 μ L of this solution is equivalent to 7 to 13% of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 2.0%.

Water <2.48> Not less than 5.0% and not more than 8.0% (20 mg, coulometric titration, water evaporation temperature: 140°C).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Imipenem Hydrate and Imipenem Reference Standard, equivalent to about 50 mg (potency), dissolve each in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution, within 30 minutes after preparation of these solutions, as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of imipenem of these solutions.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of imipenem } (\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S}) \\ &= W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Imipenem Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 and acetonitrile (100:1).

Flow rate: Adjust the flow rate so that the retention time of imipenem is about 6 minutes.

System suitability—

System performance: Dissolve 50 mg of Imipenem and 75 mg of resorcinol in 50 mL of 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0. When the procedure is run with 10 μ L of this solution under the above operating conditions, imipenem and resorcinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of imipenem is not more than 0.80%.

Containers and storage Containers—Hermetic containers.

Imipenem and Cilastatin Sodium for Injection

注射用イミペネム・シラスタチンナトリウム

Imipenem and Cilastatin Sodium for Injection is a preparation for injection which is dissolved or suspended before use.

It contains not less than 93.0% and not more than 115.0% of the labeled amount of imipenem ($\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S}$: 299.35) and an amount of cilastatin sodium ($\text{C}_{16}\text{H}_{25}\text{N}_2\text{NaO}_5\text{S}$: 380.43), equivalent to not less than 93.0% and not more than 115.0% of the labeled amount of cilastatin ($\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$: 358.45).

Method of preparation Prepare as directed under Injections, with Imipenem Hydrate and Cilastatin Sodium.

Description Imipenem and Cilastatin Sodium for Injection occurs as a white to light yellowish white powder.

Identification (1) To 1 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 100) add 1 mL of ninhydrin TS, heat in a water bath for 5 minutes: a purple color appears (cilastatin).

(2) To 2 mL of a solution of Imipenem and Cilastatin Sodium for Injection (1 in 1000) add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 50 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 296 nm and 300 nm (imipenem).

pH <2.54> The pH of a solution prepared by dissolving an

amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate according to the labeled amount, in 100 mL of isotonic sodium chloride solution is between 6.5 and 8.0. The pH of the Injection intended for intramuscular use is between 6.0 and 7.5.

Purity Clarity and color of solution—Dissolve an amount of Imipenem and Cilastatin Sodium for Injection, equivalent to 0.5 g (potency) of Imipenem Hydrate according to the labeled amount, in 100 mL of isotonic sodium chloride solution: the solution is clear and colorless or pale yellow.

Loss on drying <2.41> Not more than 3.0% (1 g, in vacuum, 60°C, 3 hours).

Bacterial endotoxins <4.01> Less than 0.25 EU/mg (potency).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test. Calculate the acceptance value by using the average of the limits specified in the potency definition for *T*.

Dissolve the total amount of the content of one Imipenem and Cilastatin Sodium for Injection in isotonic sodium chloride solution to make 100 mL. Measure exactly *V* mL of this solution, equivalent to about 25 mg (potency) of Imipenem Hydrate according to the labeled amount, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the sample solution. Proceed hereafter as directed in the Assay.

$$\begin{aligned} &\text{Amount [mg (potency)] of imipenem (C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S)} \\ &= W_S \times (A_{TI}/A_{SI}) \times (100/V) \end{aligned}$$

W_S : Amount [mg (potency)] of Imipenem Reference Standard

$$\begin{aligned} &\text{Amount (mg) of cilastatin (C}_{16}\text{H}_{26}\text{N}_2\text{O}_5\text{S)} \\ &= W_S \times (A_{TC}/A_{SC}) \times (100/V) \times 0.955 \end{aligned}$$

W_S : Amount (mg) of cilastatin ammonium for assay, calculated on the anhydrous basis and corrected on the amount of the residual solvent

Foreign insoluble matter <6.06> Perform the test according to the Method 2: the Injection which is dissolved before use meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: the Injection which is dissolved before use meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Imipenem and Cilastatin Sodium for Injections. Weigh accurately an amount of the content, equivalent to one Imipenem and Cilastatin Sodium for Injection, dissolve in isotonic sodium chloride solution to make exactly 100 mL. Measure exactly an amount of this solution, equivalent to about 25 mg (potency) of imipenem, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately an amount of Imipenem Reference Standard, equivalent to about 25 mg (potency), and weigh accurately about 25 mg of cilastatin ammonium for assay, separately determined the water content, dissolve in 10

mL of isotonic sodium chloride solution, add 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of imipenem, A_{TI} and A_{SI} , and that of cilastatin, A_{TC} and A_{SC} .

$$\begin{aligned} &\text{Amount [mg (potency)] of imipenem (C}_{12}\text{H}_{17}\text{N}_3\text{O}_4\text{S)} \\ &= W_S \times (A_{TI}/A_{SI}) \end{aligned}$$

W_S : Amount [mg (potency)] of Imipenem Reference Standard

$$\begin{aligned} &\text{Amount (mg) of cilastatin (C}_{16}\text{H}_{26}\text{N}_2\text{O}_5\text{S)} \\ &= W_S \times (A_{TC}/A_{SC}) \times 0.955 \end{aligned}$$

W_S : Amount (mg) of cilastatin ammonium for assay, calculated on the anhydrous basis and corrected on the amount of ethanol

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 0.836 g of 3-(*N*-morpholino)propanesulfonic acid, 1.0 g of sodium 1-hexane sulfonate and 50 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 800 mL of water, adjust to pH 7.0 with 0.1 mol/L sodium hydrate TS, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of imipenem is about 3 minutes.

System suitability—

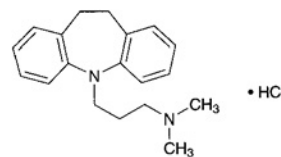
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, imipenem and cilastatin are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factors of the peak of imipenem and cilastatin are not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviations of the peak area of imipenem and cilastatin are not more than 2.0%, respectively.

Containers and storage Containers—Hermetic containers.

Imipramine Hydrochloride

イミプラミン塩酸塩



$\text{C}_{19}\text{H}_{24}\text{N}_2 \cdot \text{HCl}$: 316.87
3-(10,11-Dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)-

N,N-dimethylpropylamine monohydrochloride [113-52-0]

Imipramine Hydrochloride, when dried, contains not less than 98.5% of $C_{19}H_{24}N_2 \cdot HCl$.

Description Imipramine Hydrochloride occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

The pH of the aqueous solution (1 in 10) is between 4.2 and 5.2.

It is gradually colored by light.

Identification (1) Dissolve 5 mg of Imipramine Hydrochloride in 2 mL of nitric acid: a deep blue color develops.

(2) Dissolve 5 mg of Imipramine Hydrochloride in 250 mL of 0.01 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Imipramine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dissolve 0.05 g of Imipramine Hydrochloride in 5 mL of water, add 1 mL of ammonia TS, allow to stand for 5 minutes, filter, and acidify the filtrate with dilute nitric acid: it responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 170 – 174°C (with decomposition).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Imipramine Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: Take exactly 1.0 mL of Cobaltous Chloride Colorimetric Stock Solution, 2.4 mL of Ferric Chloride Colorimetric Solution, 0.4 mL of Cupric Sulfate Colorimetric Stock Solution and 6.2 mL of diluted hydrochloric acid (1 in 40), and mix them. Pipet 0.5 mL of this solution, and add exactly 9.5 mL of water.

(2) Iminodibenzyl—Dissolve 50 mg of Imipramine Hydrochloride in 10 mL of a mixture of hydrochloric acid and ethanol (95) (1:1) in a 25-mL brown volumetric flask. Cool the flask in ice water, add 5 mL of an ethanol (95) solution of furfural (1 in 250) and 5 mL of hydrochloric acid, and allow to stand at 25°C for 3 hours. Add a mixture of hydrochloric acid and ethanol (95) (1:1) to make 25 mL, and determine the absorbance of this solution at 565 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.16.

(3) Related substances—Dissolve 0.20 g of Imipramine Hydrochloride in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, and add ethanol (95) to make exactly 50 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetic acid (100), hydrochloric acid and water (11:7:1:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly potassium dichromate-sulfuric acid TS on the

plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Imipramine Hydrochloride, previously dried, and dissolve in 20 mL of water. Add 5 mL of sodium hydroxide TS, and extract with three 20-mL portions of chloroform. Filter each extract through a pledget of absorbent cotton on which a small quantity of anhydrous sodium sulfate is placed. Combine the chloroform extracts, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the yellow solution changes to red-purple (indicator: 10 drops of metanil yellow TS). Perform a blank determination.

Each mL of 0.1 mol/L perchloric acid VS
= 31.69 mg of $C_{19}H_{24}N_2 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Imipramine Hydrochloride Tablets

イミプラミン塩酸塩錠

Imipramine Hydrochloride Tablets contain not less than 93% and not more than 107% of the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$: 316.87).

Method of preparation Prepare as directed under Tablets, with Imipramine Hydrochloride.

Identification (1) Weigh a quantity of powdered Imipramine Hydrochloride Tablets, equivalent to 0.25 g of Imipramine Hydrochloride according to the labeled amount, add 25 mL of chloroform, shake thoroughly, and filter. Evaporate the filtrate on a water bath, and proceed with the residue as directed in the Identification (1) under Imipramine Hydrochloride.

(2) Dissolve an amount of the residue obtained in (1), equivalent to 5 mg of Imipramine Hydrochloride, in 250 mL of 0.01 mol/L hydrochloric acid TS, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 249 nm and 253 nm, and a shoulder between 270 nm and 280 nm.

(3) Dry the residue obtained in (1) at 105°C for 2 hours: the residue melts between 170°C and 174°C (with decomposition).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Imipramine Hydrochloride Tablet at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution after 60 minutes from the start of the dissolution test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent *V* mL, add 2nd fluid for dissolution test

to make exactly V' mL so that each mL of the filtrate contains about 10 μ g of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.025 g of Imipramine Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 4 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 250 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Imipramine Hydrochloride Tablets in 60 minutes should be not less than 75%.

Dissolution rate (%) with respect to the labeled amount of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$$

W_S : Amount (mg) of Imipramine Hydrochloride Reference Standard.

C : Labeled amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) in 1 tablet.

Assay Take 20 Imipramine Hydrochloride Tablets, add exactly 200 mL of 0.01 mol/L hydrochloric acid TS, and shake well until the tablets are completely disintegrated. After centrifuging the solution, pipet a volume of the supernatant liquid, equivalent to about 25 mg of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$) according to the labeled amount, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Imipramine Hydrochloride for Assay, previously dried at 105°C for 2 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 3 mL each of these solutions into separators which contain 15 mL of potassium hydrogen phthalate buffer solution, pH 5.6, 8 mL of bromocresol green-sodium hydroxide TS and 30 mL of chloroform, and shake. Filter the chloroform layer through a pledget of absorbent cotton into a 100-mL volumetric flask. Repeat the extraction with two 30-mL portions of chloroform, combine the chloroform layers in the 100-mL volumetric flask, and add chloroform to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 3 mL of 0.01 mol/L hydrochloric acid TS in the same manner as the blank. Determine the absorbances, A_T and A_S , of these solutions at 416 nm.

Amount (mg) of imipramine hydrochloride ($C_{19}H_{24}N_2 \cdot HCl$)

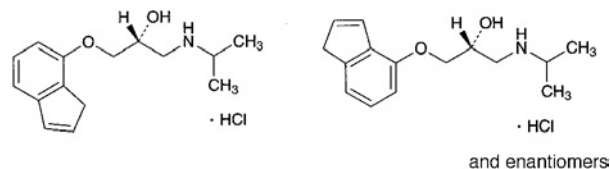
$$= W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Imipramine Hydrochloride for Assay

Containers and storage Containers—Tight containers.

Indenolol Hydrochloride

インデノロール塩酸塩



$C_{15}H_{21}NO_2 \cdot HCl$: 283.79

(2*RS*)-1-(3*H*-Inden-4-yloxy)-

3-(1-methylethyl)aminopropan-2-ol monohydrochloride

(2*RS*)-1-(3*H*-Inden-7-yloxy)-

3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[68906-88-7]

Indenolol Hydrochloride is a mixture of (2*RS*)-1-(3*H*-Inden-4-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride and (2*RS*)-1-(3*H*-Inden-7-yloxy)-3-(1-methylethyl)aminopropan-2-ol monohydrochloride.

When dried, it contains not less than 98.5% of $C_{15}H_{21}NO_2 \cdot HCl$.

Description Indenolol Hydrochloride occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95) and in chloroform, slightly soluble in acetic anhydride, very slightly soluble in ethyl acetate, and practically insoluble in diethyl ether.

The pH of a solution of Indenolol Hydrochloride (1 in 10) is between 3.5 and 5.5.

It is colored by light.

Identification (1) Dissolve 0.1 g of Indenolol Hydrochloride in 1 to 2 drops of dilute hydrochloric acid and 5 mL of water, and add 1 mL of Reinecke salt TS: a red-purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Indenolol Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Indenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Indenolol Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E_{1cm}^{1\%}$ (250 nm): 330 – 340 (after drying, 10 mg, water, 1000 mL).

Melting point <2.60> 140 – 143°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Indenolol Hydrochloride in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Indenolol Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Indenolol Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Indenolol Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, ethanol (99.5) and ammonia solution (28) (70:15:2) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Dissolve 5 mg of Indenolol Hydrochloride in 1.0 mL of a mixture of ethyl acetate and dehydrated trifluoroacetic acid (9:1), and use this solution as the sample solution. Perform the test with 2 μ L of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the areas of two adjacent peaks, A_a and A_b , having the retention times of about 16 minutes, where A_a is the peak area of shorter retention time and A_b is the peak area of longer retention time: the ratio $A_a/(A_a + A_b)$ is between 0.6 and 0.7.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 2 mm in inside diameter and about 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μ m in particle diameter) coated with 65% phenyl-methyl silicon polymer for gas chromatography at the ratio of 2%.

Column temperature: A constant temperature between 150°C and 170°C.

Carrier gas: Helium.

Flow rate: Adjust the flow rate so that the retention time of the peak showing earlier elution of the two peaks of indenolol hydrochloride is about 16 minutes.

Selection of column: Proceed with 2 μ L of the sample solution under the above operating conditions, and calculate the resolution. Use a column with the resolution between the two peaks being not less than 1.1.

Assay Weigh accurately about 0.5 g of Indenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (4:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (indi-

cator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

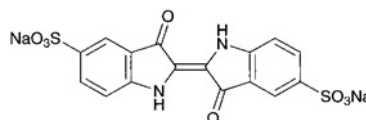
Each mL of 0.1 mol/L perchloric acid VS
= 28.38 mg of $C_{15}H_{21}NO_2 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Indigocarmine

インジゴカルミン



$C_{16}H_8N_2Na_2O_8S_2$: 466.35

Disodium 3,3'-dioxo-[1,2'-biindoline]-5,5'-disulfonate
[860-22-0]

Indigocarmine, when dried, contains not less than 95.0% of $C_{16}H_8N_2Na_2O_8S_2$.

Description Indigocarmine occurs as blue to dark blue powder or granules. It is odorless.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

When compressed, it has a coppery luster.

Identification (1) A solution of Indigocarmine (1 in 100) is dark blue in color. Perform the following tests with this solution as the sample solution: the dark blue color of each solution disappears.

(i) Add 1 mL of nitric acid to 2 mL of the sample solution;

(ii) Add 1 mL of bromine TS to 2 mL of the sample solution;

(iii) Add 1 mL of chlorine TS to 2 mL of the sample solution;

(iv) Add 2 mL of sodium hydroxide TS and 0.2 g of zinc powder to 2 mL of the sample solution, and warm.

(2) Dissolve 0.1 g of Indigocarmine in 100 mL of a solution of ammonium acetate (1 in 650). To 1 mL of the solution add a solution of ammonium acetate (1 in 650) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Ignite 1 g of Indigocarmine to carbonize. After cooling, add 20 mL of water to the residue, shake, and filter the mixture: the filtrate responds to the Qualitative Tests <1.09> for sodium salt and for sulfate.

pH <2.54> Dissolve 0.10 g of Indigocarmine in 20 mL of water: the pH of the solution is between 5.0 and 6.0.

Purity (1) Water-insoluble substances—To 1.00 g of Indigocarmine add 200 mL of water, shake, and filter through a tared glass filter (G4). Wash the residue with water until the blue color of the filtrate becomes practically colorless, and

dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 5.0 mg.

(2) Arsenic <1.11>—Place 0.8 g of Indigocarmine in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and ignite gently. Repeat the addition of 2 to 3 mL of nitric acid occasionally, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of a saturated ammonium oxalate solution, heat the solution until dense white fumes are evolved, and concentrate to 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with 5 mL of this solution as the test solution (not more than 5 ppm).

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not less than 28.0% and not more than 38.0% (after drying, 1 g).

Assay Weigh accurately about 0.5 g of Indigocarmine, previously dried, add 15 g of sodium hydrogen tartrate monohydrate, and dissolve in 200 mL of water, boil with bubbling of a stream of carbon dioxide, and titrate <2.50>, while being hot, with 0.1 mol/L titanium (III) chloride VS until the color of the solution changes from blue through yellow to orange.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.32 mg of C₁₆H₈N₂Na₂O₈S₂

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Indigocarmine Injection

インジゴカルミン注射液

Indigocarmine Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of indigocarmine (C₁₆H₈N₂Na₂O₈S₂; 466.35).

Method of preparation Prepare as directed under Injection, with Indigocarmine.

Description Indigocarmine Injection is a dark blue liquid.
pH: 3.0 – 5.0

Identification (1) To a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of nitric acid: the dark blue color of the liquid disappears, and a yellow-brown color develops.

(2) To a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of bromine TS: the dark blue color disappears, and a yellow-brown color develops.

(3) To a volume of Indigocarmine Injection, equivalent to 0.02 g of Indigocarmine according to the labeled amount, add 1 mL of chlorine TS: the dark blue color disappears, and a yellow-brown color develops.

(4) To a volume of Indigocarmine Injection, equivalent to 0.01 g of Indigocarmine according to the labeled amount, add ammonium acetate solution (1 in 650) to make 1000 mL, and determine the absorbance of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a

maximum between 610 nm and 614 nm.

Assay Measure exactly a volume of Indigocarmine Injection, equivalent to about 0.2 g of indigocarmine (C₁₆H₈N₂Na₂O₈S₂), add 6 g of sodium hydrogen tartrate monohydrate, and dissolve in water to make 200 mL. Then boil under a carbon dioxide stream, and proceed as directed in the Assay under Indigocarmine.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 23.32 mg of C₁₆H₈N₂Na₂O₈S₂

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Indium (¹¹¹In) Chloride Injection

塩化インジウム (¹¹¹In) 注射液

Indium (¹¹¹In) Chloride Injection is an aqueous solution for injection

It contains indium-111 (¹¹¹In) in the form of indium chloride.

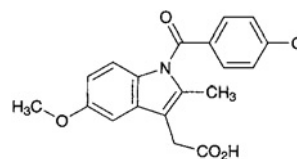
It conforms to the requirements of Indium (¹¹¹In) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Indium (¹¹¹In) Chloride Injection is a clear, colorless liquid.

Indometacin

インドメタシン



C₁₉H₁₆ClNO₄; 357.79

[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid [53-86-1]

Indometacin, when dried, contains not less than 98.0% of C₁₉H₁₆ClNO₄.

Description Indometacin occurs as a white to light yellowish white, very fine crystalline powder.

It is sparingly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

It is colored by light.

Melting point: 155 – 162°C

Identification (1) Dissolve 2 mg of Indometacin in 100 mL of methanol. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry

<2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Indometacin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Indometacin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Indometacin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample and the Reference Standard with diethyl ether, filter and dry the crystals, and perform the test with the crystals.

(3) Perform the test with Indometacin as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Acidity—To 1.0 g of Indometacin add 50 mL of water, shake for 5 minutes, and filter. To the filtrate add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: a red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Indometacin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Indometacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Indometacin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dehydrated diethyl ether and acetic acid (100) (100:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Indometacin, previously dried, dissolve in 60 mL of methanol, add 30 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 35.78 \text{ mg of } C_{19}H_{16}ClNO_4 \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Indometacin Capsules

インドメタシンカプセル

Indometacin Capsules contain not less than 90% and not more than 110% of the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$; 357.79).

Method of preparation Prepare as directed under Capsules, with Indometacin.

Identification Powder the contents of Indometacin Capsules. To a quantity of the powder, equivalent to 0.1 g of Indometacin according to the labeled amount, add 20 mL of chloroform, shake well, and centrifuge. Filter the supernatant liquid, and evaporate the filtrate to dryness. After cooling, dissolve the residue in 20 mL of methanol. To 10 mL of this solution add methanol to make 50 mL, then to 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 317 nm and 321 nm.

Purity Related substances—Powder the content of Indometacin Capsules. To a quantity of the powder, equivalent to 0.10 g of Indometacin according to the labeled amount, add exactly 10 mL of methanol, shake well, filter, and use the filtrate as the sample solution. Dissolve 25 mg of Indometacin Reference Standard in methanol to make exactly 50 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Proceed as directed in the Purity (4) under Indometacin.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Take 1 capsule of Indometacin Capsules, and perform the test using 900 mL of a mixture of water and phosphate buffer solution, pH 7.2, (4:1) as the test solution at 100 revolutions per minute as directed in the Basket method. Take 20 mL or more of the dissolved solution at 20 minutes after starting the test, and filter through a membrane filter (less than 0.8 μ m in pore size). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 30 mg of Indometacin Reference Standard, previously dried at 105°C for 4 hours, dissolve in a mixture of water and phosphate buffer solution, pH 7.2, (4:1) to make exactly 1000 mL, and use this as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 320 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Indometacin Capsules in 20 minutes should be not less than 75%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ \text{of indometacin } (C_{19}H_{16}ClNO_4) \\ = W_S \times (A_T/A_S) \times (90/C) \end{aligned}$$

W_S : Amount (mg) of Indometacin Reference Standard.

C : Labeled amount (mg) of indometacin ($C_{19}H_{16}ClNO_4$) in 1 capsule.

Assay Weigh accurately the contents of not less than 20 In-

dometacin Capsules. Powder the combined contents, and weigh accurately a portion of the powder, equivalent to about 50 mg of indometacin ($C_{19}H_{16}ClNO_4$). Dissolve in 40 mL of methanol, and add methanol to make exactly 50 mL. Filter this solution, discarding the first 10-mL portion of the filtrate. Pipet the subsequent 5 mL of the filtrate, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Indometacin Reference Standard, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of the solution, add exactly 3 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of indometacin to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of indometacin (C}_{19}\text{H}_{16}\text{ClNO}_4\text{)} \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Indometacin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7:3).

Flow rate: Adjust the flow rate so that the retention time of indometacin is about 8 minutes.

System suitability—

System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order, with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0, and between the peaks of butyl parahydroxybenzoate and indometacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Indometacin Suppositories

インドメタシン坐剤

Indometacin Suppositories contain not less than 90% and not more than 110% of the labeled amount of indometacin ($C_{19}H_{16}ClNO_4$; 357.79).

Method of preparation Prepare as directed under Suppositories, with Indometacin.

Identification Dissolve a quantity of Indometacin Suppositories, equivalent to 0.05 g of Indometacin according to the labeled amount, in 20 mL of methanol by warming, add methanol to make 50 mL, and filter if necessary. To 2 mL of this solution add methanol to make 100 mL, and use this solution as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 317 nm and 321 nm.

Assay Weigh accurately not less than 20 Indometacin Suppositories, cut into small pieces carefully, and mix well. Weigh accurately a portion of the mass, equivalent to about 50 mg of indometacin ($C_{19}H_{16}ClNO_4$), add 40 mL of tetrahydrofuran, warm at 40°C, dissolve by shaking, cool, and add tetrahydrofuran to make exactly 50 mL. Filter the solution, discard the first 10 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 100 mL. Allow the solution to stand for 30 minutes, filter through a membrane filter (0.5 μ m pore size), discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Indometacin Reference Standard, previously dried at 105°C for 4 hours, and dissolve in tetrahydrofuran to make exactly 50 mL. Pipet 5 mL of the solution, proceed in the same manner as the sample solution, and use as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of indometacin to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of indometacin (C}_{19}\text{H}_{16}\text{ClNO}_4\text{)} \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Indometacin Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted phosphoric acid (1 in 1000) (7:3).

Flow rate: Adjust the flow rate so that the retention time of

indometacin is about 8 minutes.

System suitability—

System performance: Dissolve 50 mg of 4-chlorobenzoic acid, 30 mg of butyl parahydroxybenzoate and 50 mg of indometacin in 50 mL of methanol. To 5 mL of this solution add the mobile phase to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, 4-chlorobenzoic acid, butyl parahydroxybenzoate and indometacin are eluted in this order with the resolution between the peaks of 4-chlorobenzoic acid and butyl parahydroxybenzoate being not less than 2.0 and between the peaks of parahydroxybenzoate and indometacin being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of indometacin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant, and in a cold place.

Influenza HA Vaccine

インフルエンザ HA ワクチン

Influenza HA Vaccine is a liquid for injection containing hemagglutinin of influenza virus.

It conforms to the requirements of Influenza HA Vaccine in the Minimum Requirements for Biological Products.

Description Influenza HA Vaccine is a clear liquid or a slightly whitish turbid liquid.

Insulin

インスリン

Insulin is obtained from the pancreas of healthy bovine or porcine, that has blood sugar-decreasing activity.

Its potency, calculated on the dried basis, is not less than 26 Insulin Units in each mg.

It is labeled to indicate the animal species from which it is derived.

Description Insulin occurs as a white, crystalline powder. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in diluted hydrochloric acid (1 in 360) and in dilute sodium hydroxide TS.

It is hygroscopic.

Identification Dissolve 0.01 g of Insulin in 10 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Adjust the pH of the sample solution to between 5.1 and 5.3 with a solution of sodium hydroxide (1 in 100): a precipitate is produced. Adjust the solution to a pH between

2.5 and 3.5 with dilute hydrochloric acid: the precipitate dissolves.

Purity Clarity and color of solution—Dissolve 0.10 g of Insulin in 10 mL of diluted hydrochloric acid (1 in 360): the solution is clear and colorless to light yellow.

Zinc content Weigh accurately about 10 mg of Insulin, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS and water to make exactly 50 mL. If necessary, dilute the solution with water so as to contain 0.4 to 1.0 μ g of zinc (Zn: 65.41) per mL, and use as the sample solution. Add water to an accurately measured volume of Standard Zinc Solution for atomic absorption spectrophotometry to make a solution containing 0.3 to 1.2 μ g of zinc (Zn: 65.41) per mL, and use as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution: the amount of zinc is not less than 0.27% and not more than 1.08%, calculated on the dried basis.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

Loss on drying <2.41> Not more than 10.0% (0.2 g, 105°C, 16 hours).

Residue on ignition <2.44> Weigh accurately 0.02 to 0.04 g of Insulin in a tared platinum dish, add 2 drops of nitric acid, and heat the dish at first very gently and then strongly to incinerate. Place the dish in a muffle furnace, heat at 600°C for 15 minutes, cool in a desiccator (silica gel), and weigh: the mass of the residue is not more than 2.5%.

Nitrogen content Weigh accurately about 20 mg of Insulin, dissolve in 10 mL of 0.1 mol/L hydrochloric acid TS, and perform the test as directed under Nitrogen Determination <1.08>: not less than 14.5% and not more than 16.5% of nitrogen (N: 14.01) is found, calculated on the dried basis.

Assay

(i) Animals: Select healthy rabbits each weighing not less than 1.8 kg. Keep the rabbits in the laboratory not less than 1 week before use in the assay by feeding them with an appropriate uniform diet and water.

(ii) Diluent for insulin: Dissolve 1.0 to 2.5 g of phenol or *m*-cresol in 500 mL of 0.01 mol/L hydrochloric acid VS, and add 14 to 18 g of glycerin and 0.01 mol/L hydrochloric acid VS to make 1000 mL.

(iii) Standard stock solution: Weigh accurately about 20 mg of Insulin Reference Standard, and dissolve it in the diluent for insulin to make a standard stock solution containing exactly 20.0 Units in each mL. Preserve this solution between 1°C and 15°C, and use within 6 months.

(iv) Standard solution: Dilute two portions of the standard stock solution to make two standard solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose standard solution S_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose standard solution S_L .

(v) Sample solution: Weigh accurately about 20 mg of Insulin according to the labeled Units, dissolve with the diluent

for insulin to make two different sample solutions, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution, T_H, and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution, T_L.

(vi) Dose for injection: Select the dose for injection on the basis of trial or experience. Inject a fixed identical volume, usually 0.3 to 0.5 mL, of the standard solutions and the sample solutions throughout the whole run.

(vii) Procedure: Divide the animals into 4 equal groups of not less than 6 animals each, with least difference in body mass. Withhold all food, except water, for not less than 14 hours before the injections, and withhold water during the assay until the final blood sample is taken. Handle the animals with care in order to avoid undue excitement.

Inject into each of the animals subcutaneously the dose of the standard solutions and the sample solutions indicated in the following design.

First group	S _H	Third group	T _H
Second group	S _L	Fourth group	T _L

The second injection should be made on the day after the first injection or within 1 week, using the dose of the standard solutions and the sample solutions indicated in the following design.

First group	T _L	Third group	S _L
Second group	T _H	Fourth group	S _H

At 1 hour and 2.5 hours after the time of injection, obtain a sufficient blood sample to perform the test from a marginal ear vein of each animal, and determine the blood sugar content of the blood samples according to (viii).

(viii) Blood sugar determination: Place 5.0 mL of a solution of zinc sulfate heptahydrate (9 in 2000) in a test tube 18 mm in outside diameter and 165 mm in length, add 1.0 mL of a solution of sodium hydroxide (1 in 250), and add gently 0.10 mL of the blood sample to the mixture in the test tube using a blood sugar pipet. Suck up the supernatant liquid into the pipet, wash out the remaining blood in the inner wall of the pipet, and repeat this procedure. Shake thoroughly the contents in the test tube, and heat the test tube in a water bath for 3 minutes. Filter the mixture through a funnel 30 to 40 mm in diameter in which a pledget of absorbent cotton, previously washed with two 3-mL portions of warm water, has been placed, receive the filtrate into a test tube 30 mm in inside diameter and 90 mm in length, wash the test tube and the funnel with two 3-mL portions of water, and combine the washings with the filtrate. Add 2.0 mL of alkaline potassium hexacyanoferrate (III) TS, heat in a water bath for 15 minutes, cool immediately, add 3.0 mL of potassium iodide-zinc sulfate TS and 2.0 mL of diluted acetic acid (100) (3 in 100), and titrate <2.50> the liberated iodine with 0.005 mol/L sodium thiosulfate VS (indicator: 2 to 4 drops of starch-sodium chloride TS). Perform a blank determination. From the consumed volume (mL) of 0.005 mol/L sodium thiosulfate VS, obtain the blood sugar content (%) according to the following table.

Conversion Table for Blood sugar content (%)

mL*	0	1	2	3	4	5	6	7	8	9
0.0	0.385	0.382	0.379	0.376	0.373	0.370	0.367	0.364	0.361	0.358
0.1	0.355	0.352	0.350	0.348	0.345	0.343	0.341	0.338	0.336	0.333
0.2	0.331	0.329	0.327	0.325	0.323	0.321	0.318	0.316	0.314	0.312
0.3	0.310	0.308	0.306	0.304	0.302	0.300	0.298	0.296	0.294	0.292
0.4	0.290	0.288	0.286	0.284	0.282	0.280	0.278	0.276	0.274	0.272
0.5	0.270	0.268	0.266	0.264	0.262	0.260	0.259	0.257	0.255	0.253
0.6	0.251	0.249	0.247	0.245	0.243	0.241	0.240	0.238	0.236	0.234
0.7	0.232	0.230	0.228	0.226	0.224	0.222	0.221	0.219	0.217	0.215
0.8	0.213	0.211	0.209	0.208	0.206	0.204	0.202	0.200	0.199	0.197
0.9	0.195	0.193	0.191	0.190	0.188	0.186	0.184	0.182	0.181	0.179
1.0	0.177	0.175	0.173	0.172	0.170	0.168	0.166	0.164	0.163	0.161
1.1	0.159	0.157	0.155	0.154	0.152	0.150	0.148	0.146	0.145	0.143
1.2	0.141	0.139	0.138	0.136	0.134	0.132	0.131	0.129	0.127	0.125
1.3	0.124	0.122	0.120	0.119	0.117	0.115	0.113	0.111	0.110	0.108
1.4	0.108	0.104	0.102	0.101	0.099	0.097	0.095	0.093	0.092	0.090
1.5	0.088	0.086	0.084	0.083	0.081	0.079	0.077	0.075	0.074	0.072
1.6	0.070	0.068	0.066	0.065	0.063	0.061	0.059	0.057	0.056	0.054
1.7	0.052	0.050	0.048	0.047	0.045	0.043	0.041	0.039	0.038	0.036
1.8	0.034	0.032	0.031	0.029	0.027	0.025	0.024	0.022	0.020	0.019
1.9	0.017	0.015	0.014	0.012	0.010	0.008	0.007	0.005	0.003	0.002

*Indicates the volume of 0.005 mol/L sodium thiosulfate VS required in titration. For example, if the amount was 1.28 mL, the blood sugar content would be 0.127% from the above table.

(ix) Calculation: Sum up the two blood sugar values of each animal after each injection. Subtract the blood sugar value effected by the first injection from that effected by the second injection of each animal in the first group and the third group. The differences are symbolized as y₁ and y₃, respectively. Subtract the blood sugar value effected by the second injection from that effected by the first injection of each animal in the second group and the fourth group. The differences are symbolized as y₂ and y₄, respectively. Sum up not less than 6 values of individual differences in the blood sugar values y₁, y₂, y₃, and y₄ to obtain Y₁, Y₂, Y₃, and Y₄, respectively.

Units in each mg of Insulin
= antilog M × (Units in each mL of S_H) × (b/a)

$$M = 0.301 \times (Y_a / Y_b)$$

$$Y_a = -Y_1 + Y_2 + Y_3 - Y_4$$

$$Y_b = Y_1 + Y_2 + Y_3 + Y_4$$

a: Amount (mg) of the sample.
b: Total volume (mL) of the high-dose sample solution prepared by dissolving the sample with the diluent for insulin.

Compute L (P=0.95) by using the following equation: L should be not more than 0.1212. If L exceeds 0.1212, repeat the assay by increasing the number of animals or improving the assay conditions in a better way until L becomes not more than 0.1212.

$$L = 2\sqrt{(C-1)(CM^2 + 0.09062)}$$

$$C = Y_b^2 / (Y_b^2 - 4fs^2t^2)$$

f: Number of the animals of each group.

$$s^2 = \{ \sum y_2 - (Y/f) \} / n$$

∑ y²: The sum of squares of y₁, y₂, y₃, and y₄ in each group.

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

$$n = 4(f-1)$$

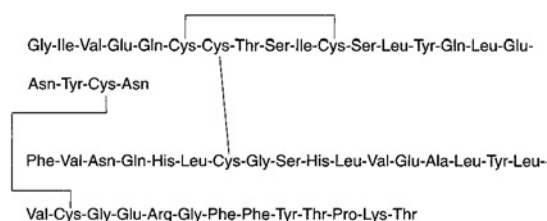
t^2 : Value shown in the following table against n for which s^2 is calculated.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

Containers and storage Containers—Tight containers.
Storage—Not exceeding 8°C.

Insulin Human (Genetical Recombination)

ヒトインスリン(遺伝子組換え)



$C_{257}H_{383}N_{65}O_{77}S_6$: 5807.57
[11061-68-0]

Insulin Human (Genetical Recombination) is a human insulin prepared by genetical recombinant technology.

It has an activity to reduce the blood sugar concentration. It contains not less than 27.5 Insulin Units per mg, calculated on the dried basis.

Description Insulin Human (Genetical Recombination) occurs as a white powder.

It is practically insoluble in water and in ethanol (95).

It dissolves in 0.01 mol/L hydrochloric acid TS and in sodium hydroxide TS with decomposition.

It is hygroscopic.

Identification Weigh accurately a suitable amount of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains 2.0 mg. Transfer 500 μ L of this solution into a clean test tube, add 2.0 mL of HEPES buffer solution, pH 7.5 and 400 μ L of V8-protease TS, react at 25°C for 6 hours, then add 2.9 mL of ammonium sulfate buffer solution to stop the reaction, and use this solution as the sample solution.

Separately, proceed with Human Insulin Reference Standard in the same manner as above, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and compare the chromatograms obtained from these solutions: the peak appears just after the peak of the solvent and the succeeding three peaks with apparently higher peak height show the same retention time and similar peak height each other on both chromatograms.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Solution A—A mixture of water, ammonium sulfate buffer solution and acetonitrile (7:2:1). Solution B—A mixture of water, acetonitrile and ammonium sulfate buffer solution (2:2:1).

Change the mixing ratio of the solutions A and B linearly from 9:1 to 3:7 in 60 minutes after sample injection, further change to 0:10 linearly in 5 minutes, and then flow the solution B only for 5 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the symmetry factor of the two larger peaks which appear next to the first peak just after the solvent peak are not more than 1.5, and the resolution between these peaks is not less than 3.4.

Purity (1) Related substances—Perform this procedure rapidly. Dissolve 7.5 mg of Insulin Human (Genetical Recombination) in 2 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak area of human insulin, A_1 , the peak area of the desamide substance at the relative retention time of 1.3 to the human insulin, A_D , and the total area of the peaks other than the solvent peak, A_T : the amounts of the desamide substance and related substances other than the desamide substance are each not more than 2.0%. Previously, perform the test with 0.01 mol/L hydrochloric acid TS in the same manner to confirm the solvent peak.

Amount (%) of the desamide substance = $(A_D/A_T) \times 100$

Amount (%) of related substances other than the desamide substance = $\{[A_T - (A_1 + A_D)]/A_T\} \times 100$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Solution A—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (41:9). Solution B—A mixture of phosphoric acid-sodium sulfate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (1:1).

Flow a mixture of the solution A and the solution B (78:22) for 36 minutes before and after the sample injection, then change the mixing ratio to 33:67 linearly in 25 minutes, and maintain this ratio for 6 minutes. Then flow the first mixture (78:22) for the next 15 minutes. Adjust the mixing ratio of the first mixture so that the retention time of human insulin is about 25 minutes.

Flow rate: 1.0 mL per minute.

Time span of measurement: For about 75 minutes after the sample is injected.

System suitability—

Test for required detection: Confirm that the peak height of the desamide substance obtained from 20 μ L of human insulin desamide substance-containing TS is between 30% and 70% of the full scale.

System performance: When the procedure is run with 20 μ L of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

(2) High-molecular proteins—Dissolve 4 mg of Insulin Human (Genetical Recombination) in 1 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 100 μ L of this solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate each peak area: the total of areas of the peaks having smaller retention time than human insulin is not more than 1.0% of the total area of all peaks.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 276 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 30 cm in length, packed with hydrophilic silica gel for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of a solution of L-arginine (1 in 1000), acetonitrile and acetic acid (100) (13:4:3).

Flow rate: Adjust the flow rate so that the retention time of human insulin is about 20 minutes.

Time span of measurement: Until the peak of human insulin monomer has appeared.

System suitability—

Test for required detection: Confirm that the peak height of the dimer obtained from 100 μ L of human insulin dimer containing TS is between 10% and 50% of the full scale.

System performance: When the procedure is run with 100 μ L of human insulin dimer containing TS under the above operating conditions, polymer, dimer and monomer are eluted in this order, and the ratio, H_1/H_2 , of the peak height of the dimer H_1 to the height of the bottom between the peaks of the dimer and the monomer H_2 is not less than 2.0.

(3) Product related impurities—Within the limits specified in each application dossier.

(4) Process related impurities—Within the limits specified in each application dossier.

Zinc content Weigh accurately about 50 mg of Insulin Human (Genetical Recombination), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL. If necessary, dilute with 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains between 0.4 μ g and 1.6 μ g of zinc (Zn: 65.41), and use this solution as the sample solution. Separately, take exactly a suitable amount of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with 0.01 mol/L hydrochloric acid TS to make solutions containing 0.40 μ g, 0.80 μ g, 1.20 μ g and 1.60 μ g of zinc (Zn: 65.41) in each mL, respectively, and use these solutions as the standard solutions. Perform the test with the sample solution and standard solutions as directed under Atomic Absorption Spectrophotometry <2.23>, and determine the amount of zinc (Zn: 65.41) in the sample solution by using a calibration curve obtained from the absorbances of the standard solutions: not more than 1.0%, calculated on the dried basis.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: Zinc hollow cathode lamp

Wavelength: 213.9 nm

Loss on drying <2.41> Not more than 10.0% (0.2 g, 105°C, 24 hours).

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Assay Perform this procedure quickly. Weigh accurately about 7.5 mg of Insulin Human (Genetical Recombination), dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 5 mL, and use this solution as the sample solution. Separately, weigh accurately a suitable amount of Human Insulin Reference Standard, dissolve exactly in 0.01 mol/L hydrochloric acid TS to make a solution so that each mL contains about 40 Insulin Units, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of human insulin, A_{TI} and A_{SI} , and the peak areas of the desamide substance at the relative retention time of 1.3 to the human insulin, A_{TD} and A_{SD} , respectively, of these solutions.

Amount (Insulin Unit/mg) of human insulin

$$\begin{aligned} & (C_{257}H_{383}N_{65}O_{77}S_6) \\ & = \{(W_S \times F)/D\} \times \{(A_{TI} + A_{TD})/(A_{SI} + A_{SD})\} \\ & \quad \times (5/W_T) \end{aligned}$$

F : Label unit (Insulin Unit/mg) of Human Insulin Reference Standard.

D : Volume (mL) of 0.01 mol/L hydrochloric acid TS used to dissolve the reference standard.

W_T : Amount (mg) of the sample calculated on the dried basis.

W_S : Amount (mg) of Human Insulin Reference Standard.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 214 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of phosphoric acid-sodium sul-

fate buffer solution, pH 2.3 and acetonitrile for liquid chromatography (3:1). Adjust the mixing ratio of the component of the mobile phase so that the retention time of human insulin is between 10 minutes and 17 minutes.

Flow rate: 1.0 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μ L of human insulin desamide substance-containing TS under the above operating conditions, human insulin and human insulin desamide substance are eluted in this order with the resolution between these peaks being not less than 2.0, and the symmetry factor of the peak of human insulin is not more than 1.8.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of human insulin is not more than 1.6%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at -20°C or below.

Insulin Injection

インスリン注射液

Insulin Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled Insulin Units.

Method of preparation Suspend Insulin in Water for Injection, dissolve by adding Hydrochloric Acid, and prepare as directed under Injections. It contains 0.10 to 0.25 g of Phenol or Cresol and 1.4 to 1.8 g of Concentrated Glycerin for each 100 mL of Insulin Injection. It should not contain sodium chloride.

Description Insulin Injection is a clear, colorless or pale yellow liquid.

Identification Adjust Insulin Injection to pH between 5.1 and 5.3 with a solution of sodium hydroxide (1 in 100): a precipitate is produced. Adjust the solution to a pH between 2.5 and 3.5 with dilute hydrochloric acid: the precipitate dissolves.

pH <2.54> 2.5 – 3.5

Residue on ignition <2.44> Measure exactly a volume of Insulin Injection, equivalent to 500 to 1000 Units according to the labeled Units, in a tared platinum dish, and evaporate slowly by heating on a water bath to dryness. Add 2 drops of nitric acid to the residue, and heat at first very gently, then strongly to incinerate. Place in a muffle furnace, and heat at 600°C for 15 minutes, cool in a desiccator (silica gel), and weigh: the mass of the residue is not more than 1 mg for each labeled 1000 Units.

Extractable volume <6.05> It meets the requirement.

Nitrogen content Perform the test as directed under Nitrogen Determination <1.08>: not less than 0.50 mg and not more than 0.64 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

Assay Proceed with Insulin Injection as directed in the As-

say under Insulin with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute two portions of Insulin Injection to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, replacing the equation as follows.

Units in each mL of Insulin Injection

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a: Volume (mL) of the sample.

b: Total volume (mL) of the high-dose sample solution prepared by diluting the volume of the sample with diluent for insulin.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Isophane Insulin Injection (Aqueous Suspension)

イソフェンインスリン水性懸濁注射液

Isophane Insulin Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.01 mg and not more than 0.04 mg of zinc (Zn: 65.41) for each labeled 100 Units.

When Sodium Chloride is used in the preparation of Isophane Insulin Injection (Aqueous Suspension), this should be stated on the label.

Method of preparation Prepare as directed under Injections, with Insulin and Protamine Sulfate. To each 100 mL of Isophane Insulin Injection (Aqueous Suspension) add either 0.38 to 0.63 g of Dibasic Sodium Phosphate, 1.4 to 1.8 g of Concentrated Glycerin, 0.15 to 0.17 g of Cresol, and 0.06 to 0.07 g of Phenol, or 0.38 to 0.63 g of Dibasic Sodium Phosphate, 0.42 to 0.45 g of Sodium Chloride, 0.7 to 0.9 g of Concentrated Glycerin, and 0.18 to 0.22 g of Cresol.

Description Isophane Insulin Injection (Aqueous Suspension) is a white aqueous suspension. When allowed to stand, it separates into a white precipitate and colorless supernatant liquid, and the precipitate returns easily to the suspension state on gentle shaking.

When examined microscopically, the precipitate mostly consists of fine, oblong crystals of 5 to 30 μm in major axis, and does not contain amorphous substances or large aggregates.

Identification Proceed as directed in the Identification under Insulin Zinc Protamine Injection (Aqueous Suspension).

pH <2.54> 7.0 – 7.4

Purity (1) Protein—Perform the test as directed under Nitrogen Determination <1.08>: not exceeding 0.85 mg of

nitrogen (N: 14.01) is found for each labeled 100 Units.

(2) Isophane ratio—(i) Buffer solution A: Dissolve 2.0 g of anhydrous disodium hydrogenphosphate, 16 g of glycerin, 1.6 g of *m*-cresol, and 0.65 g of phenol in water to make exactly 200 mL.

(ii) Buffer solution B: Dissolve 2.0 g of anhydrous disodium hydrogenphosphate, 4.35 g of sodium chloride, 8.0 g of glycerin, and 2.0 g of *m*-cresol in water to make exactly 200 mL.

(iii) Insulin solution: Weigh accurately 1000 Units of Insulin Reference Standard, dissolve in 1.5 mL of diluted hydrochloric acid (1 in 360), and add 5.0 mL of buffer solution A and water to make 20 mL. Adjust the pH to 7.2 with dilute hydrochloric acid or sodium hydroxide TS. The solution is clear. Dilute with water to make exactly 25 mL. The solution is clear, and the pH is between 7.1 and 7.4. When it is stated on the label that Sodium Chloride is used in the preparation, use 5.0 mL of buffer solution B instead of buffer solution A in the above procedure.

(iv) Protamine solution: Weigh accurately 50 mg of Protamine Sulfate Reference Standard, and dissolve in 2 mL of buffer solution A and water to make 8 mL. Adjust the pH to 7.2 with dilute hydrochloric acid or sodium hydroxide TS, and dilute with water to exactly 10 mL. The solution is clear, and the pH is between 7.1 and 7.4. When it is stated on the label that Sodium Chloride is used in the preparation, use 2 mL of buffer solution B instead of buffer solution A in the above procedure.

(v) Procedure: When Isophane Insulin Injection (Aqueous Suspension) contains 40 Units per mL, centrifuge a portion of the suspension, measure exactly two 10-mL portions of the supernatant liquid in two tubes A and B, respectively, add exactly 1 mL of the insulin solution to tube A, and 1 mL of the protamine solution to tube B, mix the contents of each tube, allow to stand for 10 minutes, and determine the turbidity of each mixture by using a photometer or a nephelometer: the turbidity of the mixture in tube B is not greater than that in tube A. When Isophane Insulin Injection (Aqueous Suspension) contains 80 Units per mL, measure exactly 5 mL of the supernatant liquid, and proceed in the same manner.

Extractable volume <6.05> It meets the requirement.

Assay (1) Insulin—To Isophane Insulin Injection (Aqueous Suspension) add diluted hydrochloric acid (1 in 1000) to adjust pH to about 2.5, and proceed with the clear solution as directed in the Assay under Insulin, with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute Insulin Injection (Aqueous Suspension) to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, using the following equation,

Units in each mL of Isophane Insulin Injection (Aqueous Suspension)

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Volume (mL) of the sample,

instead of the following equation,

Units in each mg of Insulin

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Mass (mg) of the sample.

(2) Zinc—Pipet a volume of Isophane Insulin Injection (Aqueous Suspension), equivalent to about 400 Units according to the labeled Units, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make exactly 100 mL, dilute, if necessary, with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.41) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.41) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the amount of zinc in the sample solution using the analytical curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow – cathode lamp

Wavelength: 213.9 nm

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Insulin Zinc Injection (Aqueous Suspension)

インスリン亜鉛水性懸濁注射液

Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.41) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. It contains 0.15 to 0.17 g of Sodium Acetate Hydrate, 0.65 to 0.75 g of Sodium Chloride and 0.09 to 0.11 g of Methyl Parahydroxybenzoate for each 100 mL of Insulin Zinc Injection (Aqueous Suspension).

Description Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When it is examined microscopically, the majority of the particles in the suspension are crystals, the dimension of which is 10 to 40 μm . The rest is amorphous and does not exceed 2 μm in dimension.

Identification Adjust the pH of Amorphous Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH <2.54> 7.1 – 7.5

Purity Dissolved insulin—Perform the following test with a

clear liquid obtained by centrifuging Insulin Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the labeled units of Insulin Zinc Injection (Aqueous Suspension) by proceeding as directed in the Assay (iv) under Insulin. Divide the healthy rabbits weighing not less than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Extractable volume <6.05> It meets the requirement.

Nitrogen content Perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not less than 0.50 mg and not more than 0.64 mg for each labeled 100 Units.

Assay (1) Insulin—Proceed as directed in the Assay under Insulin with the clear liquid obtained from Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 1000), with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute two portions of Insulin Zinc Injection (Aqueous Suspension) to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, using the following equation,

Units in each mL of Insulin Zinc Injection (Aqueous Suspension)

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Volume (mL) of the sample,

instead of the following equation,

Units in each mg of Insulin

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Mass (mg) of the sample.

(2) Zinc—Measure exactly a volume of Insulin Zinc Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.41) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.41) per mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to

the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

(3) Crystalline insulin—Measure exactly a volume of Amorphous Insulin Zinc Injection (Aqueous Suspension), equivalent to about 600 Units according to the labeled units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not less than 55% and not more than 70% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of the sample taken from the values of nitrogen obtained in the Nitrogen content.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Amorphous Insulin Zinc Injection (Aqueous Suspension)

無晶性インスリン亜鉛水性懸濁注射液

Amorphous Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.41) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. Each 100 mL of Amorphous Insulin Zinc Injection (Aqueous Suspension) contains 0.15 to 0.17 g of Sodium Acetate Hydrate, 0.65 to 0.75 g of Sodium Chloride, and 0.09 to 0.11 g of Methyl Parahydroxybenzoate.

Description Amorphous Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When examined microscopically, most of the particles in the suspension are amorphous and have no uniform shape, and most of the dimension does not exceed 2 μm .

Identification Adjust the pH of Amorphous Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH <2.54> 7.1 – 7.5

Purity Dissolved insulin—Perform the following test with a clear liquid obtained by centrifuging Amorphous Insulin

Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Amorphous Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the labeled units of Amorphous Insulin Zinc Injection (Aqueous Suspension) by proceeding as directed in the Assay (iv) under Insulin. Divide healthy rabbits weighing more than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Extractable volume <6.05> It meets the requirement.

Nitrogen content Perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not less than 0.50 mg and not more than 0.64 mg for each labeled 100 Units.

Assay (1) Insulin—Proceed as directed in the Assay under Insulin with the clear liquid obtained from Amorphous Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 1000), with alteration in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute two portions of Amorphous Insulin Zinc Injection (Aqueous Suspension) to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, using the following equation,

Units in each mL of Amorphous Insulin Zinc Injection (Aqueous Suspension)

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Volume (mL) of the sample,

instead of the following equation,

Units in each mg of Insulin

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Mass (mg) of the sample.

(2) Zinc—Measure exactly a volume of Amorphous Insulin Zinc Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.41) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.41) per mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as direct-

ed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

(3) Crystalline insulin—Measure exactly a volume of Amorphous Insulin Zinc Injection (Aqueous Suspension), equivalent to about 1000 Units according to the labeled units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not more than 10% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of the sample taken from the values of nitrogen obtained in the Nitrogen content.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Crystalline Insulin Zinc Injection (Aqueous Suspension)

結晶性インスリン亜鉛水性懸濁注射液

Crystalline Insulin Zinc Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.12 mg and not more than 0.30 mg of zinc (Zn: 65.41) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin and Zinc Chloride. Each 100 mL of Crystalline Insulin Zinc Injection (Aqueous Suspension) contains 0.15 to 0.17 g of Sodium Acetate Hydrate, 0.65 to 0.75 g of Sodium Chloride, and 0.09 to 0.11 g of Methyl Parahydroxybenzoate.

Description Crystalline Insulin Zinc Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless supernatant liquid, and it readily becomes a suspension again on gentle shaking.

When it is examined microscopically, most part of the particles in the suspension are crystals, the dimension of which is mostly 10 to 40 μm .

Identification Adjust the pH of Crystalline Insulin Zinc Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH <2.54> 7.1 – 7.5

Purity Dissolved insulin—Perform the following test with a

clear liquid obtained by centrifuging Crystalline Insulin Zinc Injection (Aqueous Suspension): not more than 2.5% of the labeled units is found.

Use the clear liquid of Crystalline Insulin Zinc Injection (Aqueous Suspension) as the sample solution. Prepare the standard solution having a concentration of 2.5% of the labeled units of Crystalline Insulin Zinc Injection (Aqueous Suspension) by proceeding as directed in the Assay (iv) under Insulin. Divide healthy rabbits weighing more than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass to the animals of each group. Collect blood before and 1 hour and 2.5 hours after injection, then proceed as directed in the Assay (viii) under Insulin, and calculate the ratio of the average blood sugar level of 1 hour and 2.5 hours after to that of before injection of each animal: the mean value for the group injected the sample solution is not less than that for the group injected the standard solution.

Extractable volume <6.05> It meets the requirement.

Nitrogen content Perform the test as directed under Nitrogen Determination <1.08>: not less than 0.50 mg and not more than 0.64 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

Assay (1) Insulin—Proceed as directed in the Assay under Insulin with the clear liquid obtained from Crystalline Insulin Zinc Injection (Aqueous Suspension) by adjusting the pH to about 2.5 with diluted hydrochloric acid (1 in 1000), with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute two portions of Crystalline Insulin Zinc Injection (Aqueous Suspension) to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, using the following equation,

Units in each mL of Crystalline Insulin Zinc Injection (Aqueous Suspension)

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Volume (mL) of the sample,

instead of the following equation,

Units in each mg of Insulin

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Mass (mg) of the sample.

(2) Zinc—Measure exactly a volume of Crystalline Insulin Zinc Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, then dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.41) per mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for atomic absorption spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.41) per mL, and use this solution as the standard solution. Perform the test

with the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the amount of zinc in the sample solution using the calibration curve obtained from the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

(3) Crystalline insulin—Measure accurately a volume of Crystalline Insulin Zinc Injection (Aqueous Suspension), equivalent to about 400 Units according to the labeled Units, centrifuge, discard the supernatant liquid, suspend the residue in 5 mL of water, add 10 mL of sodium acetate-acetone TS, shake for 3 minutes, and centrifuge. Discard the supernatant liquid, and repeat the above treatment on the residue. Wash down the residue into a Kjeldahl flask with 15 mL of sulfuric acid, and perform the test as directed under the Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not less than 85% of the total nitrogen content. Calculate the total nitrogen content for insulin Units of a sample from the values of nitrogen obtained in the Nitrogen content.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Insulin Zinc Protamine Injection (Aqueous Suspension)

プロタミンインスリン亜鉛水性懸濁注射液

Insulin Zinc Protamine Injection (Aqueous Suspension) is an aqueous suspension for injection.

It contains not less than 90% and not more than 110% of the labeled Insulin Units, and not less than 0.20 mg and not more than 0.30 mg of zinc (Zn: 65.41) for each labeled 100 Units.

Method of preparation Prepare as directed under Injections, with Insulin, Protamine Sulfate and Zinc Chloride. It contains 0.38 to 0.63 g of Dibasic Sodium Phosphate Hydrate, 1.4 to 1.8 g of Concentrated Glycerin, and 0.18 to 0.22 g of Cresol or 0.22 to 0.28 g of Phenol for each 100 mL of Insulin Zinc Protamine Injection (Aqueous Suspension).

Description Insulin Zinc Protamine Injection (Aqueous Suspension) is a white suspension. When allowed to stand, it separates into a white precipitate and a colorless, supernatant liquid, and it readily becomes suspension again on gentle shaking.

When it is examined microscopically, no large particles are seen.

Identification Adjust the pH of Insulin Zinc Protamine Injection (Aqueous Suspension) to between 2.5 and 3.5 with dilute hydrochloric acid: the particles dissolve, and the solution is clear and colorless.

pH <2.54> 7.0 – 7.4

Purity (1) Protein—Perform the test as directed under

Nitrogen Determination <1.08>: not exceeding 1.25 mg of nitrogen (N: 14.01) is found for each labeled 100 Units.

(2) Dissolved insulin—Perform the following test with the clear liquid obtained by centrifuging Insulin Zinc Protamine Injection (Aqueous Suspension): not more than 2.5% of the labeled Units is found.

Use a clear liquid of Insulin Zinc Protamine Injection (Aqueous Suspension) as the sample solution, and prepare the standard solution by proceeding as directed in the Assay (iv) under Insulin to adjust its concentration to 2.5% of the labeled units of Insulin Zinc Protamine Injection (Aqueous Suspension). Divide the healthy rabbits weighing not less than 1.8 kg, fasted for not less than 14 hours before injection, into 2 equal groups of not less than 3. Inject subcutaneously an amount of the standard solution or the sample solution equivalent to 0.3 units per kg of body mass. Collect blood before and 1 hour and 2.5 hours after injection, proceed as directed in the Assay (viii) under Insulin, and calculate the ratios of the average blood sugar content in each rabbit measured 1 hour and 2.5 hours after injection to the content before injection: the mean value for the group injected with the sample solution is not less than the mean value for the group injected with the standard solution.

Extractable volume <6.05> It meets the requirement.

Assay (1) Insulin—Proceed as directed in the Assay under Insulin with the clear liquid obtained by adjusting the pH to 2.5 with diluted hydrochloric acid (1 in 1000), with alterations in (v) Sample solution and (ix) Calculation as follows.

(v) Sample solution: According to the labeled Units, dilute Insulin Zinc Protamine Injection (Aqueous Suspension) to make two sample solutions with the diluent for insulin, one to contain exactly 2.0 Units in each mL which is designated as the high-dose sample solution T_H , and the other to contain exactly 1.0 Unit in each mL which is designated as the low-dose sample solution T_L .

(ix) Calculation: Proceed as directed in the Assay (ix) Calculation under Insulin, using the following equation,

Units in each mL of Insulin Zinc Protamine Injection (Aqueous Suspension)

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Volume (mL) of the sample,

instead of the following equation,

Units in each mg of Insulin

$$= \text{antilog } M \times (\text{Units in each mL of } S_H) \times (b/a)$$

a : Mass (mg) of the sample.

(2) Zinc—Measure accurately a volume of Insulin Zinc Protamine Injection (Aqueous Suspension), equivalent to about 200 Units according to the labeled units, add 1 mL of 0.1 mol/L hydrochloric acid TS and sufficient water to make exactly 200 mL, dilute with water to contain 0.6 to 1.0 μg of zinc (Zn: 65.41) in 1 mL, and use this solution as the sample solution. Separately, pipet a volume of Standard Zinc Solution for Atomic Absorption Spectrophotometry, dilute with water to contain 0.4 to 1.2 μg of zinc (Zn: 65.41) per mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the amount of zinc in the sample solution using the analytical curve obtained from

the absorbance of the standard solution.

Gas: Combustible gas—Acetylene gas

Supporting gas—Air

Lamp: Zinc hollow-cathode lamp

Wavelength: 213.9 nm

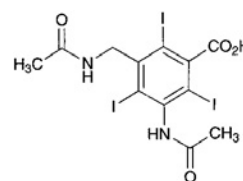
Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Expiration date 24 months after preparation.

Iodamide

ヨ一ダミド



$C_{12}H_{11}I_3N_2O_4$: 627.94

3-Acetylamino-5-acetylamino-2,4,6-triiodobenzoic acid [440-58-4]

Iodamide, calculated on the dried basis, contains not less than 98.5% of $C_{12}H_{11}I_3N_2O_4$.

Description Iodamide occurs as a white, crystalline powder. It is odorless.

It is slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

It gradually changes in color by light.

Identification (1) To 0.01 g of Iodamide add 5 mL of hydrochloric acid, and heat in a water bath for 5 minutes: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Heat 0.1 g of Iodamide over a flame: a purple gas evolves.

(3) Determine the infrared absorption spectrum of Iodamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of Iodamide in 100 mL of water by heating, and concentrate the solution to about 30 mL by gentle boiling. After cooling, collect the formed crystals by filtration, dry, and repeat the test on the dried crystals.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Iodamide in 10 mL of diluted sodium hydroxide TS (1 in 5): the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Iodamide in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and allow to stand for 2 minutes. To this solution add 5 mL of ammonium amidosulfate TS, shake thoroughly, allow to stand for 1 minute, add 0.4 mL of a solution of 1-naphthol in ethanol

(95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL, and determine the absorbance at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner, as the blank: the absorbance of the solution is not more than 0.12.

(3) Soluble halide—Dissolve 2.5 g of Iodamide in 20 mL of water and 2.5 mL of ammonia TS, then add 20 mL of dilute nitric acid and water to make 100 mL. Allow to stand for 15 minutes with occasional shaking, and filter. Discard the first 10 mL of the filtrate, transfer 25 mL of the subsequent filtrate to a Nessler tube, and add ethanol (95) to make 50 mL. Use this solution as the test solution, and proceed as directed under Chloride Limit Test <1.03>. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of dilute nitric acid, and dilute with water to 25 mL, then with ethanol (95) to 50 mL.

(4) Iodine—Dissolve 0.20 g of Iodamide in 2 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, allow to stand for 10 minutes with occasional shaking, then add 5 mL of chloroform, shake vigorously and allow to stand: the chloroform layer remains colorless.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Iodamide according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Iodamide according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iodamide in a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, connect the flask with a reflux condenser, boil for 30 minutes, cool, and filter. Wash the flask and filter paper with 50 mL of water, and combine the washings with the filtrate. Add 5 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L silver nitrate VS until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS
= 20.93 mg of C₁₂H₁₁I₃N₂O₄

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Iodinated (¹³¹I) Human Serum Albumin Injection

ヨウ化人血清アルブミン (¹³¹I) 注射液

Iodinated (¹³¹I) Human Serum Albumin Injection is an aqueous solution for injection containing normal human serum albumin iodinated by iodine-131 (¹³¹I).

It conforms to the requirements of Iodinated (¹³¹I) Human Serum Albumin Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injec-

tions are not applied to this injection.

Description Iodinated (¹³¹I) Human Serum Albumin Injection is a clear, colorless or light yellow liquid.

Iodine

ヨウ素

I: 126.90

Iodine contains not less than 99.5% of I.

Description Iodine occurs as grayish black plates or heavy, granular crystals, having a metallic luster and a characteristic odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), sparingly soluble in chloroform, and very slightly soluble in water.

It dissolves in potassium iodide TS.

Iodine sublimes at room temperature.

Identification (1) A solution of Iodine in ethanol (95) (1 in 50) shows a red-brown color.

(2) A solution of Iodine in chloroform (1 in 1000) shows a red-purple to purple color.

(3) Add 0.5 mL of starch TS to 10 mL of a saturated solution of Iodine: a dark blue color is produced. When the mixture is boiled, the color disappears, and it reappears on cooling.

Purity (1) Non-volatile residue—Sublime 2.0 g of Iodine on a water bath, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

(2) Chloride or bromide—Mix 1.0 g of finely powdered Iodine with 20 mL of water, and filter the mixture. To 10 mL of the filtrate add dropwise diluted sulfurous acid solution (1 in 5) until the yellow color disappears. Add 1 mL of ammonia TS, followed by 1 mL of silver nitrate TS in small portions, and add water to make 20 mL. Shake well, filter, and after discarding the first 2 mL of the filtrate, take 10 mL of the subsequent filtrate. To the filtrate add 2.0 mL of nitric acid and water to make 20 mL: the solution so obtained has no more turbidity than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of water, 2.5 mL of ammonia TS, 1 mL of silver nitrate TS, 2.0 mL of nitric acid and water to make 20 mL.

Assay Place 1 g of potassium iodide and 1 mL of water in a glass-stoppered flask, weigh accurately, add about 0.3 g of Iodine to the flask, and weigh accurately again. Dissolve the iodine by gentle shaking, add 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

Containers and storage Containers—Tight containers.

Iodine Tincture

ヨードチンキ

Iodine Tincture contains not less than 5.7 w/v% and not more than 6.3 w/v% of iodine (I: 126.90), and not less than 3.8 w/v% and not more than 4.2 w/v% of potassium iodide (KI: 166.00).

Method of preparation

Iodine	60 g
Potassium Iodide	40 g
70 vol% Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Tinctures, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol or Ethanol for Disinfectant and Purified Water in place of 70 vol% Ethanol.

Description Iodine Tincture is a dark red-brown liquid, and has a characteristic odor.

Specific gravity d_{20}^{20} : about 0.97

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine Tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of Iodine Tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the Qualitative Tests <1.09> for potassium salt and iodide.

Alcohol number <1.01> Not less than 6.6 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay (1) Iodine—Pipet 5 mL of Iodine Tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

(2) Potassium iodide—Pipet 5 mL of Iodine Tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate <2.50> with 0.05 mol/L potassium iodate VS until the red-purple color disappears from the chloroform layer, with agitating the mixture vigorously and continuously. After the chloroform layer has been decolorized, allow the mixture to stand for 5 minutes. If the color reappears, the mixture should be titrated <2.50> further with 0.05 mol/L potassium iodate VS. Calculate the amount (mg) of potassium iodide from the number of mL (*a*) of 0.05 mol/L potassium iodate VS used as above and the number of mL (*b*) of 0.1 mol/L sodium thiosulfate VS used in the titration under the Assay (1).

Amount (mg) of potassium iodide (KI)
= $16.600 \times \{a - (b/2)\}$

Containers and storage Containers—Tight containers.

Dilute Iodine Tincture

希ヨードチンキ

Dilute Iodine Tincture contains not less than 2.8 w/v% and not more than 3.2 w/v% of iodine (I: 126.90), and not less than 1.9 w/v% and not more than 2.1 w/v% of potassium iodide (KI: 166.00).

Method of preparation

Iodine	30 g
Potassium Iodide	20 g
70 vol% Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol or Ethanol for Disinfection and Purified Water in place of 70 vol% Ethanol. It may also be prepared by adding 70 vol% Ethanol to 500 mL of Iodine Tincture to make 1000 mL.

Description Dilute Iodine Tincture is a dark red-brown liquid, and has a characteristic odor.

Specific gravity d_{20}^{20} : about 0.93

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Dilute Iodine Tincture: a dark blue-purple color develops.

(2) Evaporate 3 mL of Diluted Iodine Tincture to dryness on a water bath, and heat gently over a free flame: a white residue is formed which responds to the Qualitative Tests <1.09> for potassium salt and iodide.

Alcohol number <1.01> Not less than 6.7 (Method 2). Perform the pretreatment (ii) in the Method 1.

Assay (1) Iodine—Pipet exactly 10 mL of Dilute Iodine Tincture, add 0.5 g of potassium iodide, 20 mL of water and 1 mL of dilute hydrochloric acid, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

Each mL of 0.1 mol/L sodium thiosulfate VS
= 12.69 mg of I

(2) Potassium iodide—Pipet exactly 10 mL of Dilute Iodine Tincture into an iodine flask, add 20 mL of water, 50 mL of hydrochloric acid and 5 mL of chloroform. Cool to room temperature, and titrate <2.50> with 0.05 mol/L potassium iodate VS until the red-purple color in the chloroform layer disappears while agitating vigorously and continuously. After the chloroform layer has been decolorized, allow the mixture to stand for 5 minutes. If the color reappears, the mixture should be titrated <2.50> further with 0.05 mol/L potassium iodate VS. Calculate the amount (mg) of potassium iodide from the volume (*a* mL) of 0.05 mol/L potassium iodate VS consumed as above and the volume (*b* mL) of 0.1 mol/L sodium thiosulfate VS consumed in the titration under Assay (1).

Amount (mg) of potassium iodide (KI)
= $16.600 \times \{a - (b/2)\}$

Containers and storage Containers—Tight containers.

Compound Iodine Glycerin

複方ヨード・グリセリン

Compound Iodine Glycerin contains not less than 1.1 w/v% and not more than 1.3 w/v% of iodine (I: 126.90), not less than 2.2 w/v% and not more than 2.6 w/v% of potassium iodide (KI: 166.00), not less than 2.7 w/v% and not more than 3.3 w/v% of total iodine (as I), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Iodine	12 g
Potassium Iodide	24 g
Glycerin	900 mL
Mentha Water	45 mL
Liquefied Phenol	5 mL
Purified Water	a sufficient quantity
To make 1000 mL	

Dissolve Potassium Iodide and Iodine in about 25 mL of Purified Water. After adding Glycerin, add Mentha Water, Liquefied Phenol and sufficient Purified Water to make 1000 mL, mixing thoroughly. It may be prepared with an appropriate quantity of Concentrated Glycerin and Purified Water in place of Glycerin.

Description Compound Iodine Glycerin is a red-brown, viscous liquid. It has a characteristic odor.

Specific gravity d_{20}^{20} : about 1.23

Identification (1) The colored solution obtained in the Assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the Assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) The colored solution obtained in the Assay (4) has a yellow color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 401 nm and 405 nm (phenol).

(4) Take 1 mL of Compound Iodine Glycerin in a glass-stoppered test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

Assay (1) Iodine—Measure the specific gravity <2.56> of Compound Iodine Glycerin according to Method 2. Weigh exactly about 7 mL of it, add ethanol (95) to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 80 mg of iodine for assay and about 0.17 g of potassium iodide for assay, previously dried at 105°C for 4 hours, dissolve in ethanol (95) to make exactly 200 mL, and use this solution as the standard solution.

Pipet 3 mL each of the sample solution and the standard solution into 50-mL separators, to each add exactly 10 mL of a mixture of chloroform and hexane (2:1) and 15 mL of water successively, and shake immediately and vigorously. Separate the chloroform-hexane layers [use the water layers in (2)], and filter through a pledget of cotton. Determine the absorbances of the filtrates, A_T and A_S , at 512 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of iodine for assay

(2) Potassium iodide—Separate the water layers of the sample solution and the standard solution obtained in (1), pipet 10 mL of each of the water layers, and to each add 1 mL of diluted dilute hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and exactly 10 mL of a mixture of chloroform and hexane (2:1). Shake immediately and vigorously, separate the chloroform-hexane layers, and filter through a pledget of cotton. Determine the absorbances, A_T and A_S , of both solutions at 512 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of potassium iodide (KI)} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of potassium iodide for assay

(3) Total iodine—Measure the specific gravity <2.56> of Compound Iodine Glycerin according to Method 2. Weigh exactly about 5 mL of it, and add water to make exactly 50 mL. Pipet 5 mL of this solution into a 50-mL flask, and add 0.5 g of zinc powder and 5 mL of acetic acid (100). Shake until the color of iodine disappears, and heat under a reflux condenser on a water bath for 30 minutes. Wash the condenser with 10 mL of hot water, and filter through a glass filter (G3). Wash the flask with two 10-mL portions of warm water, and combine the filtrate and the washings. After cooling, add water to make exactly 50 mL, and use this solution as the sample solution. On the other hand, dissolve about 0.2 g of potassium iodide for assay, previously dried at 105°C for 4 hours and accurately weighed, in water to make exactly 50 mL. Pipet 5 mL of this solution, add 5 mL of acetic acid (100) and water to make exactly 50 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and standard solution into 30-mL separators, and to each add 5 mL of water, 1 mL of diluted dilute hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and 10 mL of a mixture of chloroform and hexane (2:1). Shake well immediately, and proceed as directed in (2).

$$\text{Amount (mg) of total iodine (I)} = W_S \times (A_T/A_S) \times 0.7644$$

W_S : Amount (mg) of potassium iodide for assay

(4) Phenol—Measure the specific gravity <2.56> of Compound Iodine Glycerin according to Method 2. Weigh exactly about 2 mL of it, add 3 mL of 0.1 mol/L sodium thiosulfate VS, and shake. Add 2 mL of dilute hydrochloric acid, and shake with two 10-mL portions of chloroform. Separate the chloroform layer, and shake with two 10-mL portions of 0.5 mol/L sodium hydroxide TS. Separate the water layer, add water to make exactly 500 mL, and use this solution as the sample solution. Dissolve about 0.5 g of phenol for assay, accurately weighed, in ethanol (95) to make exactly 100 mL,

pipet 2 mL of this solution, proceed in the same manner as the sample solution, and use so obtained solution as the standard solution. Pipet 3 mL each of the sample solution and standard solution, to each add 2 mL of dilute hydrochloric acid, and place in a water bath at 30°C. Allow to stand for 10 minutes, and add exactly 2 mL of a solution of sodium nitrite (1 in 100), shake, and allow to stand at 30°C for 60 minutes. Add dilute potassium hydroxide-ethanol TS to make exactly 25 mL, and determine the absorbances of these solutions, A_T and A_S , at 403 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 3 mL of water instead of the sample solution as the blank.

$$\begin{aligned} &\text{Amount (mg) of phenol (C}_6\text{H}_6\text{O)} \\ &= W_S \times (A_T/A_S) \times (1/50) \end{aligned}$$

W_S : Amount (mg) of phenol for assay

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Dental Iodine Glycerin

歯科用ヨード・グリセリン

Dental Iodine Glycerin contains not less than 9.0 w/v% and not more than 11.0 w/v% of iodine (I: 126.90), not less than 7.2 w/v% and not more than 8.8 w/v% of potassium iodide (KI: 166.00), and not less than 0.9 w/v% and not more than 1.1 w/v% of zinc sulfate hydrate (ZnSO₄·7H₂O: 287.58).

Method of preparation

Iodine	10 g
Potassium Iodide	8 g
Zinc Sulfate Hydrate	1 g
Glycerin	35 mL
Purified Water	a sufficient quantity
To make 100 mL	

Dissolve and mix the above ingredients.

Description Dental Iodine Glycerin is a dark red-brown liquid, having the odor of iodine.

Identification (1) The colored solution obtained in the Assay (1) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (iodine).

(2) The colored solution obtained in the Assay (2) acquires a red color. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 510 nm and 514 nm (potassium iodide).

(3) Put 1 mL of Dental Iodine Glycerin in a glass-stoppered, test tube, add 10 mL of ethanol (95), and mix. Then add 2 mL of sodium hydroxide TS, add 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color develops (glycerin).

(4) The colored solution obtained in the Assay (3) acquires a red-purple to purple color. Determine the absorption

spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 618 nm and 622 nm (zinc sulfate hydrate).

Assay (1) Iodine—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 0.5 g of iodine for assay and about 0.4 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add exactly 20 mL of a mixture of chloroform and hexane (2:1), shake immediately, and separate the chloroform-hexane layer [use the water layer in (2)]. Filter through a pledget of cotton. Determine the absorbances, A_T and A_S , of the filtrates obtained from the sample solution and standard solution, respectively, at 512 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of iodine for assay

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in (1), pipet 7 mL each of the water layers, and to each add exactly 1 mL of diluted hydrochloric acid (1 in 2), 1 mL of sodium nitrite TS and 10 mL of a mixture of chloroform and hexane (2:1), and shake immediately. Separate the chloroform-hexane layer, and filter through a pledget of cotton. Determine the absorbances, A_T and A_S , of the filtrates obtained from the sample solution and standard solution, respectively, at 512 nm as directed under Ultraviolet-visible Spectrophotometry, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of potassium iodide (KI)} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of potassium iodide for assay

(3) Zinc sulfate Hydrate—Pipet 5 mL of Dental Iodine Glycerin, and add diluted ethanol (3 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. On the other hand, pipet 10 mL of Standard Zinc Stock Solution, add diluted ethanol (3 in 200) to make exactly 1000 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add 10 mL of a mixture of chloroform and hexane (2:1), shake, and allow to stand. Pipet 3 mL each of the water layers, and to each add 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, 2 mL of zincon TS and water to make exactly 25 mL. Determine the absorbances, A_T and A_S , obtained from the sample solution and standard solution, respectively, at 620 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 3 mL of water as the blank.

$$\begin{aligned} &\text{Amount (mg) of zinc sulfate Hydrate (ZnSO}_4\cdot\text{7H}_2\text{O)} \\ &= W_S \times (A_T/A_S) \times 4.397 \end{aligned}$$

W_S : Amount (mg) of zinc in 10 mL of Standard Zinc Stock Solution

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Iodine, Salicylic Acid and Phenol Spirit

ヨード・サリチル酸・フェノール精

Iodine, Salicylic Acid and Phenol Spirit contains not less than 1.08 w/v% and not more than 1.32 w/v% of iodine (I: 126.90), not less than 0.72 w/v% and not more than 0.88 w/v% of potassium iodide (KI: 166.00), not less than 4.5 w/v% and not more than 5.5 w/v% of salicylic acid (C₇H₆O₃: 138.12), not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C₆H₆O: 94.11), and not less than 7.2 w/v% and not more than 8.8 w/v% of benzoic acid (C₇H₆O₂: 122.12).

Method of preparation

Iodine Tincture	200 mL
Salicylic Acid	50 g
Phenol	20 g
Benzoid Acid	80 g
Ethanol for Disinfection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients. It may be prepared with an appropriate quantity of Ethanol and Purified Water in place of Ethanol for Disinfection.

Description Iodine, Salicylic Acid and Phenol Spirit is a dark red-brown liquid, having the odor of phenol.

Identification (1) To a mixture of 1 mL of starch TS and 9 mL of water add 1 drop of Iodine, Salicylic Acid and Phenol Spirit: a dark blue-purple color develops (iodine).

(2) To 1 mL of Iodine, Salicylic Acid and Phenol Spirit add 5 mL of ethanol (95) and water to make 50 mL. To 1 mL of this solution add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 15 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 25 mL of diethyl ether. Wash the diethyl ether extract with two 25-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is developed (phenol).

(4) Shake 1 mL of Iodine, Salicylic Acid and Phenol Spirit with 1 mL of sodium thiosulfate TS, add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 10 mL of diethyl ether, and use the diethyl ether extract as the sample solution. Dissolve 25 mg of salicylic acid, 0.01 g of phenol and 0.04 g of benzoic acid in 5 mL each of diethyl ether, respectively, and use these solutions as the standard solutions (1), (2) and (3). Perform the test with the sample solution and standard solutions (1), (2) and (3) as directed under

Thin-layer Chromatography <2.03>. Spot 5 μL of each solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the 3 spots from the sample solution show the same R_f value as the corresponding spots of the standard solutions (1), (2) and (3). Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution acquires a purple color.

Assay (1) Iodine—Pipet 4 mL of Iodine, Salicylic Acid and Phenol Spirit, add ethanol (95) to make exactly 50 mL, and use this solution as the sample solution. On the other hand, weigh accurately about 1.2 g of iodine for assay and about 0.8 g of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 4 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Pipet 3 mL each of the sample solution and standard solution, to each add exactly 25 mL of a mixture of chloroform and hexane (2:1), and shake. Further add exactly 10 mL of water, shake and separate the chloroform-hexane layers [use the water layers in (2)]. Filter through a pledget of absorbent cotton, and determine the absorbances of the filtrates from the sample solution and standard solution, respectively, A_T and A_S, at 512 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a mixture of chloroform and hexane (2:1) as the blank.

$$\text{Amount (mg) of iodine (I)} = W_S \times (A_T/A_S) \times (1/25)$$

W_S: Amount (mg) of iodine for assay

(2) Potassium iodide—Separate the water layers of the sample solution and standard solution obtained in the Assay (1), pipet 8 mL each of the water layers, and add 1 mL of diluted dilute hydrochloric acid (1 in 2) and 1 mL of sodium nitrite TS. Immediately after shaking, add exactly 10 mL of a mixture of chloroform and hexane (2:1), shake, and proceed in the same manner as for the Assay (1).

$$\begin{aligned} \text{Amount (mg) of potassium iodide (KI)} \\ = W_S \times (A_T/A_S) \times (1/25) \end{aligned}$$

W_S: Amount (mg) of potassium iodide for assay

(3) Salicylic acid, phenol and benzoic acid—Pipet 2 mL of Iodine, Salicylic Acid and Phenol Spirit, add 20 mL of diluted methanol (1 in 2) and 0.1 mol/L sodium thiosulfate VS until the color of iodine disappears, add exactly 20 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 200 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, about 80 mg of phenol for assay, and 0.32 g of benzoic acid, previously dried in a desiccator (silica gel) for 3 hours, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 25 mL of this solution, add exactly 20 mL of the internal standard solution and diluted methanol (1 in 2) to make 200 mL, and use this solution as the standard solution. Perform the test with 3 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the operating conditions in the Assay under Compound Salicylic Acid Spirit. Calculate the ratios, Q_{Ta}, Q_{Tb} and Q_{Tc}, of

the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the sample solution, and the ratios, Q_{Sa} , Q_{Sb} and Q_{Sc} , of the peak areas of salicylic acid, phenol and benzoic acid to those of the internal standard of the standard solution.

$$\begin{aligned} \text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3) \\ = W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/2) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of phenol (C}_6\text{H}_6\text{O)} \\ = W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/2) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of benzoic acid (C}_7\text{H}_6\text{O}_2) \\ = W_{Sc} \times (Q_{Tc}/Q_{Sc}) \times (1/2) \end{aligned}$$

W_{Sa} : Amount (mg) of salicylic acid for assay

W_{Sb} : Amount (mg) of phenol for assay

W_{Sc} : Amount (mg) of benzoic acid

Internal standard solution—A solution of theophylline in methanol (1 in 1000).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Iodoform

ヨードホルム



CHI_3 : 393.73

Triiodomethane [75-47-8]

Iodoform, when dried, contains not less than 99.0% of CHI_3 .

Description Iodoform occurs as lustrous, yellow crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in diethyl ether, sparingly soluble in ethanol (95), and practically insoluble in water.

It is slightly volatile at ordinary temperature.

Melting point: about 120°C (with decomposition).

Identification Heat 0.1 g of Iodoform: a purple gas is evolved.

Purity (1) Water-soluble colored substances and acidity or alkalinity—Shake well 2.0 g of Iodoform, previously powdered, with 5 mL of water for 1 minute, allow to stand, and filter the supernatant liquid: the filtrate is colorless and neutral.

(2) Chloride <1.03>—Shake well 3.0 g of Iodoform, previously powdered, with 75 mL of water for 1 minute, allow to stand, and filter the supernatant liquid. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <1.14>—To 25 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel,

24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Iodoform, previously dried, in a 500-mL glass-stoppered flask, and dissolve it in 20 mL of ethanol (95). Add exactly 30 mL of 0.1 mol/L silver nitrate VS and 10 mL of nitric acid, stopper the flask, shake well, and allow to stand in a dark place over 16 hours. Add 150 mL of water, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 5 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS

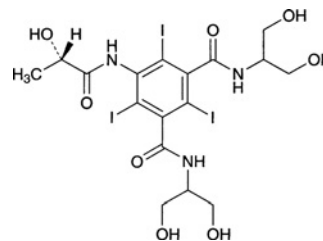
= 13.12 mg of CHI_3

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Iopamidol

イオパミドール



$\text{C}_{17}\text{H}_{22}\text{I}_3\text{N}_3\text{O}_8$: 777.09

N,N'-Bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-[(2*S*)-2-hydroxypropanoylamino]-2,4,6-triiodoisophthalamide [62883-00-5]

Iopamidol, when dried, contains not less than 99.0% of $\text{C}_{17}\text{H}_{22}\text{I}_3\text{N}_3\text{O}_8$.

Description Iopamidol occurs as a white crystalline powder.

It is very soluble in water, sparingly soluble in methanol, and very slightly soluble in ethanol (99.5).

Identification (1) To 0.05 g of Iopamidol add 5 mL of hydrochloric acid, heat for 10 minutes in a water bath: the test solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Heat 0.1 g of Iopamidol over a flame: a purple gas is evolved.

(3) Determine the infrared absorption spectrum of Iopamidol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{436}^{20}$: $-4.6 - -5.2^\circ$ (after drying, 4 g, water, warm, after cooling, 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Iopamidol in 10 mL of water: the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.60 g of

Iopamidol in 8 mL of water, add 1 mL of a solution of sodium nitrite (1 in 50) and 12 mL of 2 mol/L hydrochloric acid TS, shake, and allow to stand for 2 minutes. Add 1 mL of a solution of ammonium amidosulfate (1 in 10), shake well, allow to stand for 1 minute, and add 1 mL of naphthylethylenediamine TS and water to make exactly 50 mL. Determine the absorbance of this solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.12 (not more than 0.020%).

(3) Iodine—Dissolve 2.0 g of Iopamidol in 25 mL of water, add 5 mL of 1 mol/L sulfuric acid and 5 mL of toluene, shake well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5.0 g of Iopamidol, dissolve in 70 mL of water, and adjust the pH to about 4.5 with dilute acetic acid. To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titrate <2.50> with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate VS
= 0.1269 mg of I

Content of iodine ion in Iopamidol is not more than 0.001%.

(5) Heavy metals <1.07>—Moisten 1.0 g of Iopamidol with a small quantity of sulfuric acid, heat gradually to almost incinerate by a possibly lower temperature. After cooling, moisten again with a small quantity of sulfuric acid, heat gradually until white fumes no longer are evolved, and incinerate by ignition between 450 to 550°C. Proceed as directed in Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.10 g of Iopamidol in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetyl-amino-2,4,6-triiodoisophthalamide in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of the both solutions by the automatic integration method: each area of the peaks other than the peak of iopamidol from the sample solution is not larger than the peak area of the standard solution, and the total of these areas is not larger than 2.5 times of the peak area of the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Use water as the mobile phase A, and a mixture of water and methanol (3:1) as the mobile phase B. Change the mixed ratios of the mobile phase A and the mobile phase B stepwise as follows:

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 6	92	8
6 - 18	92 → 65	8 → 35
18 - 30	65 → 8	35 → 92
30 - 34	8	92

Flow rate: Adjust the flow rate to 1.5 mL per minute.

Time span of measurement: About 4.3 times as long as the retention time of iopamidol.

System suitability—

System performance: Dissolve 1 mL of the sample solution and 10 mg of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetyl-amino-2,4,6-triiodoisophthalamide in water to make 100 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetyl-amino-2,4,6-triiodoisophthalamide and iopamidol are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of *N,N'*-bis[2-hydroxy-1-(hydroxymethyl)ethyl]-5-hydroxyacetyl-amino-2,4,6-triiodoisophthalamide is not more than 1.0%.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Iopamidol, previously dried, transfer to a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, boil for 30 minutes under a reflux condenser, cool, and filter. Wash the flask and the filter paper with 50 mL of water, and combine the washing with the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

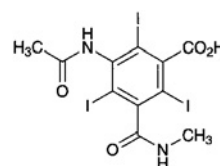
Each mL of 0.1 mol/L silver nitrate VS
= 25.90 mg of C₁₇H₂₂I₃N₃O₈

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Iotalamic Acid

イオタラム酸



C₁₁H₉I₃N₂O₄: 613.91
3-Acetyl-amino-2,4,6-triiodo-5-(methylaminocarbonyl)benzoic acid [2276-90-6]

Iotalamic Acid, when dried, contains not less than 99.0% of $C_{11}H_9I_3N_2O_4$.

Description Iotalamic Acid occurs as a white powder. It is odorless.

It is sparingly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

It gradually colored by light.

Identification (1) Heat 0.1 g of Iotalamic Acid over a flame: a purple gas is evolved.

(2) Determine the infrared spectrum of Iotalamic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Iotalamic Acid in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Primary aromatic amines—To 0.50 g of Iotalamic Acid add 15 mL of water, and dissolve it in 1 mL of sodium hydroxide TS while ice-cooling. Add 4 mL of a solution of sodium nitrite (1 in 100) to the solution, immediately add 12 mL of 1 mol/L hydrochloric acid TS, and shake gently. Then allow the mixture to stand for exactly 2 minutes, add 8 mL of ammonium amidosulfate TS, and shake occasionally for 5 minutes. Add 3 drops of a solution of 1-naphthol in ethanol (95) (1 in 10), allow to stand for 1 minute, add 3.5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, mix, and immediately add water to make 50 mL. Determine within 20 minutes the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner, as the blank: the absorbance is not more than 0.25.

(3) Soluble halides—Dissolve 0.5 g of Iotalamic Acid in 20 mL of diluted ammonia TS (1 in 40), add 6 mL of dilute nitric acid, shake, allow to stand for 5 minutes, and filter. Transfer the filtrate to a Nessler tube, wash the residue with 20 mL of water, combine the filtrate and the washings, and add water to make 50 mL. Proceed as directed for the Chloride Limit Test <1.03> using this solution as the test solution. Prepare the control solution as follows: to 0.10 mL of 0.01 mol/L hydrochloric acid VS and add 20 mL of diluted ammonia TS (1 in 40), 6 mL of dilute nitric acid and water to make 50 mL.

(4) Iodine—Dissolve 0.20 g of Iotalamic Acid in 2.0 mL of sodium hydroxide TS, add 2.5 mL of 0.5 mol/L sulfuric acid TS, and allow to stand for 10 minutes with occasional shaking. Add 5 mL of chloroform, shake well, and allow to stand: the chloroform layer remains colorless.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Iotalamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 0.6 g of Iotalamic Acid according to Method 3, and perform the test (not more than 3.3 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

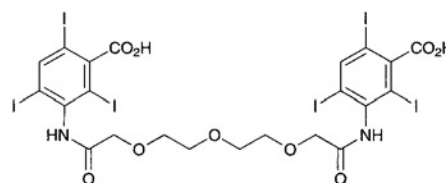
Assay Weigh accurately about 0.4 g of Iotalamic Acid, previously dried, place it in a saponification flask, dissolve in 40 mL of sodium hydroxide TS, add 1 g of zinc powder, and heat for 30 minutes under a reflux condenser. Cool, filter, wash the flask and the filter paper with 50 mL of water, and combine the washings and the filtrate. Add 5 mL of acetic acid (100) to this solution, and titrate <2.50> with 0.1 mol/L silver nitrate VS, until the color of the precipitate changes from yellow to green (indicator: 1 mL of tetrabromophenolphthalein ethyl ester TS).

Each mL of 0.1 mol/L silver nitrate VS
= 20.46 mg of $C_{11}H_9I_3N_2O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Iotroxic Acid

イオトロクス酸



$C_{22}H_{18}I_6N_2O_9$: 1215.81

3,3'-(3,6,9-Trioxaundecanedioyl)diiminobis-(2,4,6-triiodobenzoic acid) [51022-74-3]

Iotroxic Acid contains not less than 98.5% of $C_{22}H_{18}I_6N_2O_9$, calculated on the anhydrous basis.

Description Iotroxic Acid occurs as a white crystalline powder.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is gradually colored by light.

Identification (1) Heat 0.1 g of Iotroxic Acid over a flame: a purple gas evolves.

(2) Dissolve a suitable amount of Iotroxic Acid in a suitable amount of methanol, evaporate the methanol under reduced pressure, and determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Iotroxic Acid in 10 mL of diluted sodium hydroxide TS (1 in 5): the solution is clear and colorless.

(2) Primary aromatic amines—Dissolve 0.20 g of Iotroxic Acid in 5 mL of water and 1 mL of sodium hydroxide TS, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, mix, and allow to stand for 2 minutes. Add 5 mL of ammonium amidosulfate TS, shake well, allow to stand for 1 minute, then add 0.4 mL of a solution of α -naphthol in ethanol (95) (1 in 10), 15 mL of sodium hydroxide TS and water to make exactly 50 mL. Read the absorbance of this solution at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

iolet-visible Spectrophotometry <2.24>, using a blank solution obtained in the same manner as above: the absorbance is not more than 0.22.

(3) Iodine—Dissolve 0.20 g of Iotrox Acid in 2.0 mL of sodium hydrogen carbonate TS, add 5 mL of toluene, mix well, and allow to stand: the toluene layer is colorless.

(4) Free iodine ion—Weigh accurately about 5.0 g of Iotrox Acid, dissolve in 12 mL of a solution of meglumine (3 in 20), add water to make 70 mL, and adjust the pH to about 4.5 with acetic acid (100). To this solution add 2 mL of 0.1 mol/L sodium chloride TS, and titrate <2.50> with 0.001 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.001 mol/L silver nitrate
= 0.1269 mg of I

Content of iodine ion in Iotrox Acid, calculated on the anhydrous basis, is not more than 0.004%.

(5) Heavy metals <1.07>—Heat strongly 1.0 g of Iotrox Acid as directed under Residue on Ignition Test, then proceed according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Related substances—Dissolve 0.15 g of Iotrox Acid in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of toluene, acetone and formic acid (6:4:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 1.0 – 2.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

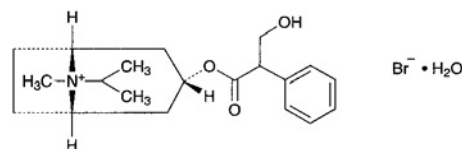
Assay Weigh accurately about 0.5 g of Iotrox Acid, dissolve in 40 mL of sodium hydroxide TS in a saponification flask, add 1 g of zinc powder, and boil for 30 minutes under a reflux condenser. After cooling, filter, wash the flask and the filter paper with 50 mL of water, and combine the washings to the filtrate. To this solution add 5 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 20.26 mg of $C_{22}H_{18}I_6N_2O_9$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ipratropium Bromide Hydrate

イプラトロピウム臭化物水和物



$C_{20}H_{30}BrNO_3 \cdot H_2O$: 430.38
(1*R*,3*r*,5*S*)-3-[(2*RS*)-3-Hydroxy-2-phenylpropanoate]-
8-methyl-8-(1-methylethyl)-8-azoniabicyclo[3.2.1]octane
bromide monohydrate [66985-17-9]

Ipratropium Bromide Hydrate, when dried, contains not less than 99.0% of ipratropium bromide ($C_{20}H_{30}BrNO_3$: 412.36).

Description Ipratropium Bromide Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, soluble in ethanol (99.5), slightly soluble in acetonitrile and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Ipratropium Bromide Hydrate (1 in 20) is between 5.0 and 7.5.

Melting point: about 223°C (with decomposition, after drying).

Identification (1) To 5 mg of Ipratropium Bromide Hydrate add 0.5 mL of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 5 mL of acetone, and add 2 drops of potassium hydroxide-ethanol TS: a purple color develops.

(2) Determine the absorption spectrum of a solution of Ipratropium Bromide Hydrate in 0.01 mol/L hydrochloric acid TS (3 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ipratropium Bromide Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Ipratropium Bromide Hydrate (1 in 100) responds to the Qualitative Tests <1.09> for bromide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ipratropium Bromide Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Ipratropium Bromide Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Ipratropium Bromide Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 2.0 g of Ipratropium Bromide Hydrate according to Method 3,

and perform the test. Use a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 1 ppm).

(5) Isopropylatropine bromide—Dissolve 25 mg of Ipratropium Bromide Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Perform the test with 25 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area, A_a , of ipratropium and the peak area, A_b , having a relative retention time to ipratropium about 1.3 by the automatic integration method: $A_b/(A_a + A_b)$ is not more than 0.01, and no peak other than the peak of ipratropium and the peak having a relative retention time to ipratropium about 1.3 appears within about 14 minutes of the retention time after the solvent peak.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and 10 to 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200), acetonitrile and methanesulfonic acid (1000:120:1).

Flow rate: Adjust the flow rate so that the retention time of ipratropium is about 7 minutes.

Selection of column: Heat a solution of Ipratropium Bromide in 1 mol/L hydrochloric acid TS (1 in 100) at 100°C for 1 hour, and cool. To 2.5 mL of this solution add the mobile phase to make 100 mL. Proceed with 25 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column showing a resolution not less than 3 between the peak of ipratropium and the peak having a relative retention time to ipratropium about 0.6.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ipratropium obtained from 25 μ L of the sample solution composes 50 to 80% of the full scale.

(6) Apo-compounds—Dissolve 0.14 g of Ipratropium Bromide in 0.01 mol/L hydrochloric acid TS to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_1 and A_2 , at 246 nm and 263 nm, respectively: A_1/A_2 is not more than 0.91.

Loss on drying <2.41> 3.9 – 4.4% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

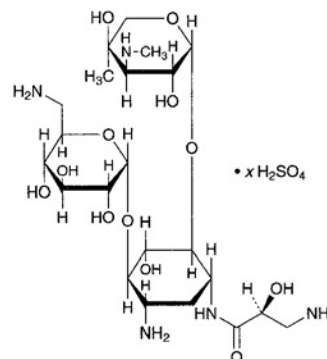
Assay Weigh accurately about 0.3 g of Ipratropium Bromide Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of 1,4-dioxane and 2.5 mL of bismuth nitrate TS, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.24 mg of $C_{20}H_{30}BrNO_3$

Containers and storage Containers—Tight containers.

Isepamicin Sulfate

イセパマイシン硫酸塩



$C_{22}H_{43}N_5O_{12} \cdot xH_2SO_4$

6-Amino-6-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-[3-deoxy-4-C-methyl-3-methylamino- β -L-arabinopyranosyl-(1 \rightarrow 6)]-2-deoxy-1-N-[(2S)-3-amino-2-hydroxypropanoyl]-D-streptamine sulfate [67814-76-0]

Isepamicin Sulfate is the sulfate of a derivative of gentamicin B, an aminoglycoside substance, having antibacterial activity produced by the growth of *Micromonospora purpurea*.

It contains not less than 680 μ g (potency) and not more than 780 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Isepamicin Sulfate is expressed as mass (potency) of isepamicin ($C_{22}H_{43}N_5O_{12}$: 569.60).

Description Isepamicin Sulfate occurs as a white to pale yellowish white powder.

It is very soluble in water, and practically insoluble in methanol and in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 0.02 g of Isepamicin Sulfate in 1 mL of water, add 3 mL of anthrone TS, shake, and allow to stand: a blue-purple color develops.

(2) Dissolve 10 mg each of Isepamicin Sulfate and Isepamicin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia water (28), ethanol (99.5), 1-butanol and chloroform (5:5:4:2) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at about 100°C for about 10 minutes: the principal spots from the sample solution and the standard solution exhibit a red-brown color and show the same R_f value.

(3) Dissolve 0.01 g of Isepamicin Sulfate in 1 mL of water, and add 1 drop of barium chloride TS: a white precipitate is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +100 – +120° (0.25 g calculated on the anhydrous bases, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of Isepamicin Sulfate in 5 mL of water: the pH of the solution is between 5.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Isepamicin Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Isepamicin Sulfate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Perform the test with 5 μ L of the sample solution obtained in the Assay as directed under Assay. Determine each peak area of the sample solution by the automatic integration method, and calculate the amounts of their peaks by the area percentage method: the amount of HAPA-gentamicin-B equivalent to about 0.4 of the relative retention time to isepamicin is not more than 5.0%, and gentamicin B equivalent to about 1.3 of that is not more than 3.0%. Correct the peak area of gentamicin B by multiplying the sensitivity coefficient, 1.11.

Operating conditions—

Apparatus, detector, column, column temperature, reaction coil, mobile phase, reagent, reaction temperature, flow rate of the mobile phase, and flow rate of the reagent: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of isepamicin.

System suitability—

Test for required detection: Pipet 1 mL of the standard solution, add water to make exactly 10 mL, and use this solution as the solution for the test for required detection. Pipet 1 mL of the solution, and add water to make exactly 10 mL. Confirm that the peak area of isepamicin obtained from 5 μ L of this solution is equivalent to 7 to 13% of that obtained from 5 μ L of the solution for the test for required detection.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 12.0% (0.2 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (2:1) instead of methanol for water determination).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Weigh accurately an amount of Isepamicin Sulfate and Isepamicin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve each in water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of isepamicin of the solutions.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of isepamicin (C}_{22}\text{H}_{43}\text{N}_5\text{O}_{12}) \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Isepamicin Sulfate Reference Standard

Operating conditions—

Apparatus: Consist of two pumps for the mobile phase and the reagent transport, inject port, column, reaction coil, detector and recorder. Use a reaction coil with thermostat.

Detector: Fluorometry (excitation wavelength: 360 nm, de-

tection wavelength: 440 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Reaction coil: A column 0.25 μ m in inside diameter and 5 m in length.

Mobile phase: Dissolve 28.41 g of anhydrous sodium sulfate and 5.23 g of sodium 1-pentane sulfonate in 900 mL of water, add 1 mL of acetic acid (100), and add water to make exactly 1000 mL.

Reagent: To 500 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, add 5 mL of a solution of *o*-phthalaldehyde in ethanol (95) (2 in 25), 1 mL of 2-mercaptoethanol and 2 mL of a solution of lauromacrogol (1 in 4).

Reaction temperature: A constant temperature of about 45°C.

Flow rate of the mobile phase: About 0.6 mL per minute.

Flow rate of the reagent: About 0.5 mL per minute.

System suitability—

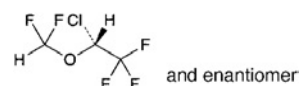
System performance: Dissolve 2 mg of Gentamicin B in 10 mL of the standard solution. When the procedure is run with 5 μ L of this solution under the above operating conditions, isepamicin and gentamicin B are eluted in this order with the resolution between these peaks being not less than 1.0.

System repeatability: When the test is repeated 5 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of isepamicin is not more than 3.0%.

Containers and storage Containers—Tight containers.

Isoflurane

イソフルラン



$\text{C}_3\text{H}_2\text{ClF}_5\text{O}$: 184.49

(2*RS*)-2-Chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane
[26675-46-7]

Isoflurane contains not less than 99.0% and not more than 101.0% of $\text{C}_3\text{H}_2\text{ClF}_5\text{O}$, calculated on the anhydrous basis.

Description Isoflurane occurs as a clear, colorless fluid liquid.

It is miscible with ethanol (99.5), with methanol and with *o*-xylene.

It is slightly soluble in water.

It is volatile, and has no inflammability.

It shows no optical rotation.

Refractive index n_D^{20} : about 1.30

Boiling point: about 47 – 50°C

Identification (1) The test solution obtained by the Oxygen Flask Combustion Method <1.06> with 50 μ L of Isoflurane, using 40 mL of water as the absorbing liquid, responds

to the Qualitative Tests <1.09> for chloride and fluoride.

(2) Determine the infrared absorption spectrum of Isoflurane as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Isoflurane Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Specific gravity <2.56> d_{20}^{20} : 1.500 – 1.520

Purity (1) Acidity or alkalinity—To 10 mL of Isoflurane add 5 mL of freshly boiled and cooled water, and shake for 1 minute: the water layer is neutral.

(2) Soluble chloride—To 60 g of Isoflurane add 40 mL of water, shake thoroughly, and separate the water layer. To 20 mL of the layer add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test with this solution as directed under Chloride Limit Test <1.03>. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 3 ppm).

(3) Soluble fluoride—To 6 g of Isoflurane add 12 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), and shake for 10 minutes. Transfer 4.0 mL of the water layer into a Nessler tube, add 30 mL of a mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), add water to make 50 mL, allow to stand for 60 minutes, and use this solution as the sample solution. Separately, to 0.4 mL of the fluorine standard solution and 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20) in a Nessler tube add 30 mL of the mixture of alizarin complexone TS, acetic acid-potassium acetate buffer solution, pH 4.3 and cerium (III) nitrate TS (1:1:1), then proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Determine the absorbances of the sample solution and standard solution at 600 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, obtained by proceeding in the same manner as above with 4.0 mL of diluted 0.01 mol/L sodium hydroxide TS (1 in 20), as the blank: the absorbance of the sample solution is not more than that of the standard solution (not more than 2 ppm).

Fluorine standard solution: Dissolve exactly 2.21 g of sodium fluoride in water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 1000 mL. Each mL of this solution contains 0.01 mg of fluorine (F).

(4) Peroxide—To 10 mL of Isoflurane add 1 mL of a freshly prepared solution of potassium iodide (1 in 10), shake vigorously, and allow to stand in a dark place for 1 hour: the water layer is not yellow.

(5) Related substances—Use Isoflurane as the sample solution. To exactly 1 mL of the sample solution add *o*-xylene to make exactly 100 mL. Pipet 1 mL of this solution, add *o*-xylene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than isoflurane is not more than the peak area of isoflurane from the standard solution, and the total area of the peaks other than isoflurane is not more than 3 times the peak area of isoflurane from the standard solution.

Operating conditions—

Detector, column, column temperature, carrier gas, and

flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 5 times as long as the retention time of isoflurane after injection of the sample solution.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add *o*-xylene to make exactly 2 mL. Confirm that the peak area of isoflurane obtained with 5 μ L of this solution is equivalent to 35 to 65% of that with 5 μ L of the standard solution.

System performance and system repeatability: Proceed as directed in the system suitability in the Assay.

(6) Residue on evaporation Pipet 65 mL of Isoflurane, evaporate on a water bath, and dry the residue at 105°C for 1 hour: not more than 1.0 mg.

Water <2.48> Not more than 0.1% (2 g, Courometric titration).

Assay To exactly 5 mL each of Isoflurane and Isoflurane Reference Standard (separately determined water content <2.48> in the same manner as Isoflurane), add exactly 3 mL of ethyl acetate as the internal standard, then add *o*-xylene to make exactly 50 mL each. To 5 mL each of these solutions add *o*-xylene to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of isoflurane to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of isoflurane (C}_3\text{H}_2\text{ClF}_5\text{O) in 5 mL of} \\ &\text{Isoflurane} \\ &= V_S \times (Q_T/Q_S) \times 1000 \times 1.506 \end{aligned}$$

V_S : Amount (mL) of Isoflurane Reference Standard, calculated on the anhydrous basis

1.506: Specific gravity (d_{20}^{20}) of isoflurane

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A stainless steel column 3 mm in inside diameter and 3.5 m in length, packed with siliceous earth for gas chromatography (125 – 149 μ m in particle diameter), coated in 10% with nonylphenoxypoly(ethyleneoxy)ethanol for gas chromatography and in 15% with polyalkylene glycol for gas chromatography.

Column temperature: A constant temperature of about 80°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of isoflurane is about 7 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, isoflurane and the internal standard are eluted in this order with the resolution between these peaks being not less than 3

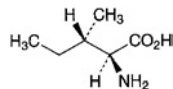
System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoflurane is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At a temperature not exceeding 30°C.

L-Isoleucine

L-イソロイシン



$C_6H_{13}NO_2$: 131.17
(2*S*,3*S*)-2-Amino-3-methylpentanoic acid [73-32-5]

L-Isoleucine, when dried, contains not less than 98.5% of $C_6H_{13}NO_2$.

Description L-Isoleucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Isoleucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +39.5 – +41.5° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Isoleucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Isoleucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Isoleucine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Isoleucine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Perform the test with 0.25 g of L-Isoleucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Isoleucine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Isoleucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Isoleucine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed un-

der Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

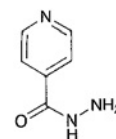
Assay Weigh accurately about 0.13 g of L-Isoleucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.12 mg of $C_6H_{13}NO_2$

Containers and storage Containers—Tight containers.

Isoniazid

イソニアジド



$C_6H_7N_3O$: 137.14
Pyridine-4-carbohydrazide [54-85-3]

Isoniazid, when dried, contains not less than 98.5% of $C_6H_7N_3O$.

Description Isoniazid occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

Identification (1) Dissolve about 20 mg of Isoniazid in water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Isoniazid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Isoniazid in 10 mL of freshly boiled and cooled water: the pH of this solution is between 6.5 and 7.5.

Melting point <2.60> 170 – 173°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of isoniazid in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Isoniazid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of Isoniazid according to Method 3, and perform the test. In this case, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite the ethanol to burn (not more than 5 ppm).

(4) Hydrazine—Dissolve 0.10 g of isoniazid in 5 mL of water, add 0.1 mL of a solution of salicylaldehyde in ethanol (95) (1 in 20), shake immediately, and allow to stand for 5 minutes: no turbidity is produced.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of isoniazid, previously dried, dissolve in 50 mL of acetic acid (100) and 10 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 13.71 \text{ mg of } C_6H_7N_3O \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Isoniazid Injection

イソニアジド注射液

Isoniazid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Injections, with Isoniazid.

Description Isoniazid Injection occurs as a clear, colorless liquid.

pH: 6.5 – 7.5.

Identification To a volume of Isoniazid Injection, equivalent to 20 mg of Isoniazid according to the labeled amount, and add water to make 200 mL. To 5 mL of the solution add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Isoniazid Injec-

tion, equivalent to about 50 mg of isoniazid ($C_6H_7N_3O$), add water to make exactly 100 mL. Pipet 5 mL of the solution, add exactly 5 mL of the internal standard and the mobile phase to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard and the mobile phase to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of isoniazid to that of the internal standard.

$$\text{Amount (mg) of isoniazid } (C_6H_7N_3O) = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of isoniazid for assay

Internal standard solution—A solution of propyl parahydroxybenzoate (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to make a solution having pH 2.5. To 500 mL of this solution add 500 mL of methanol, and add 2.86 g of sodium tridecanesulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, isoniazid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of isoniazid to that of the internal standard is not more than 1.3%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Isoniazid Tablets

イソニアジド錠

Isoniazid Tablets contain not less than 95% and not more than 105% of the labeled amount of isoniazid ($C_6H_7N_3O$: 137.14).

Method of preparation Prepare as directed under Tablets,

with Isoniazid.

Identification Take a quantity of powdered Isoniazid Tablets, equivalent to 0.02 g of Isoniazid according to the labeled amount, add 200 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 264 nm and 268 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Isoniazid Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 20 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μm . Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, then pipet 5 mL of this solution, add water to make exactly 50 mL, and then pipet 5 mL of this solution, add water to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 267 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Isoniazid Tablets in 20 minutes is not less than 75%.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ with respect to the labeled} \\ & \text{amount of isoniazid (C}_6\text{H}_7\text{N}_3\text{O)} \\ & = W_S \times (A_T/A_S) \times (90/C) \end{aligned}$$

W_S : Amount (mg) of isoniazid for assay.

C : Labeled amount (mg) of isoniazid (C₆H₇N₃O) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Isoniazid Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.1 g of isoniazid (C₆H₇N₃O), add 150 mL of water, shake for 30 minutes, then add water to make exactly 200 mL, and filter. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of isoniazid for assay, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of isoniazid of the sample solution and standard solution.

$$\begin{aligned} & \text{Amount (mg) of isoniazid (C}_6\text{H}_7\text{N}_3\text{O)} \\ & = W_S \times (A_T/A_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of isoniazid for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.80 g of potassium dihydrogen phosphate in water to make 1000 mL. Separately, to 5.76 g of phosphoric acid add water to make 1000 mL. Mix these solutions to adjust the pH to 2.5. To 400 mL of this solution add 600 mL of methanol, and dissolve 2.86 g of sodium tridecanesulfonate in this.

Flow rate: Adjust the flow rate so that the retention time of isoniazid is about 5 minutes.

System suitability—

System performance: Dissolve 5 mg of Isoniazid and 5 mg of isonicotinic acid in 100 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, isonicotinic acid and isoniazid are eluted in this order with the resolution between these peaks being not less than 1.5.

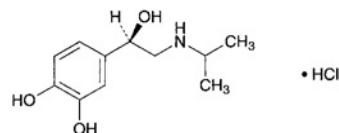
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of isoniazid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

l-Isoprenaline Hydrochloride

l-イソプレナリン塩酸塩



C₁₁H₁₇NO₃·HCl: 247.72

4-[(1*R*)-1-Hydroxy-2-[(1-methylethyl)amino]ethyl] benzene-1,2-diol monohydrochloride [51-30-9]

l-Isoprenaline Hydrochloride, when dried, contains not less than 98.0% of C₁₁H₁₇NO₃·HCl.

Description *l*-Isoprenaline Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95), and practically insoluble in acetic acid (100), in acetic anhydride, in diethyl ether and in chloroform.

It gradually changes in color by air and by light.

Identification (1) Dissolve 0.01 g of *l*-Isoprenaline Hydrochloride in 5 mL of water, and add 1 drop of iron (III) chloride TS: a deep green color develops, and changes through yellow-green to brown on standing.

(2) Dissolve 1 mg each of *l*-Isoprenaline Hydrochloride in 1 mL of water in the test tubes A and B. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5 to A, and add 10 mL of phosphate buffer solution, pH 6.5 to B. To each of the test tubes add 1 mL of iodine TS, allow to stand for 5 minutes, and add 2 mL each of sodium thiosulfate TS: a red color develops in the test tube A, and a deep red color de-

velops in the test tube B.

(3) Dissolve 0.01 g of *l*-Isoprenaline Hydrochloride in 1 mL of water, and add 1 mL of phosphotungstic acid TS: a light brown precipitate is produced.

(4) Determine the absorption spectrum of a solution of *l*-Isoprenaline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) A solution of *l*-Isoprenaline Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-36 - -41^\circ$ (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.01 g of *l*-Isoprenaline Hydrochloride in 10 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of *l*-Isoprenaline Hydrochloride in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.10 g of *l*-Isoprenaline Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of *l*-Isoprenaline Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Isoproterenone—Dissolve 50 mg of *l*-Isoprenaline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.040.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of *l*-Isoprenaline Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic acid (100) and acetic anhydride (3:2) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

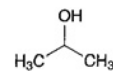
Each mL of 0.1 mol/L perchloric acid VS
= 24.77 mg of $C_{11}H_{17}NO_3 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Isopropanol

Isopropyl Alcohol

イソプロパノール



C_3H_8O : 60.10

Propan-2-ol [67-63-0]

Description Isopropanol is a clear, colorless liquid. It has a characteristic odor.

It is miscible with water, with methanol, with ethanol (95), and with diethyl ether.

It is flammable and volatile.

Identification (1) To 1 mL of Isopropanol add 2 mL of iodine TS and 2 mL of sodium hydroxide TS, and shake: a light yellow precipitate is formed.

(2) To 5 mL of Isopropanol add 20 mL of potassium dichromate and 5 mL of sulfuric acid with caution, and warm gently on a water bath: the produced gas has the odor of acetone, and the gas turns the filter paper, previously wetted with a solution of salicylaldehyde in ethanol (95) (1 in 10) and with a solution of sodium hydroxide (3 in 10), to red-brown.

Specific gravity <2.56> d_{20}^{20} : 0.785 – 0.788

Purity (1) Clarity of solution—To 2.0 mL of Isopropanol add 8 mL of water, and shake: the solution is clear.

(2) Acidity—To 15.0 mL of Isopropanol add 50 mL of freshly boiled and cooled water and 2 drops of phenolphthalein TS, and add 0.40 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Residue on evaporation—Evaporate 20.0 mL of Isopropanol on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Water <2.48> Not more than 0.75 w/v% (2 mL, direct titration).

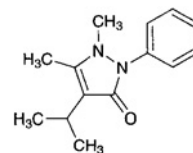
Distilling range <2.57> 81 – 83°C, not less than 94 vol%.

Containers and storage Containers—Tight containers.
Storage—Remote from fire.

Isopropylantipyrene

Propyphenazone

イソプロピルアンチピリン



$C_{14}H_{18}N_2O$: 230.31

1,5-Dimethyl-4-(1-methylethyl)-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one [479-92-5]

Isopropylantipyrine, when dried, contains not less than 98.0% of $C_{14}H_{18}N_2O$.

Description Isopropylantipyrine occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in acetone, soluble in diethyl ether, and slightly soluble in water.

Identification (1) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 1 drop of iron (III) chloride TS: a light red color develops. Further add 3 drops of sulfuric acid to this solution: the color changes to pale yellow.

(2) Add 5 mL of a solution of Isopropylantipyrine (1 in 500) to a mixture of 5 mL of potassium hexacyanoferrate (III) TS and 1 to 2 drops of iron (III) chloride TS: a dark green color gradually develops.

(3) To 2 mL of a solution of Isopropylantipyrine (1 in 500) add 2 to 3 drops of tannic acid TS: a white precipitate is produced.

Melting point <2.60> 103 – 105°C

Purity (1) Chloride <1.03> —Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid, 30 mL of dilute ethanol and water to make 50 mL (not more than 0.014%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Isopropylantipyrine in 30 mL of dilute ethanol, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 1 mL of dilute hydrochloric acid and 30 mL of dilute ethanol, and dilute with water to make 50 mL (not more than 0.019%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Isopropylantipyrine in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 25 mL of acetone, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Isopropylantipyrine according to Method 3, and perform the test (not more than 2 ppm).

(5) Antipyrine—Dissolve 1.0 g of Isopropylantipyrine in 10 mL of dilute ethanol, and add 1 mL of sodium nitrite TS and 1 mL of dilute sulfuric acid: no green color develops.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Isopropylantipyrine, previously dried, dissolve in 60 mL of a mixture of acetic acid (100) and acetic anhydride (2:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination.

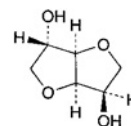
Each mL of 0.1 mol/L perchloric acid VS

= 23.03 mg of $C_{14}H_{18}N_2O$

Containers and storage Containers—Tight containers.

Isosorbide

イソソルビド



$C_6H_{10}O_4$: 146.14

1,4:3,6-Dianhydro-D-glucitol [652-67-5]

Isosorbide contains not less than 98.5% of $C_6H_{10}O_4$, calculated on the anhydrous basis.

Description Isosorbide occurs as white crystals or masses. It is odorless, or has a faint, characteristic odor, and has a bitter taste.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

It is hygroscopic.

Identification (1) To 0.1 g of Isosorbide add 6 mL of diluted sulfuric acid (1 in 2), and dissolve by heating in a water bath. After cooling, shake well with 1 mL of a solution of potassium permanganate (1 in 30), and heat in a water bath until the color of potassium permanganate disappears. To this solution add 10 mL of 2,4-dinitrophenylhydrazine TS, and heat in a water bath: an orange precipitate is formed.

(2) To 2 g of Isosorbide add 30 mL of pyridine and 4 mL of benzoyl chloride, boil under a reflux condenser for 50 minutes, cool, and pour gradually the solution into 100 mL of cold water. Filter the formed precipitate by suction through a glass filter (G3), wash with water, recrystallize twice from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: it melts <2.60> between 102°C and 103°C.

(3) Determine the infrared absorption spectrum of Isosorbide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +45.0 – +46.0° (5 g, calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Take 25 g of Isosorbide in a Nessler tube, and dissolve in 50 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 3.0 mL of Ferric Chloride Stock CS and 2.0 mL of Cupric Sulfate Stock CS add water to make 10.0 mL. To 3.0 mL of this solution add water to make 50 mL.

(2) Sulfate <1.14>—Perform the test with 2.0 g of Isosorbide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(3) Heavy metals <1.07>—Proceed with 5.0 g of Isosorbide according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution

(not more than 5 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Isosorbide according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Isosorbide in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95) and cyclohexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of ethanol (95) and sulfuric acid (9:1) on the plate, and heat at 150°C for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 1.5% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

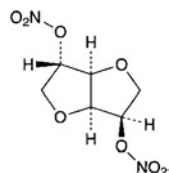
Assay Weigh accurately about 10 g of Isosorbide, calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL. Determine the optical rotation <2.49>, α_D , of this solution at $20 \pm 1^\circ\text{C}$ in a 100-mm cell.

$$\text{Amount (g) of } \text{C}_6\text{H}_{10}\text{O}_4 = \alpha_D \times 2.1978$$

Containers and storage Containers—Tight containers.

Isosorbide Dinitrate

硝酸イソソルビド



$\text{C}_6\text{H}_8\text{N}_2\text{O}_8$: 236.14

1,4:3,6-Dianhydro-D-glucitol dinitrate [87-33-2]

Isosorbide Dinitrate contains not less than 95.0% of $\text{C}_6\text{H}_8\text{N}_2\text{O}_8$, calculated on the anhydrous basis.

Description Isosorbide Dinitrate occurs as white crystals or crystalline powder. It is odorless or has a faint odor like that of nitric acid.

It is very soluble in *N,N*-dimethylformamide and in acetone, freely soluble in chloroform and in toluene, soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

It explodes if heated quickly or subjected to percussion.

Identification (1) Dissolve 0.01 g of Isosorbide Dinitrate in 1 mL of water, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes: a brown ring is produced at the zone of contact.

(2) Dissolve 0.1 g of Isosorbide Dinitrate in 6 mL of

diluted sulfuric acid (1 in 2) by heating in a water bath. After cooling, add 1 mL of a solution of potassium permanganate (1 in 30), stir well, and heat in a water bath until the color of potassium permanganate disappears. Add 10 mL of 2,4-dinitro-phenylhydrazine TS, and heat in a water bath: an orange precipitate is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +134 – +139° (1 g, calculated on the anhydrous basis, ethanol (95), 100 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Isosorbide Dinitrate in 10 mL of acetone: the solution is clear and colorless.

(2) Sulfate <1.14>—Dissolve 1.5 g of Isosorbide Dinitrate in 15 mL of *N,N*-dimethylformamide, add 60 mL of water, cool, and filter. Wash the filter paper with three 20-mL portions of water, combine the washings with the filtrate, and add water to make 150 mL. To 40 mL of this solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Nitrate—Dissolve 0.05 g of Isosorbide Dinitrate in 30 mL of toluene, and extract with three 20-mL portions of water. Combine the aqueous layers, and wash with two 20-mL portions of toluene. To the aqueous layer add water to make 100 mL, and use this solution as the sample solution. Pipet 5.0 mL of Standard Nitric Acid Solution and 25 mL of the sample solution in each Nessler tube, and add water to make 50 mL, respectively. To each of them add 0.06 g of Griss-Romijn's nitric acid reagent, stir well, allow to stand for 30 minutes, and observe from the side of the Nessler tube: the sample solution has no more color than the standard solution.

(4) Heavy metals <1.07>—Dissolve 1.0 g of Isosorbide Dinitrate in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

Water <2.48> Not more than 1.5% (0.3 g, direct titration).

Assay Weigh accurately about 0.1 g of Isosorbide Dinitrate in a Kjeldahl flask as described under the Nitrogen Determination <1.08>, dissolve in 10 mL of methanol, add 3 g of Devarda's alloy and 50 mL of water, and connect the flask with the distillation apparatus as described under the Nitrogen Determination <1.08>. Measure exactly 25 mL of 0.05 mol/L sulfuric acid VS in an absorption flask, add 5 drops of bromocresol green-methyl red TS, and immerse the lower end of the condenser tube in it. Add 15 mL of a solution of sodium hydroxide (1 in 2) through the funnel, cautiously rinse the funnel with 20 mL of water, immediately close the clamp attached to the rubber tubing, then begin the distillation with steam gradually, and continue the distillation until the distillate measures 100 mL. Remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate <2.50> the distillate and the rinsings with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red through light red-purple to light blue-green. Perform a blank determination.

Each mL of 0.05 mol/L sulfuric acid VS

= 11.81 mg of $C_6H_8N_2O_8$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and in a cold place.

Isosorbide Dinitrate Tablets

硝酸イソソルビド錠

Isosorbide Dinitrate Tablets contain not less than 93% and not more than 107% of the labeled amount of isosorbide dinitrate ($C_6H_8N_2O_8$; 236.14).

Method of preparation Prepare as directed under Tablets, with Isosorbide Dinitrate.

Identification Weigh a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 0.1 g of Isosorbide Dinitrate according to the labeled amount, add 50 mL of diethyl ether, shake well, and filter. Measure 5 mL of the filtrate, evaporate to dryness cautiously, add 1 mL of water to the residue, and dissolve by adding 2 mL of sulfuric acid cautiously. After cooling, superimpose 3 mL of iron (II) sulfate TS, and allow to stand for 5 to 10 minutes: a brown ring is produced at the zone of contact.

Purity Free nitrate ion—Weigh accurately a quantity of powdered Isosorbide Dinitrate Tablets, equivalent to 0.05 g of Isosorbide Dinitrate according to the labeled amount, transfer to a separator, add 30 mL of toluene, shake thoroughly, extract with three 20-mL portions of water, and proceed as directed in Purity (3) under Isosorbide Dinitrate.

Disintegration <6.09> It meets the requirement.

For sublingual tablets, the time limit of the test is 2 minutes, and omit the use of the disk.

Assay Weigh accurately and powder not less than 20 Isosorbide Dinitrate Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of isosorbide dinitrate ($C_6H_8N_2O_8$), add exactly 50 mL of acetic acid (100), shake for 15 minutes, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Measure exactly 10 mL of this solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and standard solution, add exactly 2.5 mL of salicylic acid TS to each, shake well, allow to stand for 15 minutes, and add 10 mL of water. Make them alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in an ice bath, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of glacial acetic in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution at 412 nm, respectively.

$$\begin{aligned} \text{Amount (mg) of isosorbide dinitrate (C}_6\text{H}_8\text{N}_2\text{O}_8\text{)} \\ = W_S \times (A_T/A_S) \times (1/20) \times 1.1678 \end{aligned}$$

W_S : Amount (mg) of potassium nitrate

Containers and storage Containers—Tight containers.

Japanese Encephalitis Vaccine

日本脳炎ワクチン

Japanese Encephalitis Vaccine is a liquid for injection containing inactivated Japanese encephalitis virus.

It conforms to the requirements of Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid.

Freeze-dried Japanese Encephalitis Vaccine

乾燥日本脳炎ワクチン

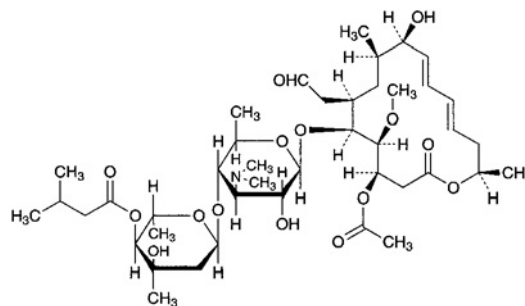
Freeze-dried Japanese Encephalitis Vaccine is a preparation for injection which is dissolved before use. It contains inactivated Japanese encephalitis virus.

It conforms to the requirements of Freeze-dried Japanese Encephalitis Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Japanese Encephalitis Vaccine is a clear or a slightly whitish turbid and colorless liquid on addition of solvent.

Josamycin

ジヨサマイシン



$C_{42}H_{69}NO_{15}$: 827.99
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide [16846-24-5]

Josamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces narboensis* var. *josamyceticus*.

It contains not less than 900 μ g (potency) and not more than 1100 μ g (potency) per mg, calculated on the

dried basis. The potency of Josamycin is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$).

Description Josamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Josamycin in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin and Josamycin Reference Standard in 1 mL of methanol, add diluted methanol (1 in 2) to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the main peak obtained from the sample solution is the same as that of the peak of josamycin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Josamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin in 5 mL of methanol, add diluted methanol (1 in 2) to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of josamycin and the related substances by the area percentage method: the amounts of the peaks other than josamycin are not more than 6%, and the total of these peaks is not more than 20%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 5 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C

Mobile phase: Dissolve 119 g of sodium perchlorate monohydrate in water to make 1000 mL, and adjust the pH to 2.5 with 1 mol/L hydrochloric acid TS. To 600 mL of this solution add 400 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin is about 10 minutes.

Time span of measurement: About 4 times as long as the retention time of josamycin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 3 mL of the sample solution, add diluted methanol (1 in 2) to make

exactly 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add diluted methanol (1 in 2) to make exactly 20 mL. Confirm that the peak area of josamycin obtained from 10 μ L of this solution is equivalent to 8 to 12% of that from 10 μ L of the solution for system suitability test.

System performance: Dissolve about 0.05 g of Josamycin in 50 mL of 0.1 mol/L potassium dihydrogen phosphate TS, pH 2.0, and allow to stand at 40°C for 3 hours. Adjust the pH of this solution to 6.8 to 7.2 with 2 mol/L sodium hydroxide TS, and add 50 mL of methanol. This solution contains both josamycin and josamycin S1. When the procedure is run with 10 μ L of this solution under the above operating conditions, the resolution between the peaks of josamycin S1, which relative retention time to josamycin is about 0.9, and josamycin is not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of josamycin is not more than 1.5%.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Josamycin Reference Standard, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add water to make solutions so that each mL contains 30 μ g (potency) and 7.5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

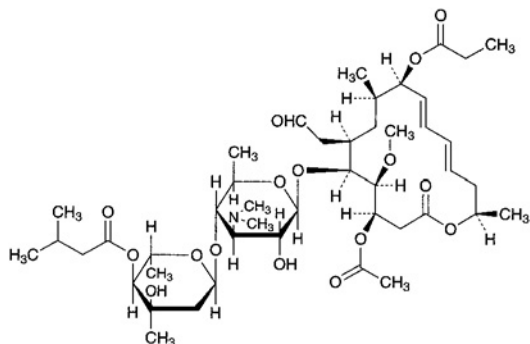
(iv) Sample solutions—Weigh accurately an amount of Josamycin, equivalent to about 30 mg (potency), dissolve in 5 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of this solution, add water to make solutions so that each mL contains 30 μ g (potency) and 7.5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Josamycin Propionate

ジヨサマイシンプロピオン酸エステル



$C_{45}H_{73}NO_{16}$: 884.06

(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[2,6-dideoxy-4-*O*-(3-methylbutanoyl)-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-9-propanoyloxyhexadeca-10,12-dien-15-olide [16846-24-5, Josamycin]

Josamycin Propionate is a derivative of josamycin.

It contains not less than 843 μ g (potency) and not more than 1000 μ g (potency) per mg, calculated on the dried basis. The potency of Josamycin Propionate is expressed as mass (potency) of josamycin ($C_{42}H_{69}NO_{15}$: 827.99).

Description Josamycin Propionate occurs as a white to light yellowish white crystalline powder.

It is very soluble in acetonitrile, freely soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Josamycin Propionate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Josamycin Propionate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Josamycin Propionate and Josamycin Propionate Reference Standard in 50 mL of diluted acetonitrile (1 in 2), and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the peak of josamycin propionate obtained from the sample solution is the same with that of the peak of josamycin propionate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (2).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Josamycin Propionate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Stan-

dard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 50 mg of Josamycin Propionate in the mobile phase to make 50 mL, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amounts of each peak other than josamycin propionate by the area percentage method: the amount of any peak other than josamycin is not more than 6%, and the total of these peaks is not more than 22%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 10 mL of triethylamine add water to make 1000 mL, and adjust the pH to 4.3 with acetic acid (100). To 500 mL of this solution add 500 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of josamycin propionate is about 24 minutes.

Time span of measurement: About 3.5 times as long as the retention time of josamycin propionate beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 3 mL of the sample solution, add the mobile phase to make exactly 50 mL, and use this solution as the solution for system suitability test. Measure exactly 2 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of josamycin propionate obtained from 10 μ L of this solution is equivalent to 8 to 12% of that from 10 μ L of the solution for system suitability test.

System performance: Dissolve 5 mg of josamycin propionate and 2 mg of josamycin in 50 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, josamycin and josamycin propionate are eluted in this order with the resolution between these peaks being not less than 25.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of josamycin propionate is not more than 1.5%.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Josamycin Propionate Reference Standard, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add

1/15 mol/L phosphate buffer solution, pH 5.6 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make solutions so that each mL contains 80 µg (potency) and 20 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

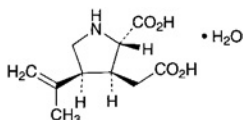
(iv) Sample solutions—Weigh accurately an amount of Josamycin Propionate, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 1/15 mol/L phosphate buffer solution, pH 5.6 to make solutions so that each mL contains 80 µg (potency) and 20 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Kainic Acid Hydrate

カイニン酸水和物



$C_{10}H_{15}NO_4 \cdot H_2O$: 231.25
(2*S*,3*S*,4*S*)-3-(Carboxymethyl)-
4-(1-methylethenyl)pyrrolidine-2-carboxylic acid
monohydrate [487-79-6, anhydride]

Kainic Acid Hydrate, when dried, contains not less than 99.0% of kainic acid ($C_{10}H_{15}NO_4$: 213.23).

Description Kainic Acid Hydrate occurs as white crystals or crystalline powder. It is odorless, and has an acid taste.

It is sparingly soluble in water and in warm water, very slightly soluble in acetic acid (100) and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

The pH of its solution (1 in 100) is between 2.8 and 3.5.

Melting point: about 252°C (with decomposition).

Identification (1) To 5 mL of a solution of Kainic Acid Hydrate (1 in 5000) add 1 mL of ninhydrin TS, and warm in a water bath at a temperature between 60°C and 70°C for 5 minutes: a yellow color is produced.

(2) Dissolve 0.05 g of Kainic Acid Hydrate in 5 mL of acetic acid (100), and add 0.5 mL of bromine TS: the color of bromine disappears immediately.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-13 - -17^\circ$ (0.5 g, water, 50 mL, 200 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Take 0.5 g of Kainic Acid Hydrate

in a platinum crucible, dissolve in 5 mL of sodium carbonate TS, and evaporate on a water bath to dryness. Heat the crucible slowly at first, and then ignite until the sample is almost incinerated. After cooling, add 12 mL of dilute nitric acid to the residue, dissolve by warming, and filter. Wash the residue with 15 mL of water, combine the washings and the filtrate, and add water to make 50 mL. Perform the test using this solution as the test solution.

Control solution: Add 5 mL of sodium carbonate TS to 0.30 mL of 0.01 mol/L hydrochloric acid VS, and proceed as directed above (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Kainic Acid Hydrate in 40 mL of water by warming. Cool, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium—Take 0.25 g of Kainic Acid Hydrate, and perform the test. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02 %).

(5) Heavy metals <1.07>—Proceed with 1.0 g of Kainic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of Kainic Acid Hydrate in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Amino acid and other imino acid—Dissolve 0.10 g of Kainic Acid Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of this solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. Spot 10 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant liquid of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 6.5 – 8.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.4 g of Kainic Acid Hydrate, previously dried, and dissolve in 50 mL of warm water, cool and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 10 drops of bromothymol blue TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 21.32 mg of $C_{10}H_{15}NO_4$

Containers and storage Containers—Tight containers.

Kainic Acid and Santonin Powder

カイニン酸・サントニン散

Kainic Acid and Santonin Powder contains not less than 9.0% and not more than 11.0% of santonin ($C_{15}H_{18}O_3$: 246.30), and not less than 1.80% and not more than 2.20% of kainic acid hydrate ($C_{10}H_{15}NO_4 \cdot H_2O$: 231.25).

Method of preparation

Santonin	100 g
Kainic Acid Hydrate	20 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Description Kainic Acid and Santonin Powder occurs as a white powder.

Identification (1) Shake 1 g of Kainic Acid and Santonin Powder with 10 mL of chloroform, and filter [use the residue for the test (2)]. Distil off the chloroform of the filtrate, and dissolve the residue in 2 mL of potassium hydroxide-ethanol TS: a red color is produced (santonin).

(2) Shake the residue obtained in (1) with 20 mL of warm water, filter, and to 1 mL of the filtrate add 10 mL of water and 1 mL of ninhydrin-L-ascorbic acid TS. Warm in a water bath between 60°C and 70°C for 5 minutes: a yellow color is produced (kainic acid).

Assay (1) Santonin—Weigh accurately about 0.25 g of Kainic Acid and Santonin Powder, add 20 mL of ethanol (95), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of ethanol (95), and filter. Combine the filtrate and the washings, and add ethanol (95) to make exactly 50 mL. Pipet 2 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 25 mg of santonin for assay, proceed in the same manner as the sample solution, and use the obtained solution as the standard solution. Determine the absorbances, A_T and A_S , of these solutions at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} &\text{Amount (mg) of santonin (C}_{15}\text{H}_{18}\text{O}_3\text{)} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of santonin for assay

(2) Kainic acid—Weigh accurately about 1.25 g of Kainic Acid and Santonin Powder, add 20 mL of diluted pyridine (1 in 10), shake thoroughly for 5 minutes, and filter. Wash the residue with three 10-mL portions of diluted pyridine (1 in 10), and filter. Combine the filtrate and the washings, and add diluted pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the sample solution. Dissolve about 25 mg of kainic acid for assay, previously dried at 105°C for 4 hours and accurately weighed, in diluted

pyridine (1 in 10) to make exactly 50 mL. Pipet 2 mL of this solution, add diluted pyridine (1 in 10) to make exactly 25 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution and standard solution, add 2 mL of ninhydrin-L-ascorbic acid TS, and heat on a water bath for 30 minutes. After cooling immediately, shake vigorously for 2 minutes, add water to make exactly 20 mL, and allow to stand for 15 minutes. Determine the absorbances, A_T and A_S , of these solutions at 425 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution prepared in the same manner with 2 mL of diluted pyridine (1 in 10) instead of the sample solution as the blank.

$$\begin{aligned} &\text{Amount (mg) of kainic acid hydrate (C}_{10}\text{H}_{15}\text{NO}_4 \cdot \text{H}_2\text{O)} \\ &= W_S \times (A_T/A_S) \times 1.0845 \end{aligned}$$

W_S : Amount (mg) of kainic acid for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Kallidinogenase

カリジノゲナーゼ

[9001-01-8]

Kallidinogenase is an enzyme obtained from healthy porcine pancreas, and has kinin-releasing activity based on cleavage of kininogen.

It contains not less than 25 Kallidinogenase Units per mg. Usually, it is diluted with Lactose Hydrate or the like.

Kallidinogenase contains not less than 90% and not more than 110% of the labeled Units.

Description Kallidinogenase occurs as a white to light brown powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Kallidinogenase (1 in 300) is between 5.5 and 7.5.

Identification (1) Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, and dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 10 Kallidinogenase Units per mL. Pipet 5 mL of this solution, and add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each test tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the sample solutions 1 and 2. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 4 mL each of this solution into two separate test tubes, add exactly 1 mL each of aprotinin TS and 0.05 mol/L phosphate buffer solution, pH 7.0 separately to each tube, allow them to stand at room temperature for 20 minutes, and use these solutions as the

sample solutions 3 and 4. Then, pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution 1 warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30.0 \pm 0.5^\circ\text{C}$ as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and determine the absorbances at 405 nm, A_{1-2} and A_{1-6} , of this solution, after having allowed it to stand for exactly 2 and 6 minutes. Perform the same test with the sample solutions 2, 3 and 4, and determine the absorbances, A_{2-2} , A_{2-6} , A_{3-2} , A_{3-6} , A_{4-2} and A_{4-6} , of these solutions. Calculate I by using the following equation: the value of I does not exceed 0.2.

$$I = \frac{(A_{1-6} - A_{1-2}) - (A_{3-6} - A_{3-2})}{(A_{2-6} - A_{2-2}) - (A_{4-6} - A_{4-2})}$$

(2) Pipet 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.1 mL of the sample solution obtained in the Assay, and start simultaneously a chronograph. Perform the test at $30.0 \pm 0.5^\circ\text{C}$ as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the change of the absorbance at 253 nm for 4 to 6 minutes. Separately, pipet 1 mL of trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Add exactly 0.1 mL of this solution to exactly 2.9 mL of substrate TS for kallidinogenase assay (2), previously warmed at $30.0 \pm 0.5^\circ\text{C}$ for 5 minutes, and use this solution as the blank. If the rate of change in the absorbance remains constant, determine the change of absorbance per 1 minute, A , and calculate R by using the following equation: the value of R is between 0.12 and 0.16.

$$R = (A/0.0383) \times \{1/(a - b)\}$$

a : Amount (mg) of Kallidinogenase in 1 mL of the sample solution.

b : Amount (Unit) of kallidinogenase in 1 mg of Kallidinogenase obtained in the Assay.

Specific activity Perform the test with Kallidinogenase as directed under Nitrogen Determination <1.08> to determine the nitrogen content, convert 1mg of nitrogen (N:14.01) into 6.25 mg of protein, and calculate the specific activity using the amount (Units) of Kallidinogenase obtained in the Assay: it is not less than 100 Kallidinogenase Units per 1mg of protein.

Purity (1) Fat—To 1.0 g of Kallidinogenase add 20 mL of diethyl ether, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue is not more than 1 mg.

(2) Kininase—

(i) Bradykinin solution: Weigh an appropriate amount of bradykinin, and dissolve in gelatin-phosphate buffer solution, pH 7.4 to prepare a solution containing $0.200 \mu\text{g}$ of bradykinin per mL.

(ii) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase according to the labeled unit, dissolve in gelatin-phosphate buffer solution, pH 7.4 to make a solution containing 1 unit of kallidinogenase per mL.

(iii) Sample solution: Pipet 0.5 mL of bradykinin solu-

tion, warm at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and mix immediately. After allow this solution to stand at $30 \pm 0.5^\circ\text{C}$ for exactly 150 seconds, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes. Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and mix. Pipet 0.1 mL of this solution, add exactly 0.9 mL of trichloroacetic acid-gelatin-tris buffer solution, and mix. Pipet 0.2 mL of this solution, add exactly 0.6 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iv) Control solution: Proceed with 0.5 mL of gelatin-phosphate buffer solution, pH 7.4 as described in (iii), and use the solution so obtained as the control solution.

(v) Procedure: Add 0.1 mL of anti-bradykinin antibody TS to anti-rabbit antibody-coated wells of a 96-well microplate, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Remove the anti-bradykinin antibody TS, add 0.3 mL of phosphate buffer solution for microplate washing to the wells, then remove. Repeat this procedure 3 times, take off the washings thoroughly, then add $100 \mu\text{L}$ each of the sample solution and control solution, and $50 \mu\text{L}$ of gelatin-phosphate buffer solution, pH 7.0, shake, and allow to stand at a constant temperature of about 25°C for 1 hour. Then add $50 \mu\text{L}$ of peroxidase-labeled bradykinin TS, shake, and allow to stand in a cold place for a night.

Take off the solution, add 0.3 mL of phosphate buffer solution for microplate washing, and remove. Repeat this procedure more 4 times, take off the washings thoroughly, add $100 \mu\text{L}$ of substrate solution for peroxidase determination, and allow to stand at a constant temperature of about 25°C for exactly 30 minutes while protecting from light. Then add $100 \mu\text{L}$ of diluted sulfuric acid (23 in 500), shake, and determine the absorbance at 490 – 492 nm.

Separately, dissolve a suitable amount of bradykinin in gelatin-phosphate buffer solution, pH 7.0 to make solutions containing exactly 100 ng, 25 ng, 6.25 ng, 1.56 ng, 0.39 ng and 0.098 ng of bradykinin per mL, and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3), the standard solution (4), the standard solution (5) and the standard solution (6), respectively. Use 1 mL of gelatin-phosphate buffer solution, pH 7.0 as the standard solution (7). To each of the well add $50 \mu\text{L}$ each of the standard solutions and $100 \mu\text{L}$ of trichloroacetic acid-gelatin-tris buffer solution, and proceed in the same manner as for the sample solution and for the control solution.

Prepare the standard curve from the amounts of bradykinin in the standard solutions and their absorbances, and determine the amount of bradykinin, B_T (pg) and B_S (pg), of the sample solution and the control solution.

The absorbance is usually determined by using a spectrophotometer for microplate. Since the wells are used as the cell for absorbance determination, take care for dirt and scratch of the well. Light pass length of the well is changeable by the amount of the liquid, exact addition of the liquid is necessary.

(vi) Judgment: The value R calculated by the following equation is not less than 0.8.

$$R = (B_T/B_S)$$

(3) Trypsin-like substances—Pipet 4 mL of the sample stock solution prepared for the Assay, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 10-mm cell, add exactly 0.5 mL of the sample solution, warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30 \pm 0.5^\circ\text{C}$ as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and determine the absorbances at 405 nm, A_2 and A_6 , of this solution after having allowed it to stand for exactly 2 and 6 minutes. Separately, pipet 4 mL of the sample stock solution prepared for the Assay, add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the control solution. Perform the same test with the control solution, and determine the absorbances, A_2' and A_6' . Calculate T by using the following equation: the value of T does not exceed 0.05.

$$T = \{(A_6' - A_2) - (A_6 - A_2)/(A_6' - A_2)\}$$

(4) Protease—Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing 1 Kallidinogenase Unit per mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, place in a test tube, and allow to stand at $35 \pm 0.5^\circ\text{C}$ for 5 minutes. Then, pipet 5 mL of substrate TS for kallidinogenase assay (3), previously warmed to $35 \pm 0.5^\circ\text{C}$, add quickly to the sample solution in the test tube, and allow to stand at $35 \pm 0.5^\circ\text{C}$ for exactly 20 minutes. Then add exactly 5 mL of trichloroacetic acid TS, shake well, allow to stand at room temperature for 1 hour, and filter through a membrane filter (5 μm in pore size). Discard the first 3 mL of the filtrate, and determine the absorbance, A , of the subsequent filtrate at 280 nm within 2 hours as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS, shake well, and add exactly 5 mL of the substrate TS for kallidinogenase assay (3). Proceed in the same manner as described for the sample solution, and determine the absorbance, A_0 , of this solution. Calculate the value of $(A - A_0)$: it is not more than 0.2.

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 3% (0.5g, 650 – 750°C).

Kinin-releasing activity

(i) Kallidinogenase solution: Weigh accurately a suitable amount of Kallidinogenase, according to the labeled unit, dissolve in 0.02 mol/L phosphate buffer solution, pH 8.0 to make a solution containing 0.1 unit of kallidinogenase per mL. Perform this procedure by using glassware.

(ii) Sample solution: Pipet 0.5 mL of kininogen TS, warm at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, then add exactly 0.5 mL of kallidinogenase solution previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and mix immediately. After allow this solution to stand at $30 \pm 0.5^\circ\text{C}$ for exactly 2 minutes, add exactly 0.2 mL of a solution of trichloroacetic acid (1 in 5), and shake. Boil for 3 minutes, then cool in ice immediately, centrifuge, and allow to stand at a room temperature for 15 minutes.

Pipet 0.5 mL of the supernatant liquid, add exactly 0.5 mL of gelatin-tris buffer solution, pH 8.0, and shake. Pipet 0.1 mL of this solution, add exactly 1.9 mL of trichloroacetic acid-gelatin-tris buffer solution, shake, and use this solution as the sample solution.

(iii) Procedure: Perform the test with the sample solution as directed in the Purity (2), and determine the amount, B (pg), of kinin per well. The kinin-releasing activity per 1 unit of Kallidinogenase calculated by the following equation is not less than 500 ng bradykinin equivalent/min/unit.

$$\text{Kinin-releasing activity (ng bradykinin equivalent/min/unit) per 1 unit of Kallidinogenase} = B \times 4.8$$

Assay Weigh accurately an appropriate amount of Kallidinogenase according to the labeled Units, dissolve in 0.05 mol/L phosphate buffer solution, pH 7.0 to prepare a solution containing about 10 Kallidinogenase Units per mL, and use this solution as the sample stock solution. Pipet 4 mL of the sample stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the sample solution. Pipet 2.5 mL of substrate TS for kallidinogenase assay (1), previously warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, place in a 1-cm cell, add exactly 0.5 mL of the sample solution, warmed at $30 \pm 0.5^\circ\text{C}$ for 5 minutes, and start simultaneously a chronograph. Perform the test at $30 \pm 0.5^\circ\text{C}$ as directed under the Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and determine the absorbances at 405 nm, A_{T2} and A_{T6} , of this solution after allowing to stand for exactly 2 and 6 minutes. Separately, dissolve Kallidinogenase Reference Standard in 0.05 mol/L phosphate buffer solution, pH 7.0 to make a solution so that each mL contains exactly 10 Units, and use this solution as the standard stock solution. Pipet 4 mL of the stock solution, add exactly 1 mL of trypsin inhibitor TS and 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL, and use this solution as the standard solution. Take exactly 0.5 mL of the standard solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{S2} and A_{S6} , of the solution after allowing to stand for exactly 2 and 6 minutes. Separately, take exactly 1 mL of the trypsin inhibitor TS, and add 0.05 mol/L phosphate buffer solution, pH 7.0 to make exactly 10 mL. Pipet 0.5 mL of this solution, perform the test in the same manner as described for the sample solution, and determine the absorbances, A_{O2} and A_{O6} , of the solution after allowing to stand for exactly 2 and 6 minutes.

Units per 1 mg of Kallidinogenase

$$= \frac{(A_{T6} - A_{T2}) - (A_{O6} - A_{O2})}{(A_{S6} - A_{S2}) - (A_{O6} - A_{O2})} \times \frac{W_S}{a} \times \frac{1}{b}$$

W_S : Amount (Units) of Kallidinogenase Reference Standard

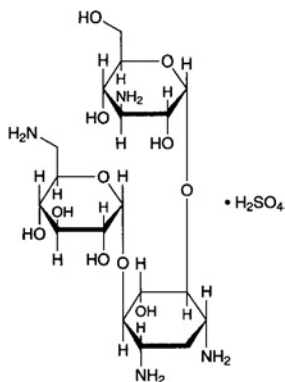
a : Volume (mL) of the standard stock solution

b : Amount (mg) of Kallidinogenase in 1 mL of the sample stock solution

Containers and storage Containers—Tight containers.

Kanamycin Monosulfate

カナマイシン—硫酸塩



$C_{18}H_{36}N_4O_{11} \cdot H_2SO_4$: 582.58

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-
[6-amino-6-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-2-deoxy-
D-streptomine monosulfate [25389-94-0]

Kanamycin Monosulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.

It contains not less than 750 μ g (potency) and not more than 832 μ g (potency) per mg, calculated on the dried basis. The potency of Kanamycin Monosulfate is expressed as mass (potency) of kanamycin ($C_{18}H_{36}N_4O_{11}$: 484.50).

Description Kanamycin Monosulfate occurs as a white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 50 mg of Kanamycin Monosulfate in 3 mL of water, and add 6 mL of anthrone TS: a blue-purple color develops.

(2) Dissolve 20 mg each of Kanamycin Monosulfate and Kanamycin Monosulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the supernatant layer of a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same *R_f* value.

(3) To a solution of Kanamycin Monosulfate (1 in 5) add 1 drop of barium chloride TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +112 – +123° (0.2 g calculated on the dried basis, water, 20 mL, 100 mm).

Sulfuric acid Weigh accurately about 0.25 g of Kanamycin Monosulfate, dissolve in 100 mL of water, adjust the pH to

11.0 with ammonia solution (28), add exactly 10 mL of 0.1 mol/L barium chloride VS, and titrate <2.50> with 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution, blue-purple, disappears (indicator: 0.5 mg of phthalein purple). At a near of the end-point add 50 mL of ethanol (99.5). Perform a blank determination in the same manner. The amount of sulfuric acid (SO_4) is not less than 15.0% and not more than 17.0%, calculated on the dried basis.

Each mL of 0.1 mol/L barium chloride VS
= 9.606 mg of SO_4

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Kanamycin Monosulfate according to Method 4, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.30 g of Kanamycin Monosulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 45 mg of Kanamycin Monosulfate Reference Standard in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 4.0% (5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5 and 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

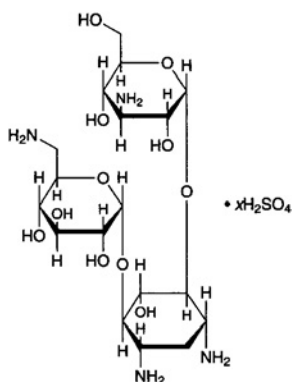
(iv) Sample solutions—Weigh accurately an amount of Kanamycin Monosulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each

mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Well-closed containers.

Kanamycin Sulfate

カナマイシン硫酸塩



$\text{C}_{18}\text{H}_{36}\text{N}_4\text{O}_{11} \cdot x\text{H}_2\text{SO}_4$

3-Amino-3-deoxy- α -D-glucopyranosyl-(1 \rightarrow 6)-
[6-amino-6-deoxy- α -D-glucopyranosyl-(1 \rightarrow 4)]-2-deoxy-
D-streptamine sulfate [133-92-6]

Kanamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces kanamyceticus*.

It contains not less than 690 μg (potency) and not more than 740 μg (potency) per mg, calculated on the dried basis. The potency of Kanamycin Sulfate is expressed as mass (potency) of kanamycin ($\text{C}_{18}\text{H}_{36}\text{N}_4\text{O}_{11}$: 484.50).

Description Kanamycin Sulfate occurs as a white to yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 20 mg each of Kanamycin Sulfate and Kanamycin Monosulfate Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ammonia solution (28) and methanol (2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and the standard solution show a purple-brown color and the same R_f value.

(2) A solution of Kanamycin Sulfate (1 in 10) responds to the Qualitative Test <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +103 – +115° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g

of Kanamycin Sulfate in 20 mL of water is between 6.0 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 1.5 g of Kanamycin Sulfate in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Kanamycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Kanamycin Sulfate according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.30 g of Kanamycin Sulfate in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 9.0 mg of Kanamycin Monosulfate Reference Standard in water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in 1-butanol (1 in 100) on the plate, and heat at 110°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer having pH 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Kanamycin Monosulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kanamycin Sulfate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Kaolin

カオリン

Kaolin is a native, hydrous aluminum silicate.

Description Kaolin occurs as white or nearly white, fragmentary masses or powder. It has a slightly clay-like odor.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It is insoluble in dilute hydrochloric acid and in sodium hydroxide TS.

When moistened with water, it darkens and becomes plastic.

Identification (1) Heat 1 g of Kaolin with 10 mL of water and 5 mL of sulfuric acid in a porcelain dish, and evaporate the mixture nearly to dryness. Cool, add 20 mL of water, boil for 2 to 3 minutes, and filter: the color of the residue is gray.

(2) The filtrate obtained in (1) responds to the Qualitative Tests <1.09> (1), (2) and (4) for aluminum salt.

Purity (1) Acid or alkali—Add 25 mL of water to 1.0 g of Kaolin, agitate thoroughly, and filter: the pH <2.54> of the filtrate is between 4.0 and 7.5.

(2) Acid-soluble substances—Add 20 mL of dilute hydrochloric acid to 1.0 g of Kaolin, agitate for 15 minutes, and filter. Evaporate 10 mL of the filtrate to dryness, and heat strongly between 450°C and 550°C to constant mass: the mass of the ignited residue is not more than 0.010 g.

(3) Carbonate—Stir 1.0 g of Kaolin with 5 mL of water, then add 10 mL of diluted sulfuric acid (1 in 2): no effervescence occurs.

(4) Heavy metals <1.07>—Boil 1.5 g of Kaolin gently with 50 mL of water and 5 mL of hydrochloric acid for 20 minutes with frequent agitation, cool, centrifuge, and separate the supernatant liquid. Wash the precipitate twice with 10 mL of water, centrifuge each time, and combine the supernatant liquid and the washings. Add dropwise ammonia solution (28) to this solution until a slight precipitate occurs, then add dilute hydrochloric acid dropwise while agitating strongly to complete solution. Add 0.45 g of hydroxylammonium chloride, and heat. Cool, add 0.45 g of sodium acetate trihydrate and 6 mL of dilute acetic acid, filter if necessary, and wash with 10 mL of water. Combine the filtrate and the washings, and add water to make 150 mL. Perform the test using 50 mL of this solution as the test solution. To 2.5 mL of Standard Lead Solution add 0.15 g of hydroxylammonium chloride, 0.15 g of sodium acetate trihydrate, 2 mL of acetic acid (31) and water to make 50 mL, and use this solution as the control solution (not more than 50 ppm).

(5) Iron <1.10>—Add 10 mL of dilute hydrochloric acid to 40 mg of Kaolin, and heat for 10 minutes with shaking in a water bath. After cooling, add 0.5 g of L-tartaric acid, dissolve with shaking, prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(6) Arsenic <1.11>—Add 5 mL of water and 1 mL of sulfuric acid to 1.0 g of Kaolin, and heat on a sand bath until white fumes begin to evolve. Cool, and add water to make 5

mL. Perform the test with this solution as the test solution (not more than 2 ppm).

(7) Foreign matter—Place 5 g of Kaolin in a beaker, add 100 mL of water, stir, and decant to leave sand. Repeat this procedure several times with 100-mL portions of water: no sandy residue remains.

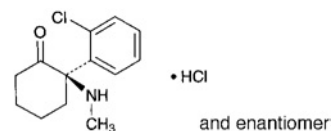
Loss on ignition <2.43> Not more than 15.0% (1 g, 600°C, 5 hours).

Plasticity Add 7.5 mL of water to 5.0 g of Kaolin, and agitate thoroughly: the resultant mass has no remarkable fluidity.

Containers and storage Containers—Well-closed containers.

Ketamine Hydrochloride

ケタミン塩酸塩



$C_{13}H_{16}ClNO \cdot HCl$: 274.19

(2*RS*)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone monohydrochloride [1867-66-9]

Ketamine Hydrochloride, when dried, contains not less than 99.0% of $C_{13}H_{16}ClNO \cdot HCl$.

Description Ketamine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in formic acid, freely soluble in water and in methanol, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

A solution of Ketamine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 258°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ketamine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 3000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketamine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ketamine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> (2) for chloride.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (269 nm): 22.0 – 24.5 (after drying, 0.03 g, 0.1 mol/L hydrochloric acid TS, 100 mL).

pH <2.54> Dissolve 1.0 g of Ketamine Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Ketamine Hydrochloride in 5 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ketamine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ketamine Hydrochloride, according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.5 g of Ketamine Hydrochloride in 10 mL of methanol and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and isopropylamine (49:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, dry the plate, and then spray evenly hydrogen peroxide TS: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

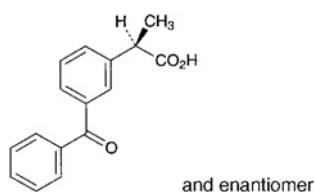
Assay Weigh accurately about 0.5 g of Ketamine Hydrochloride, previously dried, dissolve in 1 mL of formic acid, add 70 mL of a mixture of acetic anhydride and acetic acid (100) (6:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.42 mg of C₁₃H₁₆ClNO.HCl

Containers and storage Containers—Tight containers.

Ketoprofen

ケトプロフェン



C₁₆H₁₄O₃: 254.28
(2*RS*)-2-(3-Benzoylphenyl)propanoic acid [22071-15-4]

Ketoprofen, when dried, contains not less than 99.0% and not more than 100.5% of C₁₆H₁₄O₃.

Description Ketoprofen occurs as a white, crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95) and in acetone, and practically insoluble in water.

A solution of Ketoprofen in ethanol (99.5) (1 in 100) shows no optical rotation.

It is colored to pale yellow by light.

Identification (1) Determine the absorption spectrum of a solution of Ketoprofen in methanol (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ketoprofen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 94 – 97°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ketoprofen in 10 mL of acetone: the solution is clear, and has no more color than the following control solution.

Control solution: To a mixture of 0.6 mL of Cobalt (II) Chloride Colorimetric Stock Solution and 2.4 mL of Iron (III) Chloride Colorimetric Stock Solution add diluted hydrochloric acid (1 in 10) to make 10 mL. To 5.0 mL of this solution add diluted hydrochloric acid (1 in 10) to make 100 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ketoprofen according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Conduct this procedure with a minimum of exposure to light, using light-resistant vessels. Dissolve 20 mg of Ketoprofen in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the areas of the peaks, having the relative retention time of about 1.5 and about 0.3 with respect to ketoprofen, are not larger than 4.5 times and not larger than 2 times the peak area of ketoprofen from the standard solution, respectively, the area of the peak other than ketoprofen and the peaks mentioned above is not larger than the peak area of ketoprofen from the standard solution, and the total area of these peaks is not larger than 2 times the peak area of ketoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 233 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 68.0 g of potassium dihydrogen phosphate in water to make 1000 mL, and adjust the pH to 3.5 with phosphoric acid. To 20 mL of this solution add 430 mL of acetonitrile and 550 mL of water.

Flow rate: Adjust the flow rate so that the retention time of

ketoprofen is about 7 minutes.

Time span of measurement: About 7 times as long as the retention time of ketoprofen.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of ketoprofen obtained with 20 μ L of this solution is equivalent to 9 to 11% of that with 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ketoprofen are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ketoprofen is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, 60°C, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

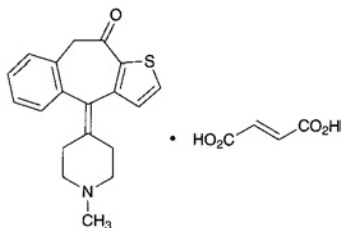
Assay Weigh accurately about 0.3 g of Ketoprofen, previously dried, dissolve in 25 mL of ethanol (95), add 25 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.43 mg of C₁₆H₁₄O₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ketotifen Fumarate

ケトチフェン fumarate



C₁₉H₁₉NOS.C₄H₄O₄: 425.50

4-(1-Methylpiperidin-4-ylidene)-4H-benzo[4,5]cyclohepta[1,2-b]thiophen-10(9H)-one monofumarate [34580-14-8]

Ketotifen Fumarate, when dried, contains not less than 99.0% and not more than 101.0% of ketotifen fumarate (C₁₉H₁₉NOS.C₄H₄O₄).

Description Ketotifen Fumarate occurs as a white to light yellowish white crystalline powder.

It is sparingly soluble in methanol and in acetic acid (100), and slightly soluble in water, in ethanol (99.5) and in acetic anhydride.

Melting point: about 190°C (with decomposition).

Identification (1) Prepare the test solution with 0.03 g of

Ketotifen Fumarate as directed under Oxygen Flask Combustion Method using 20 mL of water as the absorbing liquid: the test solution responds to the Qualitative Tests <1.09> for sulfate.

(2) Determine the absorption spectrum of a solution of Ketotifen Fumarate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Ketotifen Fumarate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <1.03>—Dissolve 0.6 g of Ketotifen Fumarate in 2.5 mL of sodium carbonate TS in a crucible, heat on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of sodium carbonate TS, the used amount of diluted nitric acid (3 in 10) for the neutralization, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.015%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ketotifen Fumarate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Ketotifen Fumarate in 10 mL of a mixture of methanol and ammonia TS (99:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of methanol and ammonia TS (99:1) to make exactly 25 mL. Pipet 1 mL of this solution, add a mixture of methanol and ammonia TS (99:1) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, water and ammonia solution (28) (90:10:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying and then hydrogen peroxide TS on the plate: the number of the spot other than the principal spot obtained from the sample solution is not more than four, and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Ketotifen Fumarate, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

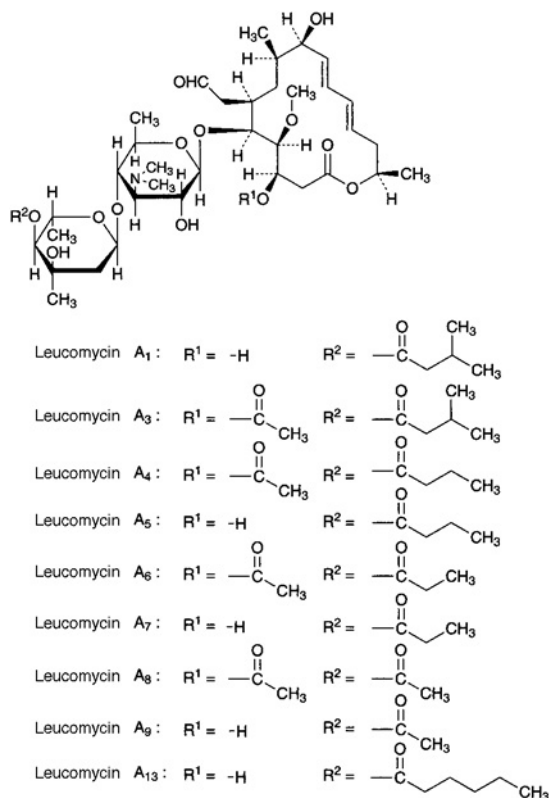
= 42.55 mg of $C_{19}H_{19}NOS \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.

Kitasamycin

Leucomycin

キサマイシン



(Leucomycins A₁, A₅, A₇, A₉ and A₁₃)
 (3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A₁: acyl = 3-methylbutanoyl
 Leucomycin A₅: acyl = butanoyl
 Leucomycin A₇: acyl = propanoyl
 Leucomycin A₉: acyl = acetyl
 Leucomycin A₁₃: acyl = hexanoyl

(Leucomycins A₃, A₄, A₆ and A₈)
 (3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide

Leucomycin A₃: acyl = 3-methylbutanoyl
 Leucomycin A₄: acyl = butanoyl
 Leucomycin A₆: acyl = propanoyl
 Leucomycin A₈: acyl = acetyl

[1392-21-8, Kitasamycin]

Kitasamycin is a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces kitasatoensis*.

It contains not less than 1450 μ g (potency) and not more than 1700 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A₅ ($C_{39}H_{65}NO_{14}$: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A₅ ($C_{39}H_{65}NO_{14}$).

Description Kitasamycin occurs as a white to light yellow-white powder.

It is very soluble in acetonitrile, in methanol and in ethanol (95), and practically insoluble in water.

Identification Determine the absorption spectrum of a solution of Kitasamycin in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Content ratio of the active principle Dissolve 0.02 g of Kitasamycin in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amounts of leucomycin A₅, leucomycin A₄ and leucomycin A₁ by the area percentage method: the amounts of leucomycin A₅, leucomycin A₄ and leucomycin A₁ are 40 to 70%, 5 to 25% and 3 to 12%, respectively. Relative retention times of leucomycin A₄ and leucomycin A₁ to that of leucomycin A₅ are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a volume of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust to pH 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A₅ is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A₅.

System suitability—

System performance: Dissolve about 20 mg each of Leucomycin A₅ Reference Standard and Josamycin Reference Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μ L of this solution under the above operating conditions, leucomycin A₅ and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μ L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leu-

comycin A₅ is not more than 1.0%.

Water <2.48> Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Leucomycin A₅ Reference Standard equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

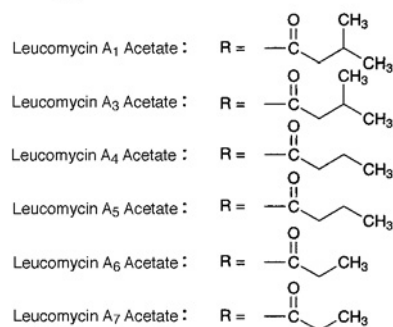
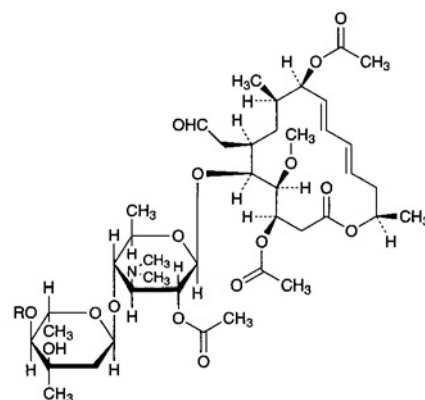
(iv) Sample solutions—Weigh accurately an amount of Kitasamycin equivalent to about 0.03 g (potency), dissolve in 10 mL of methanol, and add water to make exactly 100 mL. Take exactly a suitable amount of the solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Kitasamycin Acetate

Leucomycin Acetate

キタマイシン酢酸エステル



(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3,9-Diacetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1→4)-2-*O*-acetyl-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methylhexadeca-10,12-dien-15-olide
 Leucomycin A₁ and A₃ Acetates: acyl = 3-methylbutanoyl
 Leucomycin A₄ and A₅ Acetates: acyl = butanoyl
 Leucomycin A₆ and A₇ Acetates: acyl = propanoyl
 [178234-32-7, Kitasamycin Acetate]

Kitasamycin Acetate is a derivative of kitasamycin.

It contains not less than 680 μg (potency) and not more than 790 μg (potency) per mg, calculated on the anhydrous basis. The potency of Kitasamycin Acetate is expressed as mass (potency) of kitasamycin corresponding to the mass of leucomycin A₅ (C₃₉H₆₅NO₁₄: 771.93). One mg (potency) of kitasamycin is equivalent to 0.530 mg of leucomycin A₅ (C₃₉H₆₅NO₁₄).

Description Kitasamycin Acetate occurs as a white to light yellow-white powder.

It is very soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Kitasamycin Acetate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <2.48> Not more than 5.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solution—Weigh accurately an amount of Leucomycin A₅ Reference Standard equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

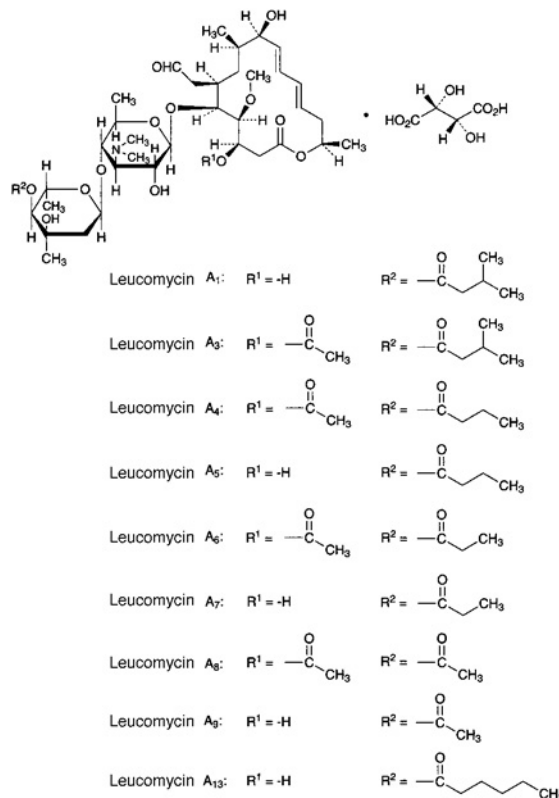
(iv) Sample solution—Weigh accurately an amount of Kitasamycin Acetate equivalent to about 30 mg (potency), dissolve in 25 mL of methanol, add water to make exactly 50 mL, shake well, and allow to stand at 37 ± 2°C for 24 hours. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Kitasamycin Tartrate

Leucomycin Tartrate

キサマイシン酒石酸塩



(Leucomycin A₁, A₅, A₇, A₉ and A₁₃ Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Acyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A₁ Tartrate: acyl = 3-methylbutanoyl

Leucomycin A₅ Tartrate: acyl = butanoyl

Leucomycin A₇ Tartrate: acyl = propanoyl

Leucomycin A₉ Tartrate: acyl = acetyl

Leucomycin A₁₃ Tartrate: acyl = hexanoyl

(Leucomycin A₃, A₄, A₆ and A₈ Tartrates)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-Acetoxy-5-[4-*O*-acyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide mono-(2*R*,3*R*)-tartrate

Leucomycin A₃ Tartrate: acyl = 3-methylbutanoyl

Leucomycin A₄ Tartrate: acyl = butanoyl

Leucomycin A₆ Tartrate: acyl = propanoyl

Leucomycin A₈ Tartrate: acyl = acetyl

[37280-56-1, Kitasamycin Tartrate]

Kitasamycin Tartrate is the tartrate of kitasamycin. It contains not less than 1300 μg (potency) per mg,

calculated on the anhydrous basis. The potency of Kitasamycin Tartrate is expressed as mass (potency) of kitasamycin based on the amount of leucomycin A₅ (C₃₉H₆₅NO₁₄: 771.93). One mg (potency) of Kitasamycin Tartrate is equivalent to 0.530 mg of leucomycin A₅ (C₃₉H₆₅NO₁₄).

Description Kitasamycin Tartrate occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Kitasamycin Tartrate in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Kitasamycin Tartrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 1 g of Kitasamycin Tartrate in 20 mL of water, add 3 mL of sodium hydroxide TS, add 20 mL of *n*-butyl acetate, shake well, and discard the *n*-butyl acetate layer. To the aqueous layer add 20 mL of *n*-butyl acetate, and shake well. The aqueous layer so obtained responds to the Qualitative Tests <1.09> (1) for tartrate.

pH <2.54> Dissolve 3.0 g of Kitasamycin Tartrate in 100 mL of water: the pH of the solution is between 3.0 and 5.0.

Content ratio of the active principle Dissolve 20 mg of Kitasamycin Tartrate in diluted acetonitrile (1 in 2) to make 20 mL, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amounts of leucomycin A₅, leucomycin A₄ and leucomycin A₁ by the area percentage method: the amount of leucomycin A₅ is 40 – 70%, leucomycin A₄ is 5 – 25%, and leucomycin A₁ is 3 – 12%. The relative retention times of leucomycin A₄ and leucomycin A₁ with respect to leucomycin A₅ are 1.2 and 1.5, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To a suitable amount of a solution of ammonium acetate (77 in 500) add diluted phosphoric acid (1 in 150) to adjust the pH to 5.5. To 370 mL of this solution add 580 mL of methanol and 50 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of leucomycin A₅ is about 8 minutes.

Time span of measurement: About 3 times as long as the retention time of leucomycin A₅.

System suitability—

System performance: Dissolve about 20 mg of Leucomycin A₅ Reference Standard and about 20 mg of Josamycin Refer-

ence Standard in 20 mL of diluted acetonitrile (1 in 2). When the procedure is run with 5 μL of this solution under the above operating conditions, leucomycin A₅ and josamycin are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of leucomycin A₅ is not more than 1.0%.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Kitasamycin Tartrate in 10 mL of water: the solution is clear and colorless or light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Kitasamycin Tartrate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Water <2.48> Not more than 3.0% (0.1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

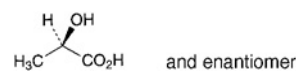
(iii) Standard solutions—Weigh accurately an amount of Leucomycin A₅ Reference Standard, equivalent to about 30 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Kitasamycin Tartrate, equivalent to about 30 mg (potency), and dissolve in water to make exactly 100 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 30 μg (potency) and 7.5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Lactic Acid

乳酸



C₃H₆O₃: 90.08

(2*RS*)-2-Hydroxypropanoic acid [50-21-5]

Lactic Acid is a mixture of lactic acid and lactic anhydride.

It contains not less than 85.0% and not more than 92.0% of C₃H₆O₃.

Description Lactic Acid occurs as a clear, colorless or light yellow, viscous liquid. It is odorless or has a faint, unpleasant odor.

It is miscible with water, with ethanol (95) and with diethyl ether.

It is hygroscopic.

Specific gravity d_{20}^{20} : about 1.20

Identification A solution of Lactic Acid (1 in 50) changes blue litmus paper to red and responds to the Qualitative Tests <1.09> for lactate.

Purity (1) Chloride <1.03>—Perform the test with 1.0 g of Lactic Acid. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—Perform the test with 2.0 g of Lactic Acid. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <1.07>—To 2.0 g of Lactic Acid add 10 mL of water and 1 drop of phenolphthalein TS, and add ammonia TS dropwise until a pale red color appears. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution from 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to 50 mL (not more than 10 ppm).

(4) Iron <1.10>—Prepare the test solution with 4.0 g of Lactic Acid according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 5 ppm).

(5) Sugars—To 1.0 g of Lactic Acid add 10 mL of water, and neutralize with sodium hydroxide TS. Boil the mixture with 10 mL of Fehling's TS for 5 minutes: no red precipitate is produced.

(6) Citric, oxalic, phosphoric and L-tartaric acid—To 1.0 g of Lactic Acid add 1.0 mL of water, followed by 40 mL of calcium hydroxide TS. Boil the mixture for 2 minutes: no change occurs.

(7) Glycerin or mannitol—Shake 10 mL of Lactic Acid with 12 mL of diethyl ether: no turbidity is produced.

(8) Volatile fatty acids—Warm Lactic Acid: it does not produce any acetic acid-like or butyric acid-like odor.

(9) Cyanide—Transfer 1.0 g of Lactic Acid to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, add dropwise a solution of sodium hydroxide (1 in 10) by shaking until a pale red color develops, add 1.5 mL of a solution of sodium hydroxide (1 in 10) and water to make 20 mL, and heat in a water bath for 10 minutes. Cool, add dropwise dilute acetic acid until a red color of the solution disappears, add 1 drop of dilute acetic acid, add 10 mL of phosphate buffer solution, pH 6.8, and 0.25 mL of sodium toluenesulfonchloramide TS, stopper immediately, mix gently, and allow to stand for 5 minutes. To the solution add 15 mL of pyridine-pyrazolone TS and water to make 50 mL, and allow to stand at 25°C for 30 minutes: the solution has no more color than the following control solution.

Control solution: Pipet 1.0 mL of Standard Cyanide Solution, and add water to make exactly 20 mL. Transfer 1.0 mL of this solution to a Nessler tube, add 10 mL of water and 1 drop of phenolphthalein TS, and then proceed as described above.

(10) Readily carbonizable substances—Superimpose slowly 5 mL of Lactic Acid, previously kept at 15°C, upon 5

mL of sulfuric acid for readily carbonizable substances, previously kept at 15°C, and allow to stand at 15°C for 15 minutes: no dark color develops at the zone of contact.

Residue on ignition <2.44> Not more than 0.1% (1 g).

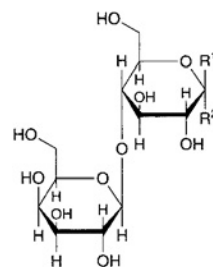
Assay Weigh accurately about 3 g of Lactic Acid, transfer in a conical flask, add accurately measured 40 mL of 1 mol/L sodium hydroxide VS, invert a watch glass over the flask, and heat on a water bath for 10 minutes. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS immediately (indicator: 2 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 90.08 mg of C₃H₆O₃

Containers and storage Containers—Tight containers.

Anhydrous Lactose

無水乳糖



α -Lactose : R¹=H, R²=OH
 β -Lactose : R¹=OH, R²=H

C₁₂H₂₂O₁₁: 342.30

β -D-Galactopyranosyl-(1→4)- β -D-glucopyranose (β -lactose)

β -D-Galactopyranosyl-(1→4)- α -D-glucopyranose (α -lactose)

[63-42-3, Anhydrous Lactose]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Anhydrous Lactose is β -lactose or a mixture of β -lactose and α -lactose.

The relative quantities of α -lactose and β -lactose in Anhydrous Lactose is indicated as the isomer ratio.

♦**Description** Anhydrous Lactose occurs as white crystals or powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).◆

Identification Determine the infrared absorption spectrum of Anhydrous Lactose, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with ♦the Reference Spectrum or ◆the spectrum of Anhydrous Lactose Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +54.4 – +55.9°. Weigh accurately about 10 g of Anhydrous Lactose, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C,

and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Anhydrous Lactose in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Anhydrous Lactose in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

♦(3) Heavy metals <1.07>—Proceed with 4.0 g of Anhydrous Lactose according to Method 2, and perform the test. Prepare the control solution with 2 mL of Standard Lead Solution (not more than 5 ppm).♦

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Anhydrous Lactose in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 2 hours).

Water <2.48> Not more than 1.0% (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

Residue on ignition <2.44> Not more than 0.1% (1 g).

♦**Microbial limit** <4.05> The total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeast is not more than 50 per g, and *Salmonella* and *Escherichia coli* should not be observed.♦

Isomer ratio Place 1 mg of Anhydrous Lactose in an 5-mL screw capped reaction vial for gas chromatography, add 0.45 mL of dimethylsulfoxide, stopper, and shake well. Add 1.8 mL of a mixture of pyridine and trimethylsilylimidazole (72:28), mix, and allow to stand for 20 minutes, and use this solution as the sample solution. Perform the test with 2 μL of the sample solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine peak areas of α-lactose and β-lactose, A_a and A_b , and calculate the content (%) of β-lactose in Anhydrous Lactose by the following equation.

$$\text{Content (\%)} \text{ of } \beta\text{-lactose} = [A_b / (A_a + A_b)] \times 100$$

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Sample injection port: about 275°C

Column: A column 4 mm in inside diameter and 0.9 m in length, packed with siliceous earth for gas chromatography coated at the ratio of 3% with 25% phenyl-25% cyanopropyl-methylsilicone polymer for gas chromatography.

Column temperature: A constant temperature of about 215°C.

Carrier gas: Helium

Flow rate: A constant flow rate of about 40 mL per minute.
System suitability—

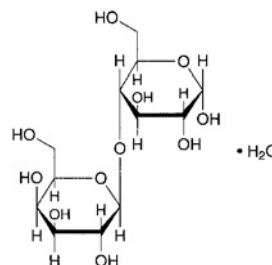
System performance: Prepare a solution with 1 mg of a mixture of α-lactose and β-lactose (1:1) in the same manner as for preparing the sample solution, and proceed with 2 μL of this solution under the above operating conditions, and determine the retention times of the peaks of α-lactose and β-lactose: a ratio of the retention time of α-lactose to that of β-lactose is about 0.7 with the resolution between these peaks being not less than 3.0.

♦**Containers and storage** Containers—Well-closed containers.♦

Lactose Hydrate

Lactose

乳糖水和物



$C_{12}H_{22}O_{11} \cdot H_2O$: 360.31

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate

[64044-51-5, Mixture of α- and β-lactose monohydrate]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Lactose Hydrate is the monohydrate of β-D-galactopyranosyl-(1→4)-α-D-glucopyranose.

♦It is a disaccharide obtained from milk, consist of one unit of glucose and one unit of galactose.♦

♦The label states the effect where it is the granulated powder.♦

♦**Description** Lactose Hydrate occurs as white, crystals, powder or granulated powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).♦

Identification Determine the infrared absorption spectrum of Lactose Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with ♦the Reference Spectrum or ♦the spectrum of Lactose Hydrate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +54.4 – +55.9°. Weigh accurately about 10 g of Lactose Hydrate, calculated on the anhydrous basis, dissolve in 80 mL of water warmed to 50°C, and add 0.2 mL of ammonia TS after cooling. After standing for 30 minutes, add water to make exactly 100 mL, and deter-

mine the optical rotation of this solution in a 100-mm cell.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lactose Hydrate in 10 mL of hot water: the solution is clear, and colorless or nearly colorless. Determine the absorbance at 400 nm of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.04.

(2) Acidity or alkalinity—Dissolve 6 g of Lactose Hydrate in 25 mL of freshly boiled and cooled water by heating, and after cooling, add 0.3 mL of phenolphthalein TS: the solution is colorless. To this solution add 0.4 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

♦(3) Heavy metals <1.07>—Dissolve 4.0 g of Lactose Hydrate in 20 mL of warm water, add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL. Proceed with this solution according to Method 1, and perform the test. Prepare the control solution with 1 mL of 0.1 mol/L hydrochloric acid TS and 2.0 mL of Standard Lead Solution (not more than 5 ppm).♦

(4) Proteins and light absorbing substances—Dissolve 1.0 g of Lactose Hydrate in water to make 100 mL, and use this solution as the sample solution. Determine the absorbances as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the control solution: not more than 0.25 at between 210 nm and 220 nm, and not more than 0.07 at between 270 nm and 300 nm.

♦Loss on drying <2.41> Not more than 0.5%. For the granulated powder, not more than 1.0% (1 g, 80°C, 2 hours).♦

Water <2.48> 4.5 – 5.5%. ♦For the granulated powder, 4.0 – 5.5% ♦ (1 g, direct titration. Use a mixture of methanol for Karl Fischer method and formamide for Karl Fischer method (2:1) instead of methanol for Karl Fischer method).

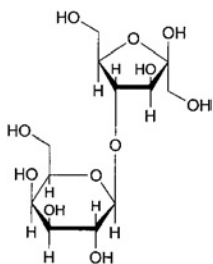
Residue on ignition <2.44> Not more than 0.1% (1 g).

♦**Microbial limit <4.05>** The total viable aerobic microbial count is not more than 100 per g, and the total count of fungi and yeast is not more than 50 per g. *Salmonella* and *Escherichia coli* should not be observed.♦

♦**Containers and storage** Containers—Well-closed containers.♦

Lactulose

ラクツロース



$C_{12}H_{22}O_{11}$: 342.30

β -D-Galactopyranosyl-(1→4)-D-fructose [4618-18-2]

Lactulose is a solution of lactulose prepared by

isomerizing lactose under the existing of alkaline and purified by ion-exchange resin.

It contains not less than 50.0% and not more than 56.0% of $C_{12}H_{22}O_{11}$.

Description Lactulose occurs as a clear, colorless or light yellow, viscous liquid. It is odorless, and has a sweet taste.

It is miscible with water and with formamide.

Identification (1) To 0.7 g of Lactulose add 10 mL of water, 10 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 25) and 0.2 mL of acetic acid (100), and heat in a water bath for 5 to 10 minutes: a blue color develops.

(2) Mix 0.3 g of Lactulose and 30 mL of water, add 16 mL of 1 mol/L iodine TS, then immediately add 2.5 mL of 8 mol/L sodium hydroxide TS, allow to stand for 7 minutes, and add 2.5 mL of diluted sulfuric acid (3 in 20). To this solution add a saturated solution of sodium sulfite heptahydrate until the solution turns light yellow, then add 3 drops of methyl orange TS, neutralize with a solution of sodium hydroxide (4 in 25), and add water to make 100 mL. To 10 mL of this solution add 5 mL of Fehling's TS, and boil for 5 minutes: a red precipitate is produced.

pH <2.54> To 2.0 g of Lactulose add water to make 15 mL: the pH of the solution is between 3.5 and 5.5.

Specific gravity <2.56> d_{20}^{20} : 1.320 – 1.360

Purity (1) Heavy metals <1.07>—Proceed with 5.0 g of Lactulose according to Method 4, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lactulose according to Method 1, and perform the test (not more than 2 ppm).

(3) Galactose and lactose—Determine the heights of the peaks corresponding to D-galactose and lactose respectively, on the chromatogram obtained in Assay from the sample solution and the standard solution, and calculate the ratios of the peak heights of D-galactose and lactose to that of the internal standard from the sample solution, Q_{Ta} and Q_{Tb} , and then from the standard solution, Q_{Sa} and Q_{Sb} : it contains D-galactose of not more than 11%, and lactose of not more than 6%.

$$\begin{aligned} &\text{Amount (mg) of D-galactose (C}_6\text{H}_{12}\text{O}_6) \\ &= W_S \times (Q_{Ta}/Q_{Sa}) \end{aligned}$$

W_S : Amount (mg) of D-galactose

$$\begin{aligned} &\text{Amount (mg) of lactose (C}_{12}\text{H}_{22}\text{O}_{11}\cdot\text{H}_2\text{O}) \\ &= W_S \times (Q_{Tb}/Q_{Sb}) \end{aligned}$$

W_S : Amount (mg) of lactose Hydrate

Loss on drying <2.41> Not more than 35% (0.5 g, in vacuum, 80°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Lactulose, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.5 g of Lactulose Reference Standard, accurately about 80 mg of D-galactose and accurately about 40 mg of lactose monohydrate, add exactly 10 mL of the internal standard solution and water to make 50 mL, and

use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of lactulose to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of } \text{C}_{12}\text{H}_{22}\text{O}_{11} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Lactulose Reference Standard

Internal standard solution—A solution of D-mannitol (1 in 20).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column 8 mm in inside diameter and 50 cm in length, packed with gel type strong acid ion-exchange resin for liquid chromatography (degree of crosslinkage: 6%) (11 μm in particle diameter).

Column temperature: A constant temperature of about 75°C.

Mobile phase: Water.

Flow rate: Adjust the flow rate so that the retention time of lactulose is about 18 minutes.

System suitability—

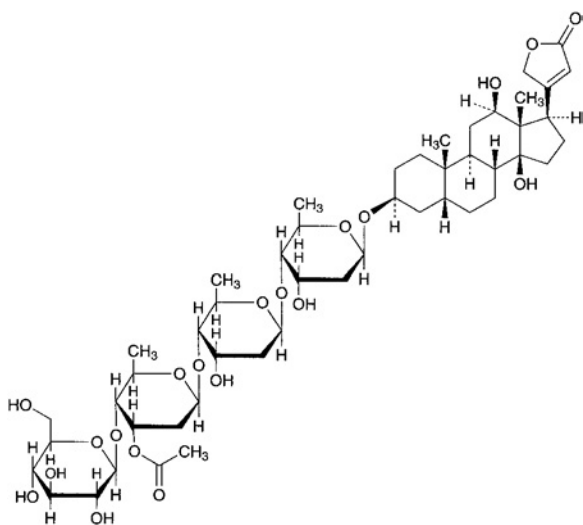
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lactulose and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak heights of lactulose, galactose and lactose to the height of the internal standard are not more than 2.0%, respectively.

Containers and storage Containers—Tight containers.

Lanatoside C

ラナトシド C



$\text{C}_{49}\text{H}_{76}\text{O}_{20}$: 985.12

3 β -[β -D-Glucopyranosyl-(1 \rightarrow 4)-3-O-acetyl-2,6-dideoxy- β -D-ribohexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribohexopyranosyl-(1 \rightarrow 4)-2,6-dideoxy- β -D-ribohexopyranosyloxy]-12 β ,14-dihydroxy-5 β ,14 β -card-20(22)-enolide [I7575-22-3]

Lanatoside C, when dried, contains not less than 90.0% and not more than 102.0% of $\text{C}_{49}\text{H}_{76}\text{O}_{20}$.

Description Lanatoside C occurs as colorless or white crystals or a white, crystalline powder. It is odorless.

It is soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is hygroscopic.

Identification Place 1 mg of Lanatoside C to a small test tube having an internal diameter of about 10 mm, dissolve in 1 mL of a solution of iron (III) chloride hexahydrate in acetic acid (100) (1 in 10,000), and underlay gently with 1 mL of sulfuric acid: at the zone of contact of the two liquids, a brown ring is produced, and the color of the upper layer near the contact zone gradually changes to blue through purple. Finally the color of the entire acetic acid layer changes to blue-green through deep blue.

Purity Related substances—Dissolve 10 mg of Lanatoside C in exactly 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1.0 mg of Lanatoside C Reference Standard in exactly 5 mL of methanol, and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with these solutions. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: any spots other than the principal spot from the sample solution are neither larger nor darker than the spot from the standard solution.

Optical rotation <2.49> $[\alpha]_D^{20}$: +32 – +35° (after drying, 0.5 g, methanol, 25 mL, 100 mm).

Loss on drying <2.41> Not more than 7.5% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 50 mg each of Lanatoside C and Lanatoside C Reference Standard, previously dried, and dissolve in methanol to make exactly 25 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and standard solution into 25-mL light-resistant, volumetric flasks, and add 5 mL of 2,4,6-trinitrophenol TS and 0.5 mL of a solution of sodium hydroxide (1 in 10), shake well, and add methanol to make 25 mL. Allow these solutions to stand between 18°C and 22°C for 25 minutes, and determine the absorbances, A_T and A_S , of the solutions at 485 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of methanol in the same manner as the blank solution.

$$\begin{aligned} &\text{Amount (mg) of } \text{C}_{49}\text{H}_{76}\text{O}_{20} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Lanatoside C Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Lanatoside C Tablets

ラナトシド C 錠

Lanatoside C Tablets contain not less than 90% and not more than 110% of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$: 985.12).

Method of preparation Prepare as directed under Tablets, with Lanatoside C.

Identification (1) Shake a quantity of powdered Lanatoside C Tablets, equivalent to 1 mg of Lanatoside C according to the labeled amount, with 3 mL of diethyl ether, and filter. Wash the residue with two 3-mL portions of diethyl ether, and air-dry. To the remaining residue add 10 mL of a mixture of chloroform and methanol (9:1), shake, and filter. Wash the residue with two 5-mL portions of a mixture of chloroform and methanol (9:1), combine the filtrate and washings, and evaporate on a water bath to a smaller volume. Transfer the solution to a small test tube having an internal diameter of about 10 mm, further evaporate on a water bath to dryness, and proceed as directed in the Identification under Lanatoside C.

(2) Perform the test with the sample solution and the standard solution obtained in the Assay as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, methanol and water (84:15:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 10 minutes: the spots obtained from the sample solution and the standard solution show a black color, and have the same *R_f* values.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Warm 1 tablet of Lanatoside C Tablets with 5 mL of water until the tablet is disintegrated, add 30 mL of ethanol (95), disperse finely the particles with the aid of ultrasonic radiation, add ethanol (95) to make exactly *V* mL of a solution containing about 5 μ g of lanatoside C ($C_{49}H_{76}O_{20}$) in each mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Lanatoside C Reference Standard, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 2 mL of this solution, add 10 mL of water, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of the sample solution, the standard solution and diluted ethanol (95) (17 in 20) into three brown glass-stoppered test tubes T, S and B, previously containing exactly 10 mL of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, add exactly 1 mL each of dilute hydrogen peroxide TS immediately, shake vigorous-

ly, and allow to stand at a constant temperature between 25°C and 30°C for 40 minutes. Determine the fluorescence intensities, F_T , F_S and F_B , of the subsequent solutions from the sample solution and the standard solution and the diluted ethanol (95) (17 in 20) at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>, respectively.

$$\begin{aligned} & \text{Amount (mg) of lanatoside C (C}_{49}\text{H}_{76}\text{O}_{20}\text{)} \\ & = W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (V/5000) \end{aligned}$$

W_S : Amount (mg) of Lanatoside C Reference Standard

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Take 1 tablet of Lanatoside C Tablets, and perform the test with 500 mL of diluted hydrochloric acid (3 in 500) deaired by a suitable method as the test solution at 100 revolutions per minute as directed in the Paddle method. Take 20 mL of the dissolved solution at 60 minutes after starting the test, and filter through a membrane filter (not more than 0.8 μ m). Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Lanatoside C Reference Standard in vacuum over phosphorus (V) oxide at 60°C for 4 hours, weigh accurately a portion of it, equivalent to 100 times an amount of the labeled amount of lanatoside C ($C_{49}H_{76}O_{20}$), dissolve in ethanol (95) to make exactly 100 mL. Pipet 1 mL of this solution, add the test solution to make exactly 500 mL, warm at 37 \pm 0.5°C for 60 minutes, and use this solution as the standard solution. Pipet 3 mL each of the sample solution, the standard solution and the test solution, and transfer to glass-stoppered brown test tubes T, S and B, respectively. To these solutions add exactly 10 mL each of 0.012 w/v% L-ascorbic acid-hydrochloric acid TS, and shake. Immediately add exactly 0.2 mL each of diluted hydrogen peroxide TS (1 in 100), shake well, and allow to stand at a constant temperature between 30°C and 37°C for 45 minutes. Determine immediately the fluorescence intensities, F_T , F_S and F_B , of the sample solution and the standard solution at 355 nm of the excitation wavelength and at 490 nm of the fluorescence wavelength as directed under Fluorometry <2.22>.

Dissolution rate of each of six Lanatoside C Tablets after 60 minutes should be not less than 65%.

Requirement of retest is not applied to Lanatoside C Tablets.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ to labeled amount of} \\ & \text{lanatoside C (C}_{49}\text{H}_{76}\text{O}_{20}\text{)} \\ & = W_S \times \{(F_T - F_B)/(F_S - F_B)\} \times (1/C) \end{aligned}$$

W_S : Amount (mg) of Lanatoside C Reference Standard.

C: Labeled amount (mg) of lanatoside C ($C_{49}H_{76}O_{20}$) in each tablet.

Assay Weigh accurately and powder not less than 20 Lanatoside C Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of lanatoside C ($C_{49}H_{76}O_{20}$), into a 100-mL light-resistant volumetric flask, add 50 mL of ethanol (95), and shake for 15 minutes. Then dilute with ethanol (95) to make exactly 100 mL. Filter this solution, discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 5 mg of Lanatoside C Reference Standard, previously dried in vacuum over phosphorus (V) oxide at 60°C for 4 hours, dissolve in ethanol (95) to make exactly 100

mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into light-resistant, glass-stoppered test tubes, add 3 mL each of alkaline 2,4,6-trinitrophenol TS, shake well and allow these solutions to stand between 22°C and 28°C for 25 minutes. Determine the absorbances, A_T and A_S , of the subsequent sample solution and the subsequent standard solution at 490 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by the same manner with 5 mL of ethanol (95), as the blank.

$$\begin{aligned} &\text{Amount (mg) of lanatoside C (C}_{49}\text{H}_{76}\text{O}_{20}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Lanatoside C Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Hydrous Lanolin

加水ラノリン

Hydrous Lanolin is Purified Lanolin to which water is added. It contains not less than 70% and not more than 75% of Purified Lanolin (as determined by the test for Residue on evaporation).

Description Hydrous Lanolin is a yellowish white, ointment-like substance, and has a slight, characteristic odor, which is not rancid.

It is soluble in diethyl ether and in cyclohexane, with the separation of water.

When melted by heating on a water bath, it separates into a clear oily layer and a clear water layer.

Melting point: about 39°C

Identification Dissolve 1 g of Hydrous Lanolin in 50 mL of cyclohexane, and remove the separated water. Superimpose carefully 1 mL of the cyclohexane solution on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and sulfuric acid layer shows a green fluorescence.

Acid value <1.13> Not more than 1.0.

Iodine value 18 – 36 Heat a suitable amount of Hydrous Lanolin on a water bath to remove its almost moisture, then weigh accurately about 0.8 g of the treated Hydrous Lanolin in a glass-stoppered 500-mL flask, and add 10 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus's TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in a light-resistant, well-closed container while occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination in the same manner.

$$\text{Iodine value} = [(a - b) \times 1.269] / W$$

W : amount (g) of sample.

a : Volume (mL) of 0.1 mol/L sodium thiosulfate VS consumed in the blank determination.

b : Volume (mL) of 0.1 mol/L sodium thiosulfate VS con-

sumed in the titration.

Purity (1) Acidity or alkalinity—To 5 g of Hydrous Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride <1.03>—To 2.0 g of Hydrous Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of the dried residue obtained in the Residue on evaporation in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. Add dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observed in the same level with the spot of standard solution. For this test use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Residue on evaporation Weigh accurately about 12.5 g of Hydrous Lanolin, dissolve in 50 mL of diethyl ether, place it in a separator, transfer the separated aqueous layer to another separator, add 10 mL of diethyl ether, shake, and combine the diethyl ether layer and diethyl ether in the first separator. Shake the diethyl ether layer with 3 g of anhydrous sodium sulfate, and filter through dry filter paper. Wash the separator and the filter paper with two 20-mL portions of diethyl ether, combine the washings with the filtrate, evaporate on a water bath until the odor of diethyl ether is no longer perceptible, and dry in a desiccator (in vacuum, silica gel) for 24 hours: the content is not less than 70% and not more than 75%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Purified Lanolin

Adeps Lanae Rurificatus

精製ラノリン

Purified Lanolin is the purified product of the fat-like substance obtained from the wool of *Ovis aries* Linné (*Bovidae*).

Description Purified Lanolin is a light yellow to yellowish brown, viscous, ointment-like substance, and has a faint, characteristic but not rancid odor.

It is very soluble in diethyl ether and in cyclohexane, freely soluble in tetrahydrofuran and in toluene, and very slightly soluble in ethanol (95). It is practically insoluble in water, but miscible without separation with about twice its mass of water, retaining ointment-like viscosity.

Melting point: 37 – 43°C

Identification Superimpose carefully 1 mL of a solution of Purified Lanolin in cyclohexane (1 in 50) on 2 mL of sulfuric acid: a red-brown color develops at the zone of contact, and the sulfuric acid layer shows a green fluorescence.

Acid value <1.13> Not more than 1.0.

Iodine value 18 – 36 Weigh accurately about 0.8 g of Purified Lanolin in a glass-stoppered 500-mL flask, add 20 mL of cyclohexane to dissolve, and add exactly 25 mL of Hanus' TS, and mix well. If a clear solution is not obtained, add more cyclohexane to make clear, and allow the mixture to stand for 1 hour between 20°C and 30°C in light-resistant, well-closed containers, with occasional shaking. Add 20 mL of a solution of potassium iodide (1 in 10) and 100 mL of water, shake, and titrate the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\text{Iodine value} = [(a - b) \times 1.269] / W$$

W: amount (g) of sample.

a: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the blank determination.

b: Volume (mL) of 0.1 mol/L sodium thiosulfate VS used in the titration of the sample.

Purity (1) Acid or alkali—To 5 g of Purified Lanolin add 25 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and separate the aqueous layer: the aqueous layer is neutral.

(2) Chloride <1.03>—To 2.0 g of Purified Lanolin add 40 mL of water, boil for 10 minutes, and cool. Add water to restore the previous mass, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(3) Ammonia—To 10 mL of the aqueous layer obtained in (1) add 1 mL of sodium hydroxide TS, and boil: the gas evolved does not turn moistened red litmus paper to blue.

(4) Water-soluble organic substances—To 5 mL of the aqueous layer obtained in (1) add 0.25 mL of 0.002 mol/L

potassium permanganate VS, and allow to stand for 5 minutes: the red color of the solution does not disappear.

(5) Petrolatum—Dissolve 1.0 g of Purified Lanolin in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the sample solution. And dissolve 20 mg of vaseline in 10 mL of a mixture of tetrahydrofuran and isooctane (1:1), and use this solution as the standard solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 25 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with isooctane to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) on the plate, heat the plate at 80°C for 5 minutes, cool, and examine under ultraviolet light (main wavelength: 365 nm): no fluorescent spot is observable same level of the spot of standard solution. Use a thin-layer plate previously developed with isooctane to the upper end, dried in air, and heated at 110°C for 60 minutes.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Total ash <5.01> Not more than 0.1%.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Lard

Adeps Suillus

豚脂

Lard is the fat obtained from *Sus scrofa* Linné var. *domesticus* Gray (*Suidae*).

Description Lard occurs as a white, soft, unctuous mass, and has a faint, characteristic odor and a bland taste.

It is freely soluble in diethyl ether and in petroleum ether, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: 36 – 42°C

Congealing point of the fatty acids: 36 – 42°C

Acid value <1.13> Not more than 2.0.

Saponification value <1.13> 195 – 203

Iodine value <1.13> 46 – 70

Purity (1) Moisture and coloration—Melt 5 g of Lard by heating on a water bath: it forms a clear liquid, from which no water separates. Observe the liquid in a layer 10 mm thick: the liquid is colorless to slightly yellow.

(2) Alkalinity—To 2.0 g of Lard add 10 mL of water, melt by warming on a water bath, and shake vigorously. After cooling, add 1 drop of phenolphthalein TS to the separated water layer: the layer is colorless.

(3) Chloride <1.03>—To 1.5 g of Lard add 30 mL of ethanol (95), boil for 10 minutes under a reflux condenser, and filter after cooling. To 20 mL of the filtrate add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50): the opalescence of the mixture does not exceed that of the follow-

ing control solution.

Control solution: To 1.0 mL of 0.01 mol/L hydrochloric acid VS add ethanol (95) to make 20 mL, and add 5 drops of a solution of silver nitrate in ethanol (95) (1 in 50).

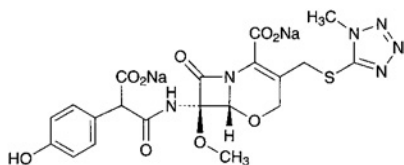
(4) Beef tallow—Dissolve 5 g of Lard in 20 mL of diethyl ether, stopper lightly with absorbent cotton, and allow to stand at 20°C for 18 hours. Collect the separated crystals, moisten them with ethanol (95), and examine under a microscope of 200 magnifications: the crystals are in the form of rhomboidal plates grouped irregularly, and do not contain prisms or needles grouped in fan-shaped clusters.

Containers and storage Containers—Well-closed containers.

Storage—Not exceeding 30°C.

Latamoxef Sodium

ラタモキセフナトリウム



$C_{20}H_{18}N_6Na_2O_9S$: 564.44

Disodium (6*R*,7*R*)-7-[2-carboxylato-2-(4-hydroxyphenyl)acetamino]-7-methoxy-3-(1-methyl-1*H*-tetrazol-5-ylsulfanylmethyl)-8-oxo-5-oxa-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate [64953-12-4]

Latamoxef Sodium contains not less than 830 μ g (potency) and not more than 940 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Latamoxef Sodium is expressed as mass (potency) of latamoxef ($C_{20}H_{20}N_6O_9S$: 520.47).

Description Latamoxef Sodium occurs as white to light yellowish white, powder or masses.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Latamoxef Sodium (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Latamoxef Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Latamoxef Sodium in heavy water for nuclear magnetic resonance spectroscopy (1 in 10) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits single signals, A and B, at around δ 3.5 ppm and at around δ 4.0 ppm. The ratio of the integrated intensity of

these signals, A:B, is about 1:1.

(4) Latamoxef Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-32 - -40^\circ$ (0.5 g calculated on the anhydrous basis, phosphate buffer solution, pH 7.0, 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Latamoxef Sodium in 10 mL of water is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Latamoxef Sodium in 10 mL of water: the solution is clear and pale yellow.

(2) Heavy metals <1.07>—Carbonize 1.0 g of Latamoxef Sodium by heating gently, previously powdered if it is masses. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (1 in 10), and burn the ethanol. After cooling, add 1 mL of sulfuric acid. Proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution by dissolving 1.0 g of Latamoxef Sodium in 20 mL of water, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve an amount of Latamoxef Sodium, equivalent to about 25 mg (potency), in water to make exactly 50 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of 1-methyl-1*H*-tetrazole-5-thiol, having the relative retention time of about 0.5 with respect to the first eluted peak of the two peaks of latamoxef, obtained from the sample solution is not more than the peak area of latamoxef from the standard solution, and the peak area of decarboxylatamoxef, having the relative retention time of about 1.7 with respect to the first peak of the two peaks of latamoxef, is not more than 2 times that of latamoxef from the standard solution. For this calculation, use the peak area for 1-methyl-1*H*-tetrazole-5-thiol after multiplying by its response factor, 0.52.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of latamoxef is not more than 2.0%.

Water <2.48> Not more than 5.0% (0.5 g, volumetric titration, back titration).

Isomer ratio Dissolve 25 mg of Latamoxef Sodium in water to make 50 mL, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, A_a and A_b , of the

two peaks in order of elution, which appear close to each other at the retention time of about 10 minutes: A_a/A_b is between 0.8 and 1.4.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.7 g of ammonium acetate in water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of the first eluted peak of latamoxef is about 8 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the sample solution under the above operating conditions, the resolution between the two peaks of latamoxef is not less than 3.

System repeatability: When the test is repeated 3 times with 5 μ L of the sample solution under the above operating conditions, the relative standard deviation of the area of the first eluted peak of latamoxef is not more than 2.0%.

Assay Weigh accurately an amount of Latamoxef Sodium and Latamoxef Ammonium Reference Standard, equivalent to about 25 mg (potency) each, dissolve in exactly 5 mL of the internal standard solution, add water to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of latamoxef to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of latamoxef (C}_{20}\text{H}_{20}\text{N}_6\text{O}_9\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Latamoxef Ammonium Reference Standard

Internal standard solution—A solution of *m*-cresol (3 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.94 g of potassium dihydrogen phosphate, 3.22 g of disodium hydrogen phosphate dodecahydrate and 1.60 g of tetra *n*-butylammonium bromide in water to make exactly 1000 mL. To 750 mL of this solution add 250 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of latamoxef is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, latamoxef and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of latamoxef to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Not exceeding 5°C.

Lauromacrogol

Polyoxyethylene Lauryl Alcohol Ether

ラウロマクロゴール

Lauromacrogol is a polyoxyethylene ether prepared by the polymerization of ethylene oxide with lauryl alcohol.

Description Lauromacrogol is a colorless or light yellow, clear liquid or a white, petrolatum-like or waxy solid. It has a characteristic odor, and a somewhat bitter and slightly irritative taste.

It is very soluble in ethanol (95), in diethyl ether and in carbon tetrachloride.

It is freely soluble or dispersed as fine oily drops in water.

Identification (1) Shake well 0.5 g of Lauromacrogol with 10 mL of water and 5 mL of ammonium thiocyanate-cobalt nitrate TS, then shake with 5 mL of chloroform, and allow to stand: the chloroform layer becomes blue in color.

(2) Dissolve 0.35 g of Lauromacrogol in 10 mL of carbon tetrachloride, and perform the test as directed in the Solution method under Infrared Spectrophotometry <2.25> using a 0.1-mm fixed cell: it exhibits absorption at the wave numbers of about 1347 cm^{-1} , 1246 cm^{-1} and 1110 cm^{-1} .

Purity (1) Acidity—Transfer 10.0 g of Lauromacrogol into a flask, and add 50 mL of neutralized ethanol. Heat on a water bath nearly to boil, shaking once or twice while heating. Cool, and add 5.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

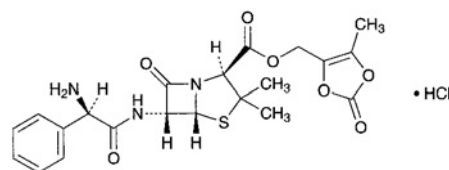
(2) Unsaturated compound—Shake 0.5 g of Lauromacrogol with 10 mL of water, and add 5 drops of bromine TS: the color of the solution does not disappear.

Residue on ignition <2.44> Not more than 0.2% (1 g).

Containers and storage Containers—Tight containers.

Lenampicillin Hydrochloride

レナンピシリン塩酸塩



$\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_7\text{S} \cdot \text{HCl}$: 497.95

5-Methyl-2-oxo[1,3]dioxol-4-ylmethyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetyl-amino]-3,3-dimethyl-7-

oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [80734-02-7]

Lenampicillin Hydrochloride is the hydrochloride of ampicillin methyloxodioxolenylmethyl ester.

It contains not less than 653 μg (potency) and not more than 709 μg (potency) per mg, calculated on the anhydrous basis and corrected by the amount of the residual solvents. The potency of Lenampicillin Hydrochloride is expressed as mass (potency) of ampicillin ($\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}$: 349.40).

Description Lenampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in water, in methanol and in ethanol (95), and freely soluble in *N,N*-dimethylformamide.

Identification (1) Determine the infrared absorption spectrum of Lenampicillin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Lenampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 mL of a solution of Lenampicillin Hydrochloride (1 in 100) add 0.5 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +174 – +194° (0.2 g calculated on the anhydrous de-residual solventization basis, ethanol (95), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Lenampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lenampicillin Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(3) Free ampicillin—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Ampicillin Reference Standard, equivalent to about 25 mg (potency), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. The sample solution should be used to the following test immediately after the solution is prepared. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_{T} and Q_{S} , of the peak height of ampicillin to that of the internal standard: the amount of ampicillin is not more than 1.0%.

$$\begin{aligned} &\text{Amount (\%)} \text{ of ampicillin } (\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S}) \\ &= (W_{\text{S}}/W_{\text{T}}) \times (Q_{\text{T}}/Q_{\text{S}}) \times 2 \end{aligned}$$

W_{S} : Amount [mg (potency)] of Ampicillin Reference Standard

W_{T} : Amount (mg) of the sample

Internal standard solution—A solution of anhydrous caffeine in the mobile phase (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.22 g of potassium dihydrogen phosphate in water to make 900 mL, and add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of ampicillin to that of the internal standard is not more than 5%.

(4) Penicilloic acid—Weigh accurately about 0.1 g of Lenampicillin Hydrochloride, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution, add 10 mL of potassium hydrogen phthalate buffer solution, pH 4.6 and exactly 10 mL of 0.005 mol/L iodine VS, allow to stand for exactly 15 minutes while protecting from exposure to light, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction: the amount of penicilloic acid ($\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_5\text{S}$: 367.42) is not more than 3.0%.

$$\begin{aligned} &\text{Each mL of 0.01 mol/L sodium thiosulfate VS} \\ &= 0.45 \text{ mg of } \text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_5\text{S} \end{aligned}$$

(5) Residual solvent <2.46>—Weigh accurately about 0.25 g of Lenampicillin Hydrochloride, dissolve in exactly 1 mL of the internal standard solution, add *N,N*-dimethylformamide to make 5 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of 2-propanol and about 0.12 g of ethyl acetate, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL and 3 mL of this solution, add exactly 1 mL each of the internal standard solution, add *N,N*-dimethylformamide to make 5 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with 4 μL each of the sample solution, standard solution (1) and standard solution (2) as directed under Gas Chromatography <2.02> according to the following conditions, and determine the ratios, Q_{Ta} and Q_{Tb} , of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the sample solution, the ratios, Q_{Sa1} and Q_{Sb1} , of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (1) and the ratios, Q_{Sa2} and Q_{Sb2} , of the peak height of 2-propanol and ethyl acetate to that of the internal standard of the standard solution (2). Calculate the amounts of 2-propanol and ethyl acetate by the following equations: not more than 0.7% and not more than 1.7%, respectively.

$$\begin{aligned} &\text{Amount (\%)} \text{ of 2-propanol} \\ &= (W_{\text{Sa}}/W_{\text{T}}) \times \{(2Q_{\text{Ta}} - 3Q_{\text{Sa1}} + Q_{\text{Sa2}})/(Q_{\text{Sa2}} - Q_{\text{Sa1}})\} \end{aligned}$$

Amount (%) of ethyl acetate
 $= (W_{Sb}/W_T) \times \{(2Q_{Tb} - 3Q_{Sb1} + Q_{Sb2}) / (Q_{Sb2} - Q_{Sb1})\}$

W_{Sa} : Amount (g) of 2-propanol

W_{Sb} : Amount (g) of ethyl acetate

W_T : Amount (g) of the sample

Internal standard solution—A solution of cyclohexane in *N,N*-dimethylformamide (1 in 1000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 3 m in length, packed with siliceous earth for gas chromatography (180–250 μ m in particle diameter) coated with tetrakis(hydroxypropylethylenediamine) for gas chromatography at the ratio of 10 to 15%.

Column temperature: A constant temperature of about 80°C.

Injection port temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 1 minute.

System suitability—

System performance: When the procedure is run with 4 μ L of the standard solution (2) under the above operating conditions, the internal standard, ethyl acetate and 2-propanol are eluted in this order, and the resolution between the peaks of the internal standard and ethyl acetate is not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 μ L of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak height of ethyl acetate to that of the internal standard is not more than 5.0%.

Water <2.48> Not more than 1.5% (1 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately an amount of Lenampicillin Hydrochloride and Lenampicillin Hydrochloride Reference Standard, equivalent to about 0.1 g (potency), dissolve each in the internal standard solution to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of lenampicillin to that of the internal standard.

Amount [μ g (potency)] of ampicillin ($C_{16}H_{19}N_3O_4S$)
 $= W_S \times (Q_T/Q_S) \times 1000$

W_S : Amount [mg (potency)] of Lenampicillin Hydrochloride Reference Standard

Internal standard solution—A solution of ethyl aminobenzoate in the mobile phase (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about

25°C.

Mobile phase: Dissolve 9.53 g of potassium dihydrogen phosphate in water to make exactly 700 mL, and add acetonitrile to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of lenampicillin is about 6 minutes.

System suitability—

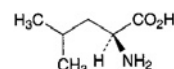
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, lenampicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lenampicillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Leucine

L-ロイシン



$C_6H_{13}NO_2$: 131.17

(2*S*)-2-Amino-4-methylpentanoic acid [61-90-5]

L-Leucine, when dried, contains not less than 98.5% of $C_6H_{13}NO_2$.

Description L-Leucine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Leucine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +14.5 – +16.0° (after drying, 1 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Leucine in 100 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Leucine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Leucine in 40 mL of water and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Leucine in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the

test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Leucine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Leucine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Leucine according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Leucine by warming, after cooling, add water to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

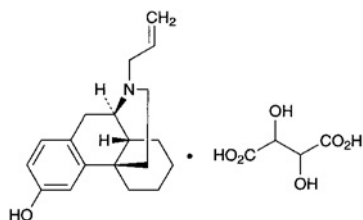
Assay Weigh accurately about 0.13 g of L-Leucine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 13.12 mg of C₆H₁₃NO₂

Containers and storage Containers—Well-closed containers.

Levallorphan Tartrate

レバロルファン酒石酸塩



C₁₉H₂₅NO·C₄H₆O₆: 433.49
17-Allylmorphinan-3-ol monotartrate [71-82-9]

Levallorphan Tartrate, when dried, contains not less than 98.5% of C₁₉H₂₅NO·C₄H₆O₆.

Description Levallorphan Tartrate occurs as a white to pale

yellow, crystalline powder. It is odorless.

It is soluble in water and in acetic acid (100), sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Levallorphan Tartrate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Levallorphan Tartrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Levallorphan Tartrate (1 in 30) responds to the Qualitative Tests <1.09> (1) and (2) for tartrate.

Optical rotation <2.49> [α]_D²⁰: -37.0 - -39.2° (after drying, 0.2 g, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.2 g of Levallorphan Tartrate in 20 mL of water: the pH of this solution is between 3.3 and 3.8.

Melting point <2.60> 174 - 178°C

Purity (1) Clarity and color of solution—Dissolve 0.2 g of Levallorphan Tartrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Levallorphan Tartrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Levallorphan Tartrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia TS (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Levallorphan Tartrate, previously dried, dissolve in 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.35 mg of C₁₉H₂₅NO·C₄H₆O₆

Containers and storage Containers—Well-closed containers.

Levallorphan Tartrate Injection

レバロルファン酒石酸塩注射液

Levallorphan Tartrate Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of levallorphan tartrate ($C_{19}H_{25}NO \cdot C_4H_6O_6$; 433.49).

Method of preparation Prepare as directed under Injection, with Levallorphan Tartrate.

Description Levallorphan Tartrate Injection is a clear, colorless liquid.

pH: 3.0 – 4.5

Identification Take an exact volume of Levallorphan Tartrate Injection, equivalent to 3 mg of Levallorphan Tartrate according to the labeled amount, add 5 mL of water and 2 drops of dilute hydrochloric acid, and wash with five 15-mL portions of diethyl ether by a vigorous shaking. Take the water layer, evaporate the diethyl ether remained by warming on a water bath, and after cooling, add 0.01 mol/L hydrochloric acid TS to make 50 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 281 nm.

Extractable volume <6.05> It meets the requirement.

Assay Take exactly a volume of Levallorphan Tartrate Injection, equivalent to about 2 mg of levallorphan tartrate ($C_{19}H_{25}NO \cdot C_4H_6O_6$), add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of levallorphan tartrate for assay, previously dried at 80°C for 4 hours on phosphorus (V) oxide under reduced pressure, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions, and calculate the ratios, Q_T and Q_S , of the peak area of levallorphan to that of the internal standard:

$$\begin{aligned} & \text{Amount (mg) of } C_{19}H_{25}NO \cdot C_4H_6O_6 \\ & = W_S \times (Q_T/Q_S) \times (1/50) \end{aligned}$$

W_S : Amount (mg) of levallorphan tartrate for assay

Internal standard solution—Dissolve 0.04 g of isobutyl parahydroxybenzoate in 10 mL of ethanol (95), add water to make 100 mL, and to 10 mL of this solution add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about

40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 300 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of levallorphan is about 12 minutes.

System suitability—

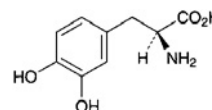
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and levallorphan are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of levallorphan to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Levodopa

レボドパ



$C_9H_{11}NO_4$; 197.19

3-Hydroxy-L-tyrosine [59-92-7]

Levodopa, when dried, contains not less than 98.5% of $C_9H_{11}NO_4$.

Description Levodopa occurs as white or slightly grayish white crystals or crystalline powder. It is odorless.

It is freely soluble in formic acid, slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

The pH of a saturated solution of Levodopa is between 5.0 and 6.5.

Melting point: about 275°C (with decomposition).

Identification (1) To 5 mL of a solution of Levodopa (1 in 1000) add 1 mL of ninhydrin TS, and heat for 3 minutes in a water bath: a purple color develops.

(2) To 2 mL of a solution of Levodopa (1 in 5000) add 10 mL of 4-aminoantipyrine TS, and shake: a red color develops.

(3) Dissolve 3 mg of Levodopa in 0.001 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (280 nm): 136 – 146 (after drying, 30 mg, 0.001 mol/L hydrochloric acid TS, 1000 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: –11.5 – –13.0° (after drying, 2.5 g, 1 mol/L hydrochloric acid TS, 50 mL, 100 nm).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Levodopa in 20 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Levodopa in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.3 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.40 g of Levodopa in 1 mL of dilute hydrochloric acid and 30 mL of water, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.005 mol/L sulfuric acid VS (not more than 0.030%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Levodopa according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 1.0 g of Levodopa in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of Levodopa in 10 mL of sodium disulfite TS, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add sodium disulfite TS to make exactly 25 mL. Pipet 1 mL of this solution, add sodium disulfite TS to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and methanol (10:5:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate and heat at 90°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

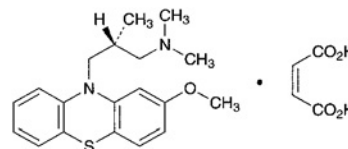
Assay Weigh accurately about 0.3 g of Levodopa, previously dried, dissolve in 3 mL of formic acid, add 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid
= 19.72 mg of C₉H₁₁NO₄

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levomepromazine Maleate

レボメプロマジンマレイン酸塩



C₁₉H₂₄N₂OS·C₄H₄O₄: 444.54

(2*R*)-3-(2-Methoxy-10*H*-phenothiazin-10-yl)-*N,N*, 2-trimethylpropylamine monomaleate [7104-38-3]

Levomepromazine Maleate, when dried, contains not less than 98.0% of C₁₉H₂₄N₂OS·C₄H₄O₄.

Description Levomepromazine Maleate occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Melting point: 184 – 190°C (with decomposition).

Identification (1) Dissolve 5 mg of Levomepromazine Maleate in 5 mL of sulfuric acid: a red-purple color develops, which slowly becomes deep red-purple. To this solution add 1 drop of potassium dichromate TS: a brownish yellow-red color is produced.

(2) To 0.2 g of Levomepromazine Maleate add 5 mL of sodium hydroxide TS and 20 mL of diethyl ether, and shake well. Separate the diethyl ether layer, wash twice with 10-mL portions of water, add 0.5 g of anhydrous sodium sulfate, filter, evaporate the diethyl ether on a water bath, and dry the residue at 105°C for 2 hours: the residue melts <2.60> between 124°C and 128°C.

(3) To 0.5 g of Levomepromazine Maleate add 5 mL of water and 2 mL of ammonia solution (28), extract with three 5-mL portions of chloroform, separate and evaporate the water layer to dryness. To the residue add 2 to 3 drops of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine all the diethyl ether extracts, evaporate the diethyl ether in a water bath at a temperature of about 35°C with the aid of a current of air: the residue melts <2.60> between 128°C and 136°C.

Optical rotation <2.49> [α]_D²⁰: –13.5 – –16.5° (after drying, 0.5 g, chloroform, 20 mL, 200 mm).

Purity (1) Clarity and color of solution—To 0.5 g of Levomepromazine Maleate add 10 mL of methanol, and dissolve by warming: the solution is clear, and colorless or pale yellow.

(2) Chloride <1.03>—Dissolve 0.5 g of Levomepromazine Maleate in 40 mL of methanol, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS, 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of

Levomepromazine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

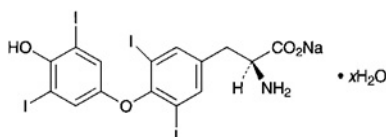
Assay Weigh accurately about 1 g of Levomepromazine Maleate, previously dried, and dissolve in a mixture of 40 mL of acetic acid (100) and 20 mL of acetone for nonaqueous titration. Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red-purple through blue-purple to blue (indicator: 5 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.45 mg of $C_{19}H_{24}N_2OS \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levothyroxine Sodium Hydrate

レボチロキシンナトリウム水和物



$C_{15}H_{10}I_4NNaO_4 \cdot xH_2O$
Monosodium *O*-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-*L*-tyrosinate hydrate [25416-65-3]

Levothyroxine Sodium Hydrate contains not less than 97.0% of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$; 798.85), calculated on the dried basis.

Description Levothyroxine Sodium Hydrate occurs as a pale yellowish white to light yellow-brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification (1) Heat 0.1 g of Levothyroxine Sodium Hydrate over a flame: a purple gas evolves.

(2) To 0.5 mg of Levothyroxine Sodium Hydrate add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and add 0.1 mL of sodium nitrite TS. Allow to stand in a dark place for 20 minutes, and add 1.5 mL of ammonia solution (28): a yellowish red color is produced.

(3) Determine the absorption spectrum of a solution of Levothyroxine Sodium Hydrate in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Moisten Levothyroxine Sodium Hydrate with sulfuric acid, and ignite: the residue responds to the Qualitative Tests

<1.09> (1) and (2) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-5 - -6^\circ$ (0.3 g, calculated on the dried basis, a mixture of ethanol (95) and sodium hydroxide TS (2:1), 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.3 g of Levothyroxine Sodium Hydrate in 10 mL of a mixture of ethanol (95) and sodium hydroxide TS (2:1) by warming: the solution is clear and pale yellow to pale yellow-brown in color.

(2) Soluble halides—Dissolve 0.01 g of Levothyroxine Sodium Hydrate in 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. To the filtrate add water to make 10 mL, then add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.20 mL of 0.01 mol/L hydrochloric acid VS add 10 mL of water and 1 drop of dilute nitric acid, and proceed as directed above.

(3) Related substances—Dissolve 20 mg of Levothyroxine Sodium Hydrate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (14:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (14:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 7 – 11% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Assay Weigh accurately about 25 mg of Levothyroxine Sodium Hydrate, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of sodium hydroxide solution (1 in 100) and 1 mL of a freshly prepared sodium bisulfate solution (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water. Bubble the solution with enough nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.6657 mg of $C_{15}H_{10}I_4NNaO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Levothyroxine Sodium Tablets

レボチロキシンナトリウム錠

Levothyroxine Sodium Tablets contain not less than 90% and not more than 110% of the labeled amount of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$; 798.85).

Method of preparation Prepare as directed under Tablets, with Levothyroxine Sodium Hydrate.

Identification (1) Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 0.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 8 mL of a mixture of water, ethanol (95), hydrochloric acid and sodium hydroxide TS (6:5:2:2), warm in a water bath for 2 minutes, cool, and filter. To the filtrate add 0.1 mL of sodium nitrite TS, and allow to stand in a dark place for 20 minutes. Add 1.5 mL of ammonia solution (28); a yellowish red color develops.

(2) To a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 1 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 10 mL of ethanol (95), shake, filter, and use the filtrate as the sample solution. Dissolve 0.01 g of levothyroxine sodium for thin-layer chromatography in 100 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and heat at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R_f* value.

Purity Soluble halides—Weigh a quantity of powdered Levothyroxine Sodium Tablets, equivalent to 2.5 mg of Levothyroxine Sodium Hydrate according to the labeled amount, add 25 mL of water, warm to 40°C, shake for 5 minutes, add 3 drops of dilute nitric acid, and filter. To the filtrate add 3 drops of silver nitrate TS, and mix: the solution has no more opalescence than the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of water and 3 drops of dilute nitric acid, and proceed as directed above.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Levothyroxine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge this solution,

pipet 5 mL of the supernatant liquid, add 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratio of the peak area of levothyroxine to that of the internal standard. Calculate the mean value from the ratios of each peak area of 10 samples: the deviation (%) of the mean value and the ratio of each peak area should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of the mean value of the 30 samples used in the 2 tests and the ratio of each peak area: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of ethinylestradiol in a mixture of acetonitrile and diluted phosphoric acid (1 in 10) (9:1) (3 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: a constant wavelength between 220 nm and 230 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilylated silica gel.

Column temperature: A constant temperature at about 25°C.

Mobile phase: A mixture of methanol, water and phosphoric acid (1340:660:1).

Flow rate: Adjust the flow rate so that the retention time of levothyroxine is about 9 minutes.

Selection of column: To 5 mL of a solution of levothyroxine sodium in 0.01 mol/L sodium hydroxide TS (1 in 200,000) add 1 mL of the internal standard solution. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of levothyroxine and the internal standard in this order with the resolution between these peaks being not less than 2.0.

Assay Weigh accurately and powder not less than 20 Levothyroxine Sodium Tablets. Weigh accurately a portion of the powder, equivalent to about 3 mg of levothyroxine sodium ($C_{15}H_{10}I_4NNaO_4$), into a crucible, and add potassium carbonate amounting to twice the mass of the powder. In the case that the weighed powder is less than 4 g, add 8 g of potassium carbonate to the crucible. Mix well, and gently tap the crucible on the bench to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again by tapping. Heat the crucible strongly at a temperature between 675°C and 700°C for 25 minutes. Cool, add 30 mL of water, heat gently to boiling, and filter into a flask. To the residue add 30 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with hot water until the filtrate measures 300 mL. Add slowly 7 mL of freshly prepared bromine TS and diluted phosphoric acid (1 in 2) in the ratio of 3.5 mL to 1 g of the added potassium carbonate, and boil until starch-potassium iodide paper is no longer colored blue by the evolved gas. Wash the inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume of not less than 250 mL. Cool, add 5 mL of a solution of phenol (1 in

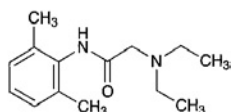
20), again rinse the inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate <2.50> immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.3329 mg of $C_{15}H_{10}I_4NNaO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Lidocaine

リドカイン



$C_{14}H_{22}N_2O$: 234.34
2-Diethylamino-*N*-(2,6-dimethylphenyl)acetamide
[137-58-6]

Lidocaine, when dried, contains not less than 99.0% of $C_{14}H_{22}N_2O$.

Description Lidocaine occurs as white to pale yellow crystals or crystalline powder.

It is very soluble in methanol and in ethanol (95), soluble in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 0.04 g of Lidocaine in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lidocaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 66 – 69°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lidocaine in 2 mL of dilute hydrochloric acid, and add water to make 10 mL: the solution is clear and colorless to light yellow.

(2) Chloride <1.03>—Dissolve 0.6 g of Lidocaine in 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.041%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Lidocaine in 5 mL of dilute hydrochloric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make

50 mL (not more than 0.096%).

(4) Heavy metals <1.07>—Carbonize 2.0 g of Lidocaine by gentle ignition. After cooling, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and fire the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.10 g of Lidocaine in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (5:3:1:1) to a distance of about 10 cm, air-dry the plate, and dry more at 80°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.5 g of Lidocaine, previously dried and accurately weighed, in 20 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 1 drop of crystal violet TS) until the color of the solution changes from purple to blue-green through blue. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 23.43 mg of $C_{14}H_{22}N_2O$

Containers and storage Containers—Tight containers.

Lidocaine Injection

Lidocaine Hydrochloride Injection

リドカイン注射液

Lidocaine Injection is an aqueous injection.

It contains not less than 95% and not more than 105% of the labeled amount of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$: 270.80).

Method of preparation Prepare as directed under Injections, with Lidocaine and an equivalent amount of Hydrochloric Acid.

No preservative is added in the case of intravenous injections.

Description Lidocaine Injection is a colorless, clear liquid.
pH: 5.0 – 7.0

Identification To a volume of Lidocaine Injection, equivalent to 0.02 g of Lidocaine Hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$) according to the labeled amount, add 1 mL of sodium hydroxide TS, and extract with 20 mL of hexane. To 10 mL

of the hexane extract add 20 mL of 1 mol/L hydrochloric acid TS, and shake vigorously. Determine the absorption spectrum of the water layer as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 265 nm.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Perform the test with Lidocaine Injection stored in a container in a volume exceeding 10 mL and intended to intravenous injection: it meets the requirements of the Pyrogen Test.

Assay To an exactly measured volume of Lidocaine Injection, equivalent to about 0.1 g of lidocaine hydrochloride ($C_{14}H_{22}N_2O \cdot HCl$), add exactly 10 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 85 mg of lidocaine for assay, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in 0.5 mL of 1 mol/L hydrochloric acid TS and a suitable volume of 0.001 mol/L hydrochloric acid TS, and add exactly 10 mL of the internal standard solution, then add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of lidocaine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of lidocaine hydrochloride} \\ & (C_{14}H_{22}N_2O \cdot HCl) \\ & = W_S \times (Q_T/Q_S) \times 1.1556 \end{aligned}$$

W_S : Amount (mg) of lidocaine for assay

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of lidocaine is about 6 minutes.

System suitability—

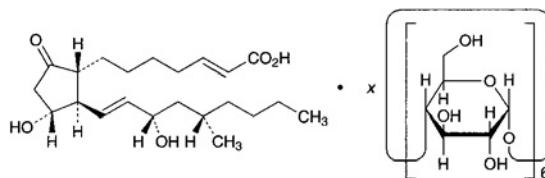
System performance: When proceed with 5 μ L of the standard solution under the above operating conditions, lidocaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of lidocaine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Limaprost Alfadex

リマプロスト アルファデクス



$C_{22}H_{36}O_5 \cdot xC_{36}H_{60}O_{30}$
(2E)-7-[(1R,2R,3R)-3-Hydroxy-2-[(1E,3S,5S)-3-hydroxy-5-methylnon-1-en-1-yl]-5-oxocyclopentyl] hept-2-enoic acid – α -cyclodextrin
[100459-01-6 (limaprost: alfadex = 1:1; clathrate compound)]

Limaprost Alfadex is a α -cyclodextrin clathrate compound of limaprost.

It contains not less than 2.8% and not more than 3.2% of limaprost ($C_{22}H_{36}O_5$; 380.52), calculated on the anhydrous basis.

Description Limaprost Alfadex occurs as a white powder.

It is freely soluble in water, slightly soluble in methanol, very slightly soluble in ethanol (99.5), and practically insoluble in ethyl acetate.

It is hygroscopic.

Identification (1) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and use the upper layer as the sample solution (1). Separately, to 20 mg of Limaprost Alfadex add 5 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution (2). Evaporate the solvent of the sample solutions (1) and (2) under reduced pressure, add 2 mL of sulfuric acid to each of the residue, and shake them for 5 minutes: the solution obtained from the sample solution (1) develops an orange-yellow color while the solution from the sample solution (2) does not develop any color.

(2) Dissolve 20 mg of Limaprost Alfadex in 5 mL of water, add 5 mL of ethyl acetate, shake, centrifuge, and evaporate the solvent of the upper layer under reduced pressure. Dissolve the residue in 2 mL of ethanol (95), 5 mL of 1,3-dinitrobenzene TS, add 5 mL of a solution of potassium hydroxide in ethanol (95) (17 in 100) while ice-cooling, and allow to stand in a dark place while ice-cooling for 20 minutes: a purple color develops.

(3) To 50 mg of Limaprost Alfadex add 1 mL of iodine TS, dissolve by heating in a water bath, and allow to stand: a dark blue precipitate is formed.

(4) Determine the absorption spectrum of a solution of Limaprost Alfadex in dilute ethanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it does not exhibit a maximum between 200 nm and 400 nm. To 10 mL of this solution add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> $[\alpha]_D^{20}$: +125 – 135° (0.1 g, calculated on the anhydrous basis, dilute ethanol, 20 mL, 100 mm)

Purity Related substances—Perform the test immediately after preparation of the sample solution. Dissolve 0.10 g of Limaprost Alfadex in 2 mL of water, add 1 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add dilute ethanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 3 mL of the standard solution (1), add dilute ethanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 3 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following operating conditions, and determine each peak area by the automatic integration method: the area of the peak of 17-epi-isomer, having the relative retention time of about 1.1 with respect to limaprost, and the area of the peak of 11-deoxy substance, having the relative retention time of about 2.1 with respect to limaprost, are not larger than the peak area of limaprost from the standard solution (2), and the area of the peak other than the principal peak and other than the peaks mentioned above is not larger than 1/3 times the peak area of limaprost from the standard solution (2). The total area of the peaks other than limaprost from the samples solution is not larger than the peak area of limaprost from the standard solution (1).

Operating conditions—

Detector, column, column temperature, mobile phase and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of limaprost beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution (1) add dilute ethanol to make exactly 10 mL. Confirm that the peak area of limaprost obtained from 3 μ L of this solution is equivalent to 8 to 12% of that of limaprost obtained from 3 μ L of the standard solution (1).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution (1) under the above conditions, the relative standard deviation of the peak area of limaprost is not more than 2.0%.

Water <2.48> Not more than 6.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Limaprost Alfadex, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 3 mg of Limaprost Reference Standard, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 3 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of limaprost to that of the internal standard.

$$\text{Amount (mg) of limaprost (C}_{22}\text{H}_{36}\text{O}_5) = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Limaprost Reference Standard

Internal standard solution—A solution of propyl para-

hydroxybenzoate in ethanol (95) (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.02 mol/L potassium dihydrogen phosphate TS, acetonitrile for liquid chromatography and 2-propanol for liquid chromatography (9:5:2)

Flow rate: Adjust the flow rate so that the retention time of limaprost is about 12 minutes.

System suitability—

System performance: When the procedure is run with 3 μ L of the standard solution under the above operating conditions, the internal standard and limaprost are eluted in this order with the resolution between these peaks being not less than 7.

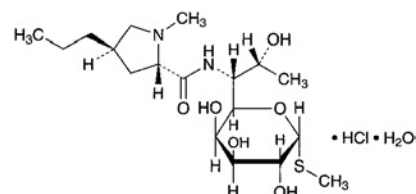
System repeatability: When the test is repeated 6 times with 3 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of limaprost to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding –10°C.

Lincomycin Hydrochloride Hydrate

リンコマイシン塩酸塩水和物



$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$: 461.01

Methyl 6,8-dideoxy-6-[(2S,4R)-1-methyl-4-propylpyrrolidine-2-carboxamido]-1-thio-D-erythro- α -D-galacto-octopyranoside monohydrochloride monohydrate [7179-49-9]

Lincomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces lincolnensis* var. *lincolnensis*.

It contains not less than 825 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Lincomycin Hydrochloride Hydrate is expressed as mass (potency) of lincomycin ($C_{18}H_{34}N_2O_6S$: 406.54).

Description Lincomycin Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in water and in methanol, sparingly soluble in ethanol (95), and very slightly soluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Lincomycin Hydrochloride Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Lincomycin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Lincomycin Hydrochloride Hydrate (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +135 – +150° (0.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Lincomycin Hydrochloride Hydrate in 1 mL of water: 3.0 – 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Lincomycin Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Lincomycin Hydrochloride Hydrate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 5 ppm).

(3) Lincomycin B—Perform the test with 20 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of lincomycin and lincomycin B, having the relative retention time of about 0.5 with respect to lincomycin, by the automatic integration method: the peak area of lincomycin B is not more than 5.0% of the sum of the peak areas of lincomycin and lincomycin B.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of lincomycin obtained from 20 μ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 20 μ L of the sample solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> 3.0 – 6.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Lincomycin Hydrochloride Hydrate and Lincomycin Hydrochloride Reference Standard, equivalent to about 10 mg (potency), dissolve each in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of lincomycin.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of lincomycin (C}_{18}\text{H}_{34}\text{N}_2\text{O}_6\text{S)} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Lincomycin Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 46°C.

Mobile phase: To 13.5 mL phosphoric acid add water to make 1000 mL, and adjust the pH to 6.0 with ammonia TS. To 780 mL of this solution add 150 mL of acetonitrile and 150 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of lincomycin is about 9 minutes.

System suitability—

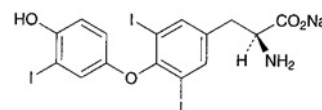
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the theoretical plates and the symmetrical factor of the peak of lincomycin are not less than 4000 and not more than 1.3, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lincomycin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Liothyronine Sodium

リオチロニンナトリウム



$\text{C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4$: 672.96

Monosodium *O*-(4-hydroxy-3-iodophenyl)-3,5-diiodo-L-tyrosinate [55-06-1]

Liothyronine Sodium contains not less than 95.0% of $\text{C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4$, calculated on the dried basis.

Description Liothyronine Sodium occurs as a white to light brown powder. It is odorless.

It is slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) To 5 mL of a solution of Liothyronine Sodium in ethanol (95) (1 in 1000) add 1 mL of ninhydrin TS, and warm in a water bath for 5 minutes: a purple color develops.

(2) Heat 0.02 g of Liothyronine Sodium with a few drops of sulfuric acid over a flame: a purple gas is evolved.

(3) Determine the absorption spectrum of a solution of Liothyronine Sodium in ethanol (95) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Ignite 0.02 g of Liothyronine Sodium until thoroughly charred. After cooling, add 5 mL of water to the residue, shake, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +18 – +22° (0.2 g, calculated on the dried basis, a mixture of ethanol (95) and 1 mol/L hydrochloric acid TS (4:1), 10 mL, 100 mm).

Purity (1) Soluble halide—To 10 mg of Liothyronine Sodium add 10 mL of water and 1 drop of dilute nitric acid, shake for 5 minutes, and filter. Add water to the filtrate to make 10 mL, and mix with 3 drops of silver nitrate TS: the solution shows no more turbidity than the following control solution.

Control solution: To 0.35 mL of 0.01 mol/L hydrochloric acid VS add 1 drop of dilute nitric acid and water to make 10 mL, and add 3 drops of silver nitrate TS.

(2) Iodine and iodide—Dissolve 0.10 g of Liothyronine Sodium in 10 mL of dilute sodium hydroxide TS and 15 mL of water, add 5 mL of dilute sulfuric acid, and allow to stand for 10 minutes with occasional shaking. Filter the mixture into a Nessler tube, add 10 mL of chloroform and 3 drops of a solution of potassium iodate (1 in 100) to the filtrate, mix for 30 seconds, and allow to stand: the chloroform layer has no more color than the following control solution.

Control solution: Weigh exactly 0.111 g of potassium iodide, and dissolve in water to make 1000 mL. Pipet 1 mL of this solution, add 10 mL of dilute hydroxide TS, 14 mL of water and 5 mL of dilute sulfuric acid, and mix. Filter the mixture into a Nessler tube, and perform the test with the filtrate in the same manner as for the sample.

(3) Related substances—Dissolve 0.15 g of Liothyronine Sodium in 5 mL of diluted ammonia TS (1 in 3), and use this solution as the sample solution. Pipet 1 mL of this solution, add diluted ammonia TS (1 in 3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 4.0% (0.2 g, 105°C, 2 hours).

Assay Weigh accurately about 25 mg of Liothyronine Sodium, and proceed as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 10 mL of a solution of sodium hydroxide (1 in 100) and 1 mL of a freshly prepared solution of sodium bisulfate (1 in 100) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and wash C, B and the inner wall of A with 40 mL of water. To the test solution add 1 mL of bromine-acetic acid TS, insert the stopper C, and shake vigorously for 1 minute. Remove the stopper, rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water, and add 0.5 mL of formic acid. Stopper the flask with C, and shake vigorously for 1 minute again. Remove the stopper, and rinse the stopper, the sample holder and the inner wall of the flask with 40 mL of water again. Bubble the solution with enough

nitrogen gas in the flask to remove the oxygen and excess bromine, add 0.5 g of potassium iodide to the solution, and dissolve. Add immediately 3 mL of dilute sulfuric acid, mix, and allow to stand for 2 minutes. Titrate <2.50> the solution with 0.02 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium thiosulfate VS
= 0.7477 mg of $C_{15}H_{11}I_3NNaO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Liothyronine Sodium Tablets

リオチロニンナトリウム錠

Liothyronine Sodium Tablets contain not less than 90% and not more than 110% of the labeled amount of liothyronine sodium ($C_{15}H_{11}I_3NNaO_4$: 672.96).

Method of preparation Prepare as directed under Tablets, with Liothyronine Sodium.

Identification (1) To a glass-stoppered centrifuge tube add a portion of finely powdered Liothyronine Sodium Tablets, equivalent to 0.1 mg of Liothyronine Sodium according to the labeled amount, add 30 mL of dilute sodium hydroxide TS, shake vigorously, and centrifuge. Transfer the supernatant liquid to a separator, add 10 mL of dilute hydrochloric acid, and extract with two 20-mL portions of ethyl acetate. Filter each extract successively through absorbent cotton previously overlaid with 8 g of anhydrous sodium sulfate. Evaporate the filtrate on a water bath to dryness with the aid of a current of nitrogen. Dissolve the residue in 0.5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 10 mg of liothyronine sodium for thin-layer chromatography in methanol to make 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *t*-butanol, *t*-amyl alcohol, water, ammonia solution (28) and 2-butanone (59:32:17:15:7) to a distance of about 12 cm, and air-dry the plate. Spray evenly a solution of 0.3 g of ninhydrin in 100 mL of a mixture of 1-butanol and acetic acid (100) (97:3) on the plate, and dry the plate at 100°C for 3 minutes: the spots obtained from the sample solution and the standard solution show a red-purple color, and has the same *R_f* value.

(2) The colored solution obtained in the Assay is blue in color.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Place 1 tablet of Liothyronine Sodium Tablets in a glass-stoppered centrifuge tube, add exactly 10 mL of 0.01 mol/L sodium hydroxide TS, warm at 50°C for 15 minutes, and shake vigorously for 20 minutes. Centrifuge for 5 minutes, and filter the supernatant liquid, if necessary. Pipet a definite volume of this solution, and add a volume of 0.01 mol/L so-

dium hydroxide VS to prepare a definite volume of a solution containing about 0.5 μg of liothyronine sodium ($\text{C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4$) per mL. Pipet 5 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the sample solution. Perform the test with 200 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratio of the peak area of the liothyronine to that of the internal standard. Calculate the mean value of the ratios of each peak area of 10 samples: the deviation (%) of each ratio of the peak area from the mean value should be not more than 15%. When the deviation (%) is more than 15%, and 1 sample shows not more than 25%, perform another test with 20 samples. Calculate the deviation (%) of each ratio of the peak area from the mean value of the 30 samples used in the two tests: there should be not more than 1 sample with the deviation more than 15% but not more than 25%, and no sample should deviate by more than 25%.

Internal standard solution—A solution of propylparahydroxybenzoate in a mixture of methanol and diluted phosphoric acid (1 in 10) (9:1) (1 in 250,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Diluted methanol (57 in 100).

Flow rate: Adjust the flow rate so that the retention time of liothyronine is about 9 minutes.

System suitability—

System performance: To 5 mL of a solution of liothyronine sodium in 0.01 mol/L sodium hydroxide TS (1 in 2,000,000) add 1 mL of the internal standard solution, and use this solution as the solution for system suitability test. When the procedure is run with 200 μL of this solution under the above operating conditions, the internal standard and liothyronine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 200 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the ratios of the peak area of liothyronine to that of the internal standard is not more than 1.0%.

Assay Weigh accurately not less than 20 Lisinopril Sodium Tablets, and finely powder. Place an accurately weighed portion of the powder, equivalent to about 50 μg of liothyronine sodium ($\text{C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4$), in an agate mortar, add 1 g of powdered potassium carbonate, and mix well. Transfer the mixture cautiously to a porcelain crucible, and compact the contents by gently tapping the crucible on a table. Add an additional 1.5 g of powdered potassium carbonate to the same agate mortar, mix well with any content adhering to the mortar, cautiously overlay the mixture on the top of the same porcelain crucible, and compact the charge again in the same manner. Ignite the combined mixture in the crucible between 675°C and 700°C for 30 minutes. Cool, add a few mL of water to the crucible, heat gently to boiling, and filter the contents of the crucible through a glass filter (G4) into a

20-mL volumetric flask. Wash the residue with water, and combine the washings with the filtrate. Cool, add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of potassium iodide for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 200 mL. Measure exactly 5 mL of the solution, and add a solution of potassium carbonate (1 in 8) to make exactly 100 mL. To 2 mL of this solution, exactly measured, add a solution of potassium carbonate (1 in 8) to make exactly 20 mL, and use the solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution into glass-stoppered test tubes, add 3.0 mL of diluted sulfuric acid (4 in 25) and 2.0 mL of potassium permanganate TS, and heat on a water bath for 15 minutes. Cool, add 1.0 mL of diluted sodium nitrite TS (1 in 10), swirl to mix, and add 1.0 mL of a solution of ammonium amidosulfate (1 in 10). Allow to stand at room temperature for 10 minutes with occasional shaking. Then add 1.0 mL of potato starch TS and 1.0 mL of a freshly prepared, diluted potassium iodide TS (1 in 40), swirl to mix, and transfer each solution to a 20-mL volumetric flask. Rinse the test tube with water, collect the washings in the volumetric flask, add water to make 20 mL, and allow to stand for 10 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of potassium carbonate (1 in 8) in the same manner as the sample solution as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at the wavelength of maximum absorption at about 600 nm, respectively.

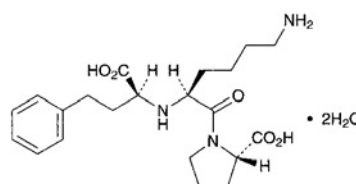
$$\text{Amount (mg) of liothyronine sodium (C}_{15}\text{H}_{11}\text{I}_3\text{NNaO}_4) = W_S \times (A_T/A_S) \times (1/2000) \times 1.3513$$

W_S : Amount (mg) of potassium iodide for assay

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Lisinopril Hydrate

リシノプリル水和物



$\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5 \cdot 2\text{H}_2\text{O}$: 441.52
(2S)-1-[(2S)-6-Amino-2-[(1S)-1-carboxy-3-phenylpropylamino]hexanoyl]pyrrolidine-2-carboxylic acid dihydrate
[83915-83-7]

Lisinopril Hydrate contains not less than 98.5% and not more than 101.0% of lisinopril ($\text{C}_{21}\text{H}_{31}\text{N}_3\text{O}_5$: 405.49), calculated on the anhydrous basis.

Description Lisinopril Hydrate occurs as a white crystalline powder, having a slight characteristic odor.

It is soluble in water, sparingly soluble in methanol, and

practically insoluble in ethanol (99.5).

Melting point: about 160°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Lisinopril Hydrate in methanol (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Lisinopril Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: $-43.0 - -47.0^\circ$ (0.25 g calculated on the anhydrous basis, 0.25 mL/L zinc acetate buffer solution, pH 6.4, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Lisinopril Hydrate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve about 0.10 g of Lisinopril Hydrate in 50 mL of water, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 1.2 with respect to lisinopril, is not larger than 1/5 times the peak area of lisinopril from the standard solution, the area of the peak other than lisinopril and the peak mentioned above is not larger than 2/15 times the peak area of lisinopril from the standard solution, and the total area of the peaks other than lisinopril is not larger than the peak area of lisinopril from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2).

Mobile phase B: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	90 → 50	10 → 50
10 – 25	50	50

Flow rate: About 1.5 mL per minute.

Time span of measurement: About 2.5 times as long as the retention time of lisinopril beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2.5 mL of the standard solution, and add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μ L of this solution is equivalent to 3.5 to 6.5% of that with 15 μ L of the standard solution.

System performance: To 10 mg of Lisinopril Hydrate and 2 mL of a solution of anhydrous caffeine (1 in 1000) add water to make 200 mL. When the procedure is run with 15 μ L of this solution under the above operating conditions, lisinopril and caffeine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 15 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Water <2.48> Not less than 8.0% and not more than 9.5% (0.3 g, volumetric titration, back titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.66 g of Lisinopril Hydrate, dissolve in 80 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.55 mg of $C_{21}H_{31}N_3O_5$

Containers and storage Containers—Well-closed containers.

Lisinopril Tablets

リシノプリル錠

Lisinopril Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$; 405.49).

Method of preparation Prepare as directed under Tablets, with Lisinopril Hydrate.

Identification To an amount of powdered Lisinopril Tablets, equivalent to 10 mg of lisinopril, add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of Lisinopril in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 30 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetonitrile, acetic acid (100), water and ethyl acetate (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 120 °C: the principal spot with the sample solution and the spot with the standard solution show a red-purple color and their R_f values are the same.

Purity Related substances Powder not less than 20 Lisinopril Tablets. Take a portion of the powder, equivalent

to about 25 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of water, shake for 20 minutes, filter, and use the filtrate as the sample solution. Pipet 3 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 15 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 2.0 with respect to lisinopril, is not more than 2/3 times the peak area of lisinopril from the standard solution.

Operating conditions—

Proceed as directed in the Purity (2) under Lisinopril Hydrate.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of lisinopril obtained with 15 μ L of this solution is equivalent to 3.5 to 6.5% of that with 15 μ L of the standard solution.

System performance: Proceed as directed in the Purity (2) under Lisinopril Hydrate.

System repeatability: When the test is repeated 6 times with 15 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Lisinopril Tablets add exactly 5 mL each of the internal standard solution per every 1 mg of lisinopril according to the labeled amount, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Hereafter, proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount (mg) of lisinopril (C}_{21}\text{H}_{31}\text{N}_3\text{O}_5) \\ &= W_S \times (Q_T/Q_S) \times (C/10) \end{aligned}$$

W_S : Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000)

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Lisinopril Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 60 minutes after starting the test for a 5-mg tablet, 90 minutes after starting the test for a 10-mg tablet and a 20-mg tablet, and filter through a membrane filter with pore size of not more than 0.5 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 5.6 μ g of lisinopril ($C_{21}H_{31}N_3O_5$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of lisinopril for assay, separately determined the water content <2.48> in the same manner as Lisinopril Hydrate, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water

to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of lisinopril. The dissolution rate for a 5-mg tablet in 60 minutes is not less than 80%, for a 10-mg tablet in 90 minutes is not less than 80%, and for a 20-mg tablet in 90 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of lisinopril ($C_{21}H_{31}N_3O_5$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$$

W_S : Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

C: Labeled amount (mg) of lisinopril ($C_{21}H_{31}N_3O_5$) in 1 tablet

Operating conditions—

Detector, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 7 minutes.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of lisinopril are not less than 1000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of lisinopril is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Lisinopril Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 5 mg of lisinopril ($C_{21}H_{31}N_3O_5$), add exactly 25 mL of the internal standard solution, shake for 20 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of lisinopril for assay, separately determined the water content <2.48> in the same manner as Lisinopril Hydrate, add exactly 50 mL of the internal standard solution to dissolve, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of lisinopril to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of lisinopril (C}_{21}\text{H}_{31}\text{N}_3\text{O}_5) \\ &= W_S \times (Q_T/Q_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of lisinopril for assay, calculated on the anhydrous basis

Internal standard solution—A solution of anhydrous caffeine (1 in 20,000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: A mixture of diluted 0.05 mol/L sodium dihydrogen phosphate TS (1 in 2) and acetonitrile for liquid chromatography (19:1).

Flow rate: Adjust the flow rate so that the retention time of lisinopril is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, lisinopril and the internal standard are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of lisinopril to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Lithium Carbonate

炭酸リチウム

Li_2CO_3 : 73.89

Lithium Carbonate, when dried, contains not less than 99.5% of Li_2CO_3 .

Description Lithium Carbonate occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in water, slightly soluble in hot water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in dilute acetic acid.

The pH of a solution of Lithium Carbonate (1 in 100) is between 10.9 and 11.5.

Identification (1) Perform the test as directed under Flame Coloration Test <1.04> (1) with Lithium Carbonate: a persistent red color appears.

(2) Dissolve 0.2 g of Lithium Carbonate in 3 mL of dilute hydrochloric acid, and add 4 mL of sodium hydroxide TS and 2 mL of disodium hydrogenphosphate TS: a white precipitate is produced. To the precipitate add 2 mL of hydrochloric acid: it dissolves.

(3) A solution of Lithium Carbonate (1 in 100) responds to the Qualitative Tests <1.09> for carbonate.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Lithium Carbonate in 10 mL of water by warming: the solution is clear and colorless.

(2) Acetic acid-insoluble substances—Take 1.0 g of Lithium Carbonate, dissolve in 40 mL of dilute acetic acid, filter the insoluble substances using filter paper for quantitative analysis, wash with five 10-mL portions of water, and ignite the insoluble substances together with the filter paper to in-

cinerate: the mass of the residue is not more than 1.5 mg.

(3) Chloride <1.03>—To 0.40 g of Lithium Carbonate add 10 mL of water and 7 mL of dilute nitric acid, and dissolve by heating to boil. After cooling, add 6 mL of dilute nitric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.022%).

(4) Sulfate <1.14>—To 0.40 g of Lithium Carbonate add 10 mL of water and 4 mL of dilute hydrochloric acid, and dissolve by heating to boil. After cooling, add 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(5) Heavy metals <1.07>—To 4.0 g of Lithium Carbonate add 5 mL of water, gradually add 10 mL of hydrochloric acid while mixing, and dissolve. Evaporate the solution on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a slight red color, then add 2 mL of dilute acetic acid, and dilute with water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Evaporate 10 mL of hydrochloric acid on a water bath to dryness. To the residue add 10 mL of water, and dissolve. Place the solution in a Nessler tube, add 1 drop of phenolphthalein TS, add ammonia TS until the solution shows a pale red color, then add 2.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and dilute with water to make 50 mL (not more than 5 ppm).

(6) Iron—Prepare the test solution with 1.0 g of Lithium Carbonate according to Method 2 using 11 mL of dilute hydrochloric acid, and perform the test according to Method B. Prepare the control solution with 1.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Aluminum—To 10 mL of solution A obtained in (6) add 10 mL of water and 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and shake. Add 1 mL of a solution of L-ascorbic acid (1 in 100), 2 mL of aluminon TS and water to make 50 mL, shake well, and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 0.1758 g of aluminum potassium sulfate 12-water in water to make 1000 mL. To 1.0 mL of this solution add 10 mL of solution B obtained in (6) and water to make 20 mL, add 5 mL of acetic acid-sodium acetate buffer solution, pH 4.5, and proceed in the same manner.

(8) Barium—To 20 mL of solution A obtained in (6) add 6 mL of water, 0.5 mL of dilute hydrochloric acid, 3 mL of ethanol (95) and 2 mL of potassium sulfate TS, and allow to stand for 1 hour: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 17.8 mg of barium chloride dihydrate in water to make 1000 mL. To 6 mL of this solution add 20 mL of solution B, 0.5 mL of dilute hydrochloric acid and 3 mL of ethanol (95), and proceed in the same manner.

(9) Calcium—Weigh accurately about 5 g of Lithium Carbonate, add 50 mL of water and 15 mL of hydrochloric acid, and dissolve. Remove carbon dioxide from the solution by boiling, add 5 mL of ammonium oxalate TS, then make alkaline with ammonia TS, and allow to stand for 4 hours. Filter the produced precipitate through a glass filter (G4),

wash with warm water until the turbidity of the washing is not produced with calcium chloride TS within 1 minute. Transfer the precipitate and the glass filter into a beaker, add water until the glass filter is covered with water, then add 3 mL of sulfuric acid, heat between 70°C and 80°C, and titrate with 0.02 mol/L potassium permanganate VS until a pale red color persists for 30 seconds: the amount of calcium (Ca: 40.08) is not more than 0.05%.

Each mL of 0.02 mol/L potassium permanganate VS
= 2.004 mg of Ca

(10) Magnesium—To 3.0 mL of solution A obtained in (6) add 0.2 mL of a solution of titan yellow (1 in 1000) and water to make 20 mL, then add 5 mL of sodium hydroxide (3 in 20), and allow to stand for 10 minutes: the solution has no more color than the following control solution.

Control solution: Dissolve 49.5 mg of magnesium sulfate heptahydrate, previously dried at 105°C for 2 hours and heated at 450°C for 3 hours, in water to make 1000 mL. To this solution add 3 mL of solution B obtained in (6), 0.2 mL of a solution of titanium yellow (1 in 1000) and water to make 20 mL, and proceed in the same manner.

(11) Potassium—Dissolve 1.0 g of Lithium Carbonate in water to make 100 mL, and use this solution as the sample solution. To 5 mL of the sample solution add 1.0 mL of dilute acetic acid, shake, add 5 mL of a solution of sodium tetraphenylborate (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water to make 1000 mL. To 5 mL of this solution add 1.0 mL of dilute acetic acid, shake, and proceed in the same manner.

(12) Sodium—Weigh accurately about 0.8 g of Lithium Carbonate, dissolve in water to make exactly 100 mL, and use this solution as the sample stock solution. Measure exactly 25 mL of the sample stock solution, add water to make exactly 100 mL, and use this solution as the sample solution (1). Separately, weigh accurately 25.4 mg of sodium chloride, dissolve in water to make exactly 1000 mL, and use this solution as the standard solution. Measure exactly 25 mL of the sample stock solution, add exactly 20 mL of the standard solution, then add water to make exactly 100 mL, and use this solution as the sample solution (2). Determine emission intensities of sodium using a flame photometer with the sample solution (1) and the sample solution (2) under the following conditions. Adjust the wavelength dial to 589 nm, atomize the sample solution (2) into the flame, then adjust the sensitivity so that the emission intensity L_S shows 100 adjustment, and determine emission intensity L_T of the sample solution (1). Then, make the other conditions identical, change the wavelength dial to 580 nm, determine emission intensity L_B of the sample solution (1): the amount of sodium, calculated from the following equation, is not more than 0.05%.

$$\text{Amount (\%)} \text{ of sodium (Na)} \\ = \{(L_T - L_B)/(L_S - L_T)\} \times (W'/W) \times 100$$

W : Amount (mg) of the sample in 25 mL of the sample stock solution

W' : Amount (mg) of sodium in 20 mL of the standard solution

(13) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lithium Carbonate, add 2 mL of water and 3 mL of

hydrochloric acid, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

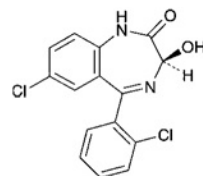
Assay Weigh accurately about 1 g of Lithium Carbonate, previously dried, add exactly 100 mL of water and 50 mL of 0.5 mol/L sulfuric acid VS, remove carbon dioxide by boiling gently, cool, and titrate <2.50> the excess sulfuric acid with 1 mol/L sodium hydroxide VS until the color of the solution changes from red to yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

Each mL of 0.5 mol/L sulfuric acid VS
= 36.95 mg of Li_2CO_3

Containers and storage Containers—Well-closed containers.

Lorazepam

ロラゼパム



and enantiomer

$\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2$: 321.16

(3*RS*)-7-Chloro-5-(2-chlorophenyl)-3-hydroxy-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one [846-49-1]

Lorazepam, when dried, contains not less than 98.5% of $\text{C}_{15}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}_2$.

Description Lorazepam occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored by light.

Identification (1) To 0.02 g of Lorazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, and cool: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Determine the absorption spectrum of a solution of Lorazepam in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Lorazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the test with Lorazepam as directed under Flame Coloration Test <1.04> (2): a green color appears.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (229 nm): 1080 – 1126 (after drying, 1 mg, ethanol (95), 200 mL).

Purity (1) Chloride <1.03>—To 1.0 g of Lorazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Lorazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Lorazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Lorazepam in 20 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 1,4-dioxane and acetic acid (100) (91:5:4) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

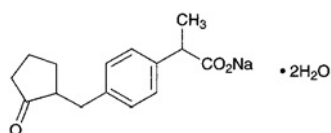
Assay Weigh accurately about 0.4 g of Lorazepam, previously dried, dissolve in 50 mL of acetone, and titrate <2.50> with 0.1 mol/L tetrabutylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L tetrabutylammonium hydroxide VS
= 32.12 mg of $C_{15}H_{10}Cl_2N_2O_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Loxoprofen Sodium Hydrate

ロキソプロフェンナトリウム水和物



$C_{15}H_{17}NaO_3 \cdot 2H_2O$: 304.31
Monosodium 2-[4-[(2-oxocyclopentyl)methyl]phenyl]propanoate dihydrate
[80382-23-6]

Loxoprofen Sodium Hydrate contains not less than 98.5% of loxoprofen sodium ($C_{15}H_{17}NaO_3$: 268.28),

calculated on the anhydrous basis.

Description Loxoprofen Sodium Hydrate occurs as white to yellowish white crystals or crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Loxoprofen Sodium Hydrate (1 in 20) does not show optical rotation.

The pH of a solution of Loxoprofen Sodium Hydrate in freshly boiled and cooled water (1 in 20) is between 6.5 and 8.5.

Identification (1) Determine the absorption spectrum of a solution of Loxoprofen Sodium Hydrate (1 in 55,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Loxoprofen Sodium Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Loxoprofen Sodium Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of water: the solution is clear and colorless or pale yellow. The color is not darker than that of diluted Matching Fluid for Color A (1 in 2).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Loxoprofen Sodium Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 1.0 g of Loxoprofen Sodium Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane and acetic acid (100) (9:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 11.0 – 13.0% (0.2 g, direct titration).

Assay Weigh accurately about 60 mg of Loxoprofen Sodium Hydrate, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, add diluted methanol (3 in 5) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Loxoprofen Reference Standard, previously dried in a desiccator (in vacuum, 60°C) for 3 hours, and dissolve in diluted methanol (3 in 5) to make exactly 100 mL. Pipet 5 mL of this solution, proceed in the same manner as directed for the preparation of the sample solution, and use so obtained solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed

under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of loxoprofen to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of loxoprofen sodium (C}_{15}\text{H}_{17}\text{NaO}_3) \\ = W_S \times (Q_T/Q_S) \times 1.089 \end{aligned}$$

W_S : Amount (mg) of Loxoprofen Reference Standard

Internal Standard Solution—A solution of ethyl benzoate in diluted methanol (3 in 5) (7 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 222 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol, water, acetic acid (100) and triethylamine (600:400:1:1).

Flow rate: Adjust the flow rate so that the retention time of loxoprofen is about 7 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, loxoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

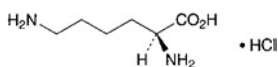
System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of loxoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

L-Lysine Hydrochloride

Lysine Hydrochloride

L-リジン塩酸塩



$\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2 \cdot \text{HCl}$: 182.65
(2S)-2,6-Diaminohexanoic acid monohydrochloride
[657-27-2]

L-Lysine Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_2 \cdot \text{HCl}$.

Description L-Lysine Hydrochloride occurs as a white powder. It is odorless, and has a slight, characteristic taste.

It is freely soluble in water and in formic acid, and practically insoluble in ethanol (95).

Identification (1) Determine the infrared absorption spectrum of L-Lysine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any differ-

ence appears between the spectra, dissolve L-Lysine Hydrochloride in water, evaporate the water to dryness at 60°C, and repeat the test with the residue.

(2) A solution of L-Lysine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +19.0 – +21.5° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Lysine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.6 g of L-Lysine Hydrochloride. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(3) Ammonium <1.02>—Perform the test with 0.25 g of L-Lysine Hydrochloride. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of L-Lysine Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of L-Lysine Hydrochloride in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 50 mL, pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol and ammonia water (28) (67:33) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50) and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of L-Lysine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat on a water bath for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 9.132 \text{ mg of } \text{C}_6\text{H}_{14}\text{N}_2\text{O}_2\text{HCl} \end{aligned}$$

Containers and storage Containers—Tight containers.

Lysozyme Hydrochloride

リゾチーム塩酸塩



$C_{616}H_{963}N_{193}O_{182}S_{10} \cdot xHCl$
[12650-88-3, egg white lysozyme]

Lysozyme Hydrochloride is a hydrochloride of a basic polypeptide obtained from albumen of hen's egg, and has an activity to hydrolyze mucopolysaccharides.

It contains not less than 0.9 mg (potency) of lysozyme per mg, calculated on the dried basis.

Description Lysozyme Hydrochloride occurs as white, crystals, or crystalline or amorphous powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

The pH of a solution of Lysozyme Hydrochloride (3 in 200) is between 3.0 and 5.0.

Identification (1) To 5 mL of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 500) add 1 mL of ninhydrin TS, and heat for 10 minutes: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Lysozyme Hydrochloride in acetate buffer solution, pH 5.4 (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity of solution—To 5 mL of a solution of Lysozyme Hydrochloride (3 in 200) add, if necessary, dilute hydrochloric acid to adjust the pH to 3: the solution is clear.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Lysozyme Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 8.0% (0.1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 2.0% (0.5 g).

Nitrogen Perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is between 16.8% and 18.6%, calculated on the dried basis.

Assay Weigh accurately an amount of Lysozyme Hydrochloride, equivalent to about 25 mg (potency), dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 2 mL of this solution, add phosphate buffer solution, pH 6.2 to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of

Lysozyme Reference Standard (separately determine its loss on drying <2.41> in the same manner as Lysozyme Hydrochloride), equivalent to about 25 mg (potency), and dissolve in phosphate buffer solution, pH 6.2 to make exactly 100 mL. Pipet 1 mL and 2 mL of this solution, add phosphate buffer solution, pH 6.2 to them to make exactly 50 mL, and use these solutions as the standard solution (1) and the solution (2), respectively. Keep the sample solution and the standard solutions in an ice-bath. Pipet 4 mL of substrate solution for lysozyme hydrochloride, previously warmed in a water bath of 35°C for about 5 minutes, add exactly 100 μ L of the sample solution, previously warmed in a water bath of 35°C for about 3 minutes, and allow to stand at 35°C for exactly 10 minutes, then add exactly 0.5 mL of 1 mol/L hydrochloric acid TS, and immediately shake. Determine the absorbance under Ultraviolet-visible Spectrophotometry <2.24>, A_T , of this solution at 640 nm, using water as the blank. Determine the absorbances, A_{S1} and A_{S2} , of the solutions obtained with the standard solution (1) and the standard solution (2) in the same manner as the sample solution.

Amount [mg (potency)] of lysozyme per mg, calculated on the dried basis

$$= (W_S/2W_T) \times \{(A_{S1} - A_T)/(A_{S1} - A_{S2}) + 1\}$$

W_S : Amount (mg) of Lysozyme Reference Standard, calculated on the dried basis.

W_T : Amount (mg) of the sample, calculated on the dried basis.

Containers and storage Containers—Tight containers.

Macrogol 400

Polyethylene Glycol 400

マクロゴール 400

Macrogol 400 is a polymer of ethylene oxide and water, represented by the formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which the value of n ranges from 7 to 9.

Description Macrogol 400 occurs as a clear, colorless and viscous liquid. It has no odor or a slight, characteristic odor.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is soluble in diethyl ether.

It is slightly hygroscopic.

Congealing point: 4 – 8°C

Specific gravity d_{20}^{20} : 1.110 – 1.140

Identification Dissolve 0.05 g of Macrogol 400 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n -hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54> Dissolve 1.0 g of Macrogol 400 in 20 mL of water: the pH of this solution is between 4.0 and 7.0.

Purity (1) Acidity—Dissolve 5.0 g of Macrogol 400 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(2) Ethylene glycol and diethylene glycol—Dissolve 4.0 g of Macrogl 400 in water to make exactly 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg each of ethylene glycol and diethylene glycol, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine the peak heights, H_{Ta} and H_{Sa} , of ethylene glycol of each solution, and the peak heights, H_{Tb} and H_{Sb} , of diethylene glycol, and calculate the amount of ethylene glycol and diethylene glycol: the sum of the contents of ethylene glycol and diethylene glycol is not more than 0.25%.

$$\begin{aligned} &\text{Amount (mg) of ethylene glycol} \\ &= W_{Sa} \times (H_{Ta}/H_{Sa}) \times (1/10) \end{aligned}$$

$$\begin{aligned} &\text{Amount (mg) of diethylene glycol} \\ &= W_{Sb} \times (H_{Tb}/H_{Sb}) \times (1/10) \end{aligned}$$

W_{Sa} : Amount (mg) of ethylene glycol for gas chromatography

W_{Sb} : Amount (mg) of diethylene glycol for gas chromatography

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column about 3 mm in inside diameter and about 1.5 m in length, packed with siliceous earth for gas chromatography, 150 to 180 μ m in particle diameter, coated with D-sorbitol at the ratio of 12%.

Column temperature: A constant temperature of about 165 °C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of diethylene glycol is about 8 minutes.

Selection of column: Proceed with 2 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column clearly dividing peaks of ethylene glycol and diethylene glycol in this order.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of diethylene glycol obtained from 2 μ L of the standard solution composes about 80% of the full scale.

Average molecular mass Add 42 g of phthalic anhydride to 300 mL of freshly distilled pyridine, exactly measured, in a 1-L light-resistant glass-stoppered bottle. Shake the bottle vigorously to dissolve the solid, and allow to stand for 16 hours or more. Pipet 25 mL of this solution into an about 200-mL glass-stoppered pressure bottle. Add about 1.5 g of Macrogl 400, accurately weighed, stopper the bottle, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of $98 \pm 2^\circ\text{C}$, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at $98 \pm 2^\circ\text{C}$ for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\begin{aligned} &\text{Average molecular mass} \\ &= (W \times 4000)/(a - b) \end{aligned}$$

W : Amount (g) of sample.

a : Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination.

b : Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample.

Average molecular mass is between 380 and 420.

Water <2.48> Not more than 1.0% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Macrogl 1500

Polyethylene Glycol 1500

マクロゴール 1500

Macrogl 1500 is a mixture containing equal amounts of lower and higher polymers of ethylene oxide and water, represented by the formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, in which the value of n is 5 or 6 for the lower polymers and from 28 to 36 for the higher.

Description Macrogl 1500 occurs as a white, smooth petrolatum-like solid. It is odorless or has a faint, characteristic odor.

It is very soluble in water, in pyridine and in diphenyl ether, freely soluble in methanol, sparingly soluble in ethanol (95), very slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

Congealing point: 37 – 41 °C

Identification Dissolve 0.05 g of Macrogl 1500 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n -hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54> Dissolve 1.0 g of Macrogl 1500 in 20 mL of water: the pH of the solution is between 4.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 5.0 g of Macrogl 1500 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogl 1500 in 20 mL of neutralized ethanol, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: the solution is red in color.

(3) Ethylene glycol and diethylene glycol—Place 50.0 g of Macrogl 1500 in a distilling flask, add 75 mL of diphenyl ether, warm to dissolve if necessary, distil slowly under a reduced pressure of 0.13 to 0.27 kPa and take 25 mL of the distillate in a 100-mL container with 1-mL graduation. To the distillate add exactly 20 mL of water, shake vigorously, cool in ice water, congeal the diphenyl ether, and filtrate into a 25-mL volumetric flask. Wash the residue with 5.0 mL of ice-cold water, combine the washings with the filtrate, warm to room temperature, and add water to make 25 mL. Transfer this solution to a glass-stoppered flask, shake with 25.0 mL of freshly distilled acetonitrile, and use this solution as the sample solution. Separately, to 62.5 mg of diethylene glycol

add a mixture of water and freshly distilled acetonitrile (1:1) to make exactly 25 mL, and use this solution as the standard solution. Take exactly 10 mL each of the sample solution and the standard solution, and add to each exactly 15 mL of cerium (IV) diammonium nitrate TS. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24> within 2 to 5 minutes: the absorbance of the solution obtained from the sample solution at the wavelength of maximum absorption at about 450 nm is not larger than the absorbance of the solution obtained from the standard solution.

Water <2.48> Not more than 1.0% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Tight containers.

Macrogol 4000

Polyethylene Glycol 4000

マクロゴール 4000

Macrogol 4000 is a polymer of ethylene oxide and water, represented by the formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, in which the value of n ranges from 59 to 84.

Description Macrogol 4000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in methanol and in pyridine, and practically insoluble in ethanol (99.5) and in diethyl ether.

Congealing point: 53 – 57°C

Identification Dissolve 0.05 g of Macrogol 4000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n -hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54> Dissolve 1.0 g of Macrogol 4000 in 20 mL of water: the pH of this solution is between 4.0 and 7.5.

Purity (1) Clarity and color of solution—A solution of 5.0 g of Macrogol 4000 in 50 mL of water is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 4000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 12.5 g of Macrogol 4000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at $98 \pm 2^\circ\text{C}$, to the level so that the mixture in the

bottle soaks completely in water. Maintain the temperature of the bath at $98 \pm 2^\circ\text{C}$ for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\begin{aligned} \text{Average molecular mass} \\ = (W \times 4000) / (a - b) \end{aligned}$$

W : Amount (g) of sample.

a : Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination.

b : Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample.

Average molecular mass is between 2600 and 3800.

Water <2.48> Not more than 1.0% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol 6000

Polyethylene Glycol 6000

マクロゴール 6000

Macrogol 6000 is a polymer of ethylene oxide and water, represented by the formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, in which the value of n ranges from 165 to 210.

Description Macrogol 6000 is a white, paraffin-like solid, occurring as flakes or powder. It is odorless or has a faint, characteristic odor.

It is very soluble in water, freely soluble in pyridine, and practically insoluble in methanol, in ethanol (95), in ethanol (99.5) and in diethyl ether.

Congealing point: 56 – 61°C

Identification Dissolve 0.05 g of Macrogol 6000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid n -hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54> Dissolve 1.0 g of Macrogol 6000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 6000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 6000 in 20 mL of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 12.5 g of Macrogol 6000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of

freshly distilled pyridine into a 1000-mL light-resistant, glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, previously heated at $98 \pm 2^\circ\text{C}$, to the level so that the mixture in the bottle soaks completely in water. Maintain the temperature of the bath at $98 \pm 2^\circ\text{C}$ for 30 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination in the same manner.

$$\begin{aligned} &\text{Average molecular mass} \\ &= (W \times 4000)/(a - b) \end{aligned}$$

W: Amount (g) of sample.

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the blank determination.

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS consumed in the test of the sample.

Average molecular mass is between 7300 and 9300.

Water <2.48> Not more than 1.0% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol 20000

Polyethylene Glycol 20000

マクロゴール 20000

Macrogol 20000 is a polymer of ethylene oxide and water, represented by the formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_n\text{CH}_2\text{OH}$, in which the value of *n* lies between 340 and 570.

Description Macrogol 20000 occurs as white, paraffin-like flakes or powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water and in pyridine, and practically insoluble in methanol, in ethanol (95), in anhydrous diethyl ether, in petroleum benzene and in macrogol 400.

Congealing point: $56 - 64^\circ\text{C}$

Identification Dissolve 0.05 g of Macrogol 20000 in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, and filter, if necessary. To the filtrate add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10): a yellow-green precipitate is formed.

pH <2.54> Dissolve 1.0 g of Macrogol 20000 in 20 mL of water: the pH of this solution is between 4.5 and 7.5.

Purity (1) Clarity and color of solution—Dissolve 5.0 g of Macrogol 20000 in 50 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of Macrogol 20000 in 20 mL

of neutralized ethanol by warming, cool, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 1 drop of phenolphthalein TS: the color of the solution is red.

Average molecular mass Weigh accurately about 15 g of Macrogol 20000, transfer to an about 200-mL glass-stoppered pressure bottle, add about 25 mL of pyridine, dissolve by warming, and allow to cool. Separately, pipet 300 mL of freshly distilled pyridine into a 1000-mL light-resistant glass-stoppered bottle, add 42 g of phthalic anhydride, dissolve with vigorous shaking, and allow to stand for 16 hours or more. Pipet 25 mL of this solution, transfer to the former pressure bottle, stopper the bottle tightly, wrap it securely with strong cloth, and immerse in a water bath, having a temperature of $98 \pm 2^\circ\text{C}$, to the same depth as the mixture in the bottle. Maintain the temperature of the bath at $98 \pm 2^\circ\text{C}$ for 60 minutes. Remove the bottle from the bath, and allow to cool in air to room temperature. Add exactly 50 mL of 0.5 mol/L sodium hydroxide VS and 5 drops of a solution of phenolphthalein in pyridine (1 in 100). Titrate <2.50> with 0.5 mol/L sodium hydroxide VS until a light red color remains for not less than 15 seconds. Perform a blank determination.

$$\begin{aligned} &\text{Average molecular mass} \\ &= (W \times 4000)/(a - b) \end{aligned}$$

W: Amount (g) of sample.

a: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the blank determination.

b: Volume (mL) of 0.5 mol/L sodium hydroxide VS used in the test of the sample.

Average molecular mass is between 15000 and 25000.

Water <2.48> Not more than 1.0% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Containers and storage Containers—Well-closed containers.

Macrogol Ointment

Polyethylene Glycol Ointment

マクロゴール軟膏

Method of preparation

Macrogol 4000	500 g
Macrogol 400	500 g

To make 1000 g

Melt Macrogol 4000 and Macrogol 400 by warming on a water bath at 65°C , and mix well until it congeals. Less than 100 g of Macrogol 4000 or Macrogol 400 may be replaced by an equal amount of Macrogol 400 or Macrogol 4000 to prepare 1000 g of a proper soft ointment.

Description Macrogol Ointment is white in color. It has a faint, characteristic odor.

Identification Dissolve 0.05 g of Macrogol Ointment in 5 mL of dilute hydrochloric acid, add 1 mL of barium chloride TS, shake, filter if necessary, and add 1 mL of a solution of phosphomolybdic acid *n*-hydrate (1 in 10) to the filtrate: a yellow-green precipitate is formed.

Containers and storage Containers—Tight containers.

Magnesium Carbonate

炭酸マグネシウム

Magnesium Carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate.

Magnesium Carbonate contains not less than 40.0% and not more than 44.0% of magnesium oxide (MgO: 40.30).

“Heavy magnesium carbonate” may be used as commonly used name for Magnesium Carbonate which shows the height of the precipitate below the 12.0-mL graduation line in the Precipitation test.

Description Magnesium Carbonate occurs as white, friable masses or powder. It is odorless.

It is practically insoluble in water, in ethanol (95), in diethyl ether and in 1-propanol.

It dissolves in dilute hydrochloric acid with effervescence. Its saturated solution is alkaline.

Identification (1) Dissolve 1 g of Magnesium Carbonate in 10 mL of dilute hydrochloric acid, boil, then cool, neutralize with sodium hydroxide TS, and filter, if necessary: the solution responds to the Qualitative Tests <1.09> for magnesium salt.

(2) Magnesium Carbonate responds to the Qualitative Tests <1.09> (1) for carbonate.

Purity (1) Soluble salts—To 2.0 g of Magnesium Carbonate add 40 mL of 1-propanol and 40 mL of water, heat to boil with constant stirring, cool, and filter. Wash the residue with water, combine the washings with the filtrate, and add water to make exactly 100 mL. Evaporate 50 mL of the solution on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue does not exceed 10.0 mg.

(2) Heavy metals <1.07>—Moisten 1.0 g of Magnesium Carbonate with 4 mL of water, dissolve by addition of 10 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid, 1 drop of ammonia TS, filter, if necessary, wash the filter paper with water, combine the washings with the filtrate, and add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 10 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 30 ppm).

(3) Iron—Prepare the test solution with 0.10 g of Magnesium Carbonate according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 200 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of Magnesium Carbonate, previously moistened with 1.5 mL of water, add 3.5 mL of dilute hydrochloric acid, and perform the test (not more than 5 ppm).

(5) Calcium oxide—Weigh accurately about 0.6 g of Magnesium Carbonate, and dissolve in 35 mL of water and 6

mL of dilute hydrochloric acid. Add 250 mL of water and 5 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2''-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The content of calcium oxide (CaO: 56.08) is not more than 0.6%.

(6) Acid-insoluble substances—Mix 5.0 g of Magnesium Carbonate and 75 mL of water, add 10 mL of hydrochloric acid dropwise while stirring, boil for 5 minutes, and cool. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the residue is not more than 2.5 mg.

Precipitation test Transfer 1.0 g of Magnesium Carbonate, previously sifted through a No. 100 (150 μm) sieve to a glass-stoppered measuring cylinder with a 50-mL graduation line at 150 mm from the bottom, and add water to make 50 mL. Shake vigorously for exactly 1 minute, allow to stand for 15 minutes, and measure the height of the precipitate (in graduation in mL).

Assay Weigh accurately about 0.4 g of Magnesium Carbonate, dissolve in 10 mL of water and 3.5 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of the solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction. From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.015 mg of MgO

Each mg of calcium oxide (CaO)
= 0.36 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS

Containers and storage Containers—Well-closed containers.

Magnesium Oxide

酸化マグネシウム

MgO: 40.30

Magnesium Oxide, when ignited, contains not less than 96.0% of MgO.

When 5 g of Magnesium Oxide has a volume not more than 30 mL, it may be labeled heavy magnesium oxide.

Description Magnesium Oxide occurs as a white powder or granules. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

It absorbs moisture and carbon dioxide in air.

Identification A solution of Magnesium Oxide in dilute hydrochloric acid (1 in 50) responds to the Qualitative Tests <1.09> for magnesium salt.

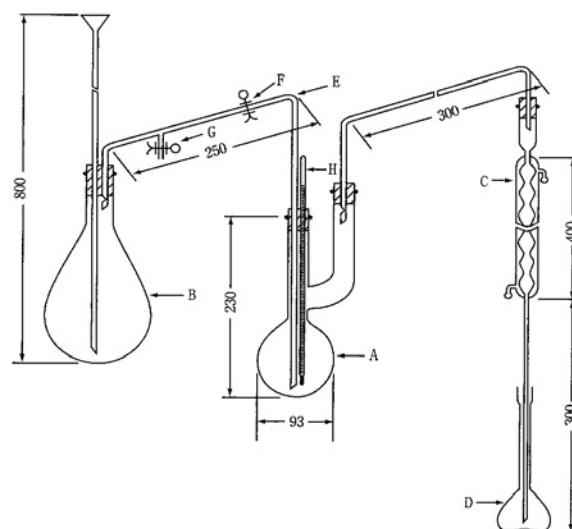
Purity (1) Alkali and soluble salts—Transfer 2.0 g of Magnesium Oxide to a beaker, add 100 mL of water, cover the beaker with a watch-glass, heat on a water bath for 5 minutes, and filter immediately. After cooling, to 50 mL of the filtrate add 2 drops of methyl red TS and 2.0 mL of 0.05 mol/L sulfuric acid VS: a red color develops. Evaporate 25 mL of the remaining filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 10 mg.

(2) Carbonate—Boil 0.10 g of Magnesium Oxide with 5 mL of water, cool, and add 5 mL of acetic acid (31): almost no effervescence occurs.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Magnesium Oxide in 20 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 1 drop of phenolphthalein TS, neutralize with ammonia TS, add 2 mL of dilute acetic acid, and filter, if necessary. Wash the filter paper with water, add water to the combined washing and the filtrate to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 20 mL of dilute hydrochloric acid add 1 drop of phenolphthalein TS, neutralize with ammonia TS, and add 2 mL of dilute acetic acid, 4.0 mL of Standard Lead Solution and water to make 50 mL (not more than 40 ppm).

(4) Iron <1.10>—Prepare the test solution with 40 mg of Magnesium Oxide according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) Calcium oxide—Weigh accurately about 0.25 g of Magnesium Oxide, previously ignited, dissolve in 6 mL of dilute hydrochloric acid by heating. Cool, add 300 mL of water and 3 mL of a solution of L-tartaric acid (1 in 5), then add 10 mL of a solution of 2,2',2''-nitrilotrisethanol (3 in 10) and 10 mL of 8 mol/L potassium hydroxide TS, allow to stand for 5 minutes, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the color of



The figures are in mm.

A: Distilling flask of about 300-mL capacity.

B: Steam generator of about 1000-mL capacity, containing a few boiling tips to prevent bumping

C: Condenser

D: Receiver: 200-mL volumetric flask

E: Steam-introducing tube having an internal diameter of about 8 mm

F, G: Rubber tube with a clamp

H: Thermometer

the solution changes from red-purple to blue (indicator: 0.1 g of NN indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 0.5608 mg of CaO

The mass of calcium oxide (CaO: 56.08) is not more than 1.5%.

(6) Arsenic <1.11>—Dissolve 0.20 g of Magnesium Oxide in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 10 ppm).

(7) Acid-insoluble substances—Mix 2.0 g of Magnesium Oxide with 75 mL of water, add 12 mL of hydrochloric acid dropwise, while shaking, and boil for 5 minutes. Collect the insoluble residue using filter paper for quantitative analysis, wash well with water until the last washing shows no turbidity with silver nitrate TS, and ignite the residue together with the filter paper: the mass of the ignited residue does not more than 2.0 mg.

(8) Fluoride—(i) Apparatus: Use a hard glass apparatus as illustrated in the figure. Ground-glass joints may be used.

(ii) Procedure: Transfer 5.0 g of Natural Aluminum Silicate to the distilling flask A with the aid of 20 mL of water, add about 1 g of glass wool and 50 mL of diluted purified sulfuric acid (1 in 2), and connect A to the distillation apparatus, previously washed with steam streamed through the steam introducing tube E. Connect the condenser C with the receiver D containing 10 mL of 0.01 mol/L sodium hydroxide VS and 10 mL of water so that the lower end of C is immersed in the solution. Heat A gradually until the temperature of the solution in A reaches 130°C, then open the

rubber tube F, close the rubber tube G, boil water in the steam generator B vigorously, and introduce the generated steam into F. Simultaneously, heat A, and maintain the temperature of the solution in A between 135°C and 145°C. Adjust the distilling rate to about 10 mL per minute. Collect about 170 mL of the distillate, then stop the distillation, wash C with a small quantity of water, combine the washings with the distillate, add water to make exactly 200 mL, and use this solution as the test solution. Perform the test with the test solution as directed in the procedure of determination for fluoride under the Oxygen Flask Combustion Method. No corrective solution is used in this procedure.

$$\begin{aligned} &\text{Amount (mg) of fluoride (F: 19.00) in the test solution} \\ &= \text{amount (mg) of fluoride in 5 mL of} \\ &\quad \text{the standard solution} \\ &\quad \times (A_T/A_S) \times (200/V) \end{aligned}$$

The content of fluoride (F) is not more than 0.08%.

Loss on ignition <2.43> Not more than 10% (0.25 g, 900°C, constant mass).

Assay Ignite Magnesium Oxide to constant mass at 900°C, weigh accurately about 0.2 g of the residue, dissolve in 10 mL of water and 4.0 mL of dilute hydrochloric acid, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

From the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed, deduct the volume of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS corresponding to the content of calcium oxide (CaO) obtained in the Purity (5).

$$\begin{aligned} &\text{Each mL of 0.05 mol/L disodium dihydrogen} \\ &\quad \text{ethylenediamine tetraacetate VS} \\ &= 2.015 \text{ mg of MgO} \end{aligned}$$

$$\begin{aligned} &\text{Each mg of calcium oxide (CaO)} \\ &= 0.36 \text{ mL of 0.05 mol/L disodium dihydrogen} \\ &\quad \text{ethylenediamine tetraacetate VS} \end{aligned}$$

Containers and storage Containers—Tight containers.

Magnesium Silicate

ケイ酸マグネシウム

Magnesium Silicate contains not less than 45.0% of silicon dioxide (SiO₂: 60.08) and not less than 20.0% of magnesium oxide (MgO: 40.30), and the ratio of percentage (%) of magnesium oxide to silicon dioxide is not less than 2.2 and not more than 2.5.

Description Magnesium Silicate occurs as a white, fine powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Mix 0.5 g of Magnesium Silicate with 10

mL of dilute hydrochloric acid, filter, and neutralize the filtrate with ammonia TS: the solution responds to the Qualitative Tests <1.09> for magnesium salt.

(2) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Place the bead in contact with Magnesium Silicate, and fuse again: an infusible matter appears in the bead, which changes to an opaque bead with a web-like structure upon cooling.

Purity (1) Soluble salts—Add 150 mL of water to 10.0 g of Magnesium Silicate, heat on a water bath for 60 minutes with occasional shaking, then cool, dilute with water to 150 mL, and centrifuge. Dilute 75 mL of the resultant transparent liquid with water to 100 mL, and use this solution as the sample solution. Evaporate 25 mL of the sample solution on a water bath to dryness, and ignite the residue at 700°C for 2 hours: the mass of the ignited residue is not more than 0.02 g.

(2) Alkalinity—To 20 mL of the sample solution obtained in (1) add 2 drops of phenolphthalein TS and 1.0 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Chloride <1.03>—Take 10 mL of the sample solution obtained in (1), add 6 mL of dilute nitric acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.75 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(4) Sulfate <1.14>—To the residue obtained in (1) add about 3 mL of dilute hydrochloric acid, and heat on a water bath for 10 minutes. Add 30 mL of water, filter, wash the residue on the filter with water, combine the washings with the filtrate, and dilute to 50 mL with water. To 4 mL of the solution add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.480%).

(5) Heavy metals <1.07>—To 1.0 g of Magnesium Silicate add 20 mL of water and 3 mL of hydrochloric acid, and boil for 2 minutes. Filter, and wash the residue on the filter with two 5-mL portions of water. Evaporate the combined filtrate and washings on a water bath to dryness, add 2 mL of dilute acetic acid to the residue, warm until solution is complete, filter, if necessary, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 30 ppm).

(6) Arsenic <1.11>—To 0.4 g of Magnesium Silicate add 5 mL of dilute hydrochloric acid, heat gently to boiling while shaking well, cool rapidly, and centrifuge. Mix the residue with 5 mL of dilute hydrochloric acid with shaking, centrifuge, then add 10 mL of water to the residue, and repeat the extraction in the same manner. Concentrate the combined extracts on a water bath to 5 mL. Use this solution as the test solution, and perform the test (not more than 5 ppm).

Loss on ignition <2.43> Not more than 34% (0.5 g, 850°C, 3 hours).

Acid-consuming capacity <6.04> Place about 0.2 g of Magnesium Silicate, accurately weighed, in a glass-stoppered flask, add exactly 30 mL of 0.1 mol/L hydrochloric acid VS and 20 mL of water, shake at 37 ± 2°C for 1 hour, and cool. Pipet 25 mL of the supernatant liquid, and titrate <2.50> the excess hydrochloric acid, while stirring well, with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5.

1 g of Magnesium Silicate, calculated on the anhydrous basis by making allowance for the observed loss on ignition determined as directed in the preceding Loss on ignition, consumes not less than 140 mL and not more than 160 mL of 0.1 mol/L hydrochloric acid VS.

Assay (1) Silicon dioxide—Weigh accurately about 0.7 g of Magnesium Silicate, add 10 mL of 0.5 mol/L sulfuric acid TS, evaporate on a water bath to dryness, add 25 mL of water to the residue, and heat on a water bath for 15 minutes with occasional stirring. Filter the supernatant liquid through filter paper for assay, add 25 mL of hot water to the residue, stir, and decant the supernatant liquid on the filter paper to filter. Wash the residue in the same manner with two 25-mL portions of hot water, transfer the residue onto the filter paper, and wash with hot water until the last washing does not respond to the Qualitative Tests <1.09> (1) for sulfate. Place the residue and the filter paper in a platinum crucible, incinerate with strong heating, and ignite between 775°C and 825°C for 30 minutes, then cool, and weigh the residue as *a* (g). Moisten the residue with water, and add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 minutes, cool, and weigh the residue as *b* (g).

$$\begin{aligned} \text{Content (\% of silicon dioxide (SiO}_2\text{))} \\ = \{(a - b)/W\} \times 100 \end{aligned}$$

W: Mass (g) of the sample

(2) Magnesium oxide—Weigh accurately about 0.3 g of Magnesium Silicate, transfer to a 50-mL conical flask, add 10 mL of 0.5 mol/L sulfuric acid VS, and heat on a water bath for 15 minutes. Cool, transfer to a 100-mL volumetric flask, wash the conical flask with water, add the washings to the volumetric flask, dilute with water to 100 mL, and filter. Pipet 50 mL of the filtrate, shake with 50 mL of water and 5 mL of diluted 2,2',2''-nitrotrisethanol (1 in 2), add 2.0 mL of ammonia TS and 10 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

$$\begin{aligned} \text{Each mL of 0.05 mol/L disodium dihydrogen} \\ \text{ethylenediamine tetraacetate VS} \\ = 2.015 \text{ mg of MgO} \end{aligned}$$

(3) Ratio of percentage (%) of magnesium oxide (MgO) to silicon dioxide (SiO₂)—Calculate the quotient from the percentages obtained in (1) and (2).

Containers and storage Containers—Well-closed containers.

Magnesium Stearate

ステアリン酸マグネシウム

Magnesium Stearate consists chiefly magnesium salts of stearic acid (C₁₈H₃₆O₂: 284.48) and palmitic acid (C₁₆H₃₂O₂: 256.42).

It contains, when dried, not less than 4.0% and not more than 5.0% of magnesium (Mg: 24.31).

Description Magnesium Stearate occurs as a white, light, bulky powder.

It is smooth to the touch and sticky to the skin. It has no odor or a faint, characteristic odor.

It is practically insoluble in water and in ethanol (95).

Identification (1) Mix 5.0 g of Magnesium Stearate with 50 mL of peroxide-free diethyl ether, 20 mL of dilute nitric acid, and 20 mL of water in a round-bottom flask, and heat to dissolve completely under a reflux condenser. After cooling, transfer the contents of the flask to a separator, shake, allow the layers to separate, and transfer the aqueous layer to a flask. Extract the diethyl ether layer with two 4-mL portions of water, and combine these extracts to the main aqueous extract. After washing the combined aqueous extract with 15 mL of peroxide-free diethyl ether, transfer to a 50-mL volumetric flask, add water to make exactly 50 mL, mix, and use this solution as the sample solution: the sample solution responds to the Qualitative Tests <1.09> for magnesium.

(2) The retention times of the peaks corresponding to stearic acid and palmitic acid in the chromatogram of the sample solution correspond to those of methyl stearate and methyl palmitate in the chromatogram of the system suitability solution, as obtained in the Purity (5).

Purity (1) Acidity or alkalinity—Heat 1.0 g of Magnesium Stearate in 20 mL of freshly boiled and cooled water on a water bath for 1 minute while shaking, and filter after cooling. To 10 mL of the filtrate add 0.05 mL of bromothymol blue TS, and add exactly 0.05 mL of 0.1 mol/L hydrochloric acid VS or 0.1 mol/L sodium hydroxide VS: the color of the solution changes.

(2) Chloride <1.03>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 1.40 mL of 0.02 mol/L hydrochloric acid VS (not more than 0.10%).

(3) Sulfate <1.14>—Perform the test with 10.0 mL of the sample solution obtained in Identification (1). Prepare the control solution with 10.2 mL of 0.01 mol/L sulfuric acid VS (not more than 1.0%).

(4) Heavy metals <1.07>—Heat 1.0 g of Magnesium Stearate weakly first, then incinerate at about 500 ± 25°C. After cooling, add 2 mL of hydrochloric acid, evaporate on a water bath to dryness, add 20 mL of water and 2 mL of dilute acetic acid to the residue, and heat for 2 minutes. After cooling, filter this solution through a filter paper, wash the filter paper with 15 mL of water, and combine the washing with the filtrate. To the filtrate add water to make 50 mL, and perform the test with this solution as the test solution. Prepare the control solution as follows: evaporate 2 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(5) Relative content of stearic acid and palmitic acid—Transfer exactly 0.10 g of Magnesium Stearate to a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and reflux for about 10 minutes to dissolve the solids. Add 4.0 mL of heptane through the condenser, and reflux for about 10 minutes. After cooling, add 20 mL of saturated sodium chloride solution, shake, and allow the layers to separate. Transfer the heptane layer through about 0.1 g of anhydrous sodium sulfate, previously washed with heptane, to another flask.

Transfer 1.0 mL of this solution to a 10-mL volumetric flask, dilute with heptane to volume, mix, and use this solution as the sample solution. Perform the test with 1 μ L of the sample solution as directed under Gas chromatography <2.02> according to the following conditions, and determine the area, *A*, of the methyl stearate peak and the total of the areas, *B*, of all of fatty acid ester peaks. Calculate the percentage of stearic acid in the fatty acid fraction of Magnesium Stearate by the following formula.

$$\text{Content (\%)} \text{ of stearic acid} = (A/B) \times 100$$

Similarly, calculate the percentage of palmitic acid in Magnesium Stearate. The methyl stearate peak, and the total of the methyl stearate and methyl palmitate peaks are not less than 40% and not less than 90% of the total area of all fatty acid ester peaks, respectively, in the chromatogram.

Operating conditions—

Detector: A hydrogen flame-ionization detector maintained at a constant temperature of about 260°C.

Sample injection port: A splitless injection system maintained at a constant temperature of about 220°C.

Column: A fused silica capillary column 0.32 mm in inside diameter and 30 m in length, the inside coated with a 0.5- μ m layer of polyethylene glycol 15000-diepoxyde for gas chromatography.

Column temperature: Maintain at 70°C for 2 minutes after injection, then program to increase the temperature at the rate of 5°C per minute to 240°C and to maintain this temperature for 5 minutes.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of methyl stearate is about 32 minutes.

Split ratio: Splitless

Time span of measurement: About 1.5 time as long as the retention time of methyl stearate beginning after the solvent peak.

System suitability—

Test for required detection: Place exactly 50 mg each of stearic acid for gas chromatography and palmitic acid for gas chromatography, each previously dried in a desiccator (silica gel) for 4 hours, in a small conical flask fitted with a reflux condenser. Add 5.0 mL of boron trifluoride-methanol TS, mix, and proceed in the same manner as directed for the preparation of the sample solution, and use the solution so obtained as the solution for system suitability test. To exactly 1 mL of the solution add heptane to make exactly 10 mL. Confirm that the peak area of methyl stearate obtained from 1 μ L of this solution is equivalent to 5 to 15% of that from the solution for system suitability test.

System performance: When the procedure is run with 1 μ L of the solution for system suitability test under the above operating conditions, methyl palmitate and methyl stearate are eluted in this order, with the relative retention time of methyl palmitate to methyl stearate being about 0.86, and with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak areas of methyl palmitate and methyl stearate are not more than 6.0%, respectively, and the relative standard deviation of the ratios of the peak area of methyl palmitate to methyl stearate is not more than 1.0%.

Loss on drying <2.41> Not more than 6.0% (2 g, 105°C,

constant mass).

Microbial limit <4.05> The total viable aerobic microbial count is not more than 1000 per g, and the total count of fungi and yeasts is not more than 500 per g. *Salmonella* and *Escherichia coli* should not be observed.

Assay Transfer about 0.5 g of previously dried Magnesium Stearate, accurately weighed, to a 250-mL flask, add 50 mL of a mixture of 1-butanol and ethanol (99.5) (1:1), 5 mL of ammonia solution (28), 3 mL of ammonium chloride buffer solution, pH 10, 30.0 mL of 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, and 1 to 2 drops of eriochrome black T TS, and mix. Heat at 45°C to 50°C to make the solution clear, and after cooling, titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.1 mol/L zinc sulfate VS until the solution changes from blue to purple in color. Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L disodium dihydrogen} \\ \text{ethylenediamine tetraacetate VS} \\ = 2.431 \text{ mg of Mg} \end{aligned}$$

Containers and storage Containers—Tight containers.

Magnesium Sulfate Hydrate

硫酸マグネシウム水和物

MgSO₄·7H₂O: 246.47

Magnesium Sulfate Hydrate, when ignited, contains not less than 99.0% of magnesium sulfate (MgSO₄: 120.37).

Description Magnesium Sulfate Hydrate occurs as colorless or white crystals. It has a cooling, saline, bitter taste.

It is very soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification A solution of Magnesium Sulfate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> for magnesium salt and for sulfate.

pH <2.54> Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the pH of this solution is between 5.0 and 8.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 1.0 g of Magnesium Sulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Zinc—Dissolve 2.0 g of Magnesium Sulfate Hydrate in 20 mL of water, and add 1 mL of acetic acid and 5 drops of potassium hexacyanoferrate (II) TS: no turbidity is produced.

(5) Calcium—Dissolve 1.0 g of Magnesium Sulfate Hydrate in 5.0 mL of dilute hydrochloric acid, add water to make 100 mL, and use this solution as the sample solution. Separately, dissolve 1.0 g of Magnesium Sulfate Hydrate in 2.0 mL of standard calcium solution and 5.0 mL of dilute hydrochloric acid, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine the absorbances, A_T and A_S , of both solutions: A_T is not bigger than $A_S - A_T$ (not more than 0.02%).

Gas: Combustible gas—Acetylene or hydrogen
Supporting gas—Air

Lamp: Calcium hollow-cathode lamp

Wavelength: 422.7 nm

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Magnesium Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Loss on ignition <2.43> 45.0 – 52.0% (1 g, after drying at 105°C for 2 hours, ignite at 450°C for 3 hours).

Assay Weigh accurately about 0.6 g of Magnesium Sulfate Hydrate, previously ignited at 450°C for 3 hours after drying at 105°C for 2 hours, and dissolve in 2 mL of dilute hydrochloric acid and water to make exactly 100 mL. Pipet 25 mL of this solution, add 50 mL of water and 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 6.018 mg of $MgSO_4$

Containers and storage Containers—Well-closed containers.

Magnesium Sulfate Injection

硫酸マグネシウム注射液

Magnesium Sulfate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of magnesium sulfate hydrate ($MgSO_4 \cdot 7H_2O$: 246.47).

Method of preparation Prepare as directed under Injections, with Magnesium Sulfate Hydrate.

Description Magnesium Sulfate Injection is a clear, colorless liquid.

Identification Measure a volume of Magnesium Sulfate Injection, equivalent to 0.5 g of Magnesium Sulfate Hydrate according to the labeled amount, and add water to make 20 mL: the solution responds to the Qualitative Tests <1.09> for magnesium salt and for sulfate.

pH <2.54> 5.5 – 7.0 When the labeled concentration exceeds 5%, prepare a solution of 5% with water, and perform

the test.

Bacterial endotoxins <4.01> Less than 0.09 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Magnesium Sulfate Injection, equivalent to about 0.3 g of magnesium sulfate hydrate ($MgSO_4 \cdot 7H_2O$), and add water to make 75 mL. Then add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and proceed as directed in the Assay under Magnesium Sulfate Hydrate.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4 \cdot 7H_2O$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Magnesium Sulfate Mixture

硫酸マグネシウム水

Magnesium Sulfate Mixture contains not less than 13.5 w/v% and not more than 16.5 w/v% of magnesium sulfate hydrate ($MgSO_4 \cdot 7H_2O$: 246.47).

Method of preparation

Magnesium Sulfate Hydrate	150 g
Bitter Tincture	20 mL
Dilute Hydrochloric Acid	5 mL
Purified Water	a sufficient quantity
<hr/>	
	To make 1000 mL

Prepare before use, with the above ingredients.

Description Magnesium Sulfate Mixture is a light yellowish clear liquid. It has a bitter and acid taste.

Identification (1) Magnesium Sulfate Mixture responds to the Qualitative Tests <1.09> for magnesium salt.

(2) Magnesium Sulfate Mixture responds to the Qualitative Tests <1.09> (2) for chloride.

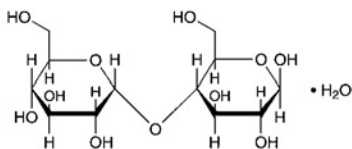
Assay Pipet 10 mL of Magnesium Sulfate Mixture, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add 50 mL of water and 5 mL of pH 10.7 ammonia-ammonium chloride buffer solution, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 12.32 mg of $MgSO_4 \cdot 7H_2O$

Containers and storage Containers—Tight containers.

Maltose Hydrate

マルトース水和物



$C_{12}H_{22}O_{11} \cdot H_2O$: 360.31

α -D-Glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranose monohydrate [6363-53-7]

Maltose Hydrate, when dried, contains not less than 98.0% of $C_{12}H_{22}O_{11} \cdot H_2O$.

Description Maltose Hydrate occurs as white crystals or crystalline powder.

It has a sweet taste.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of ammonia TS, and heat for 5 minutes on a water bath: an orange color develops.

(2) Add 2 to 3 drops of a solution of Maltose Hydrate (1 in 50) to 5 mL of boiling Fehling TS: a red precipitate is formed.

Optical rotation <2.49> $[\alpha]_{D}^{20}$: +126 – +131° Weigh accurately about 10 g of Maltose Hydrate, previously dried, dissolve in 0.2 mL of ammonia TS and water to make exactly 100 mL, and determine the optical rotation of this solution in a 100-mm cell.

pH <2.54> The pH of a solution of Maltose Hydrate (1 in 10) is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Put 10 g of Maltose Hydrate in 30 mL of water in a Nessler tube, warm at 60 °C in a water bath to dissolve, and after cooling, add water to make 50 mL: the solution is clear, and has no more color than the following control solution.

Control solution: Add water to a mixture of 1.0 mL of Cobaltous Chloride Stock CS, 3.0 mL of Ferric Chloride Stock CS and 2.0 mL of Cupric Sulfate Stock CS to make 10.0 mL. To 1.0 mL of this solution add water to make 50 mL.

(2) Chloride <1.03>—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Sulfate <1.14>—Perform the test with 2.0 g of Maltose Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 5.0 g of Maltose Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 4 ppm).

(5) Arsenic <1.11>—Dissolve 1.5 g of Maltose Hydrate in 5 mL of water, add 5 mL of dilute sulfuric acid and 1 mL of bromine TS, heat on a water bath for 5 minutes, then heat to concentrate to 5 mL, and use this solution as the test solution

after cooling. Perform the test (not more than 1.3 ppm).

(6) Dextrin, soluble starch and sulfite—Dissolve 1.0 g of Maltose Hydrate in 10 mL of water, and add 1 drop of iodine TS: a yellow color appears, and the color changes to a blue by adding 1 drop of starch TS.

(7) Nitrogen—Weigh accurately about 2 g of Maltose Hydrate, and perform the test as directed under Nitrogen Determination <1.08> using 10 mL of sulfuric acid for the decomposition and 45 mL of a solution of sodium hydroxide (2 in 5) for the addition: the amount of nitrogen (N: 14.01) is not more than 0.01%.

(8) Related substances—Dissolve 0.5 g of Maltose Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak areas from both solutions by the automatic integration method: the total area of the peaks which appear before the peak of maltose from the sample solution is not larger than 1.5 times of the peak area of maltose from the standard solution, and the total area of the peaks which appear after the peak of maltose from the sample solution is not larger than 1/2 time of the peak area of maltose from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the sensitivity so that the peak height of maltose obtained from 20 μ L of the standard solution is about 30 mm.

Time span of measurement: About 2 times as long as the retention time of maltose.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g each of Maltose Hydrate and Maltose Reference Standard, previously dried, dissolve in exactly 10 mL each of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions, and determine the ratios, Q_T and Q_S , of the peak area of maltose to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{12}H_{22}O_{11} \cdot H_2O \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Maltose Reference Standard

Internal standard solution—A solution of ethylene glycol (1 in 50).

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 8 mm in inside diameter and about 55 cm in length, packed with gel-type strong acid cation-exchange resin for liquid chromatography (degree of cross-linking: 8 %) (10 μ m in particle diameter).

Column temperature: A constant temperature of about

50°C.

Mobile phase: Water

Flow rate: Adjust the flow rate so that the retention time of maltose is about 18 minutes.

Selection of column: Dissolve 0.25 g of maltose, 0.25 g of glucose and 0.4 g of ethylene glycol in water to make 100 mL. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of maltose, glucose and ethylene glycol in this order with the resolution of between the peaks of maltose and glucose being not less than 4.

Containers and storage Containers—Tight containers.

Freeze-dried Mamushi Antivenom, Equine

乾燥まむしウマ抗毒素

Freeze-dried Mamushi Antivenom, Equine, is a preparation for injection which is dissolved before use.

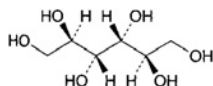
It contains *Agkistrodon Halys* antivenom in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Mamushi Antivenom, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Mamushi Antivenom, Equine, becomes a colorless or light yellow-brown, clear liquid, or a slightly white-turbid liquid on addition of solvent.

D-Mannitol

D-マンニトール



$C_6H_{14}O_6$: 182.17

D-Mannitol [69-65-8]

D-Mannitol, when dried, contains not less than 98.0% of $C_6H_{14}O_6$.

Description D-Mannitol occurs as white crystals or powder. It is odorless, and has a sweet taste with a cold sensation.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) To 5 drops of a saturated solution of D-Mannitol add 1 mL of iron (III) chloride TS and 5 drops of a solution of sodium hydroxide (1 in 5): a yellow precipitate is produced. Shake this solution vigorously: a clear solution is produced. On addition of a solution of sodium hydroxide (1 in 5), no precipitate is produced.

(2) Determine the infrared absorption spectrum of D-Mannitol as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit

similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 1 g of D-Mannitol in 3 mL of warm water, then allow to stand at 5°C for 24 hours or until crystals appear, and filter. Wash the crystals so obtained with a few amount of cold water, dry at 105°C for 4 hours, and perform the test with the crystals.

Optical rotation <2.49> $[\alpha]_D^{20}$: + 137 – + 145° Weigh accurately about 1.0 g of D-Mannitol, previously dried, dissolve in 80 mL of a solution of hexaammonium heptamolybdate tetrahydrate (1 in 20), and add diluted sulfuric acid (1 in 35) to make exactly 100 mL. Measure the optical rotation of this solution in a 100-mm cell.

Melting point <2.60> 166 – 169°C

Purity (1) Clarity and color of solution—Dissolve 2.0 g of D-Mannitol in 10 mL of water by warming: the solution is clear and colorless.

(2) Acidity—Dissolve 5.0 g of D-Mannitol in 50 mL of freshly boiled and cooled water, and add 1 drop of phenolphthalein TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Chloride <1.03>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(4) Sulfate <1.14>—Perform the test with 2.0 g of D-Mannitol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(5) Heavy metals <1.07>—Proceed with 5.0 g of D-Mannitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(6) Nickel—Dissolve 0.5 g of D-Mannitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(7) Arsenic <1.11>—Prepare the test solution with 1.5 g of D-Mannitol according to Method 1, and perform the test (not more than 1.3 ppm).

(8) Sugars—To 5.0 g of D-Mannitol add 15 mL of water and 4.0 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS), and add water to make 50 mL. Pipet 10 mL of this solution into a flask, boil gently with 10 mL of water and 40 mL of Fehling's TS for 3 minutes, and allow to stand to precipitate copper (I) oxide. Filter the supernatant liquid through a glass filter (G4), wash the precipitate with hot water until the last washing no longer shows an alkaline reaction, and filter the washings through the glass filter described above. Dissolve the precipitate in 20 mL of iron (III) sulfate TS in the flask, filter through the glass filter described above, and wash the filter with water. Combine the washings and the filtrate, heat to 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate: the consumed volume is not more than 1.0 mL.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of D-Mannitol, previously dried, and dissolve in water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly

50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. After cooling, add 2.5 g of potassium iodide, stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of C₆H₁₄O₆

Containers and storage Containers—Tight containers.

D-Mannitol Injection

D-Mannite Injection

D-マンニトール注射液

D-Mannitol Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of D-mannitol (C₆H₁₄O₆: 182.17).

Method of preparation Prepare as directed under Injections, with D-Mannitol. No preservative is added.

Description D-Mannitol Injection is a clear, colorless liquid. It has a sweet taste.

It may precipitate crystals.

Identification Concentrate D-Mannitol Injection on a water bath to make a saturated solution. Proceed with 5 drops of this solution as directed in the Identification (1) under D-Mannitol.

pH <2.54> 4.5 – 7.0

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

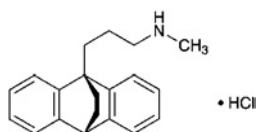
Assay Measure exactly a volume of D-Mannitol Injection, equivalent to about 5 g of D-Mannitol (C₆H₁₄O₆), and add water to make exactly 250 mL. To exactly 10 mL of this solution add water to make exactly 100 mL. Measure exactly 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under D-Mannitol.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of C₆H₁₄O₆

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Maprotiline Hydrochloride

マプロチリン塩酸塩



C₂₀H₂₃N.HCl: 313.86
3-(9,10-Dihydro-9,10-ethanoanthracen-9-yl)-

N-methylpropylamine monohydrochloride [10347-81-6]

Maprotiline Hydrochloride, when dried, contains not less than 99.0% of C₂₀H₂₃N.HCl.

Description Maprotiline Hydrochloride occurs as a white crystalline powder.

It is soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water.

Melting point: about 244°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Maprotiline Hydrochloride in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Maprotiline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample with ethanol (99.5), filter, dry the crystals so obtained, and perform the test with the crystals.

(3) To 5 mL of a solution of Maprotiline Hydrochloride (1 in 200) add 2 mL of ammonia TS, heat on a water bath for 5 minutes, cool, and filter. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Maprotiline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Maprotiline Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of 2-butanol, diluted ammonia solution (28) (1 in 3) and ethyl acetate (14:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Maprotiline Hydrochloride, previously dried, dissolve in 180 mL of acetic acid (100), add 8 mL of a solution of bismuth nitrate pentahydrate in acetic acid (100) (1 in 50), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 31.39 mg of $C_{20}H_{23}N.HCl$

Containers and storage Containers—Well-closed containers.

Freeze-dried Live Attenuated Measles Vaccine

乾燥弱毒生麻疹ワクチン

Freeze-dried Live Attenuated Measles Vaccine is a preparation for injection which is dissolved before use.

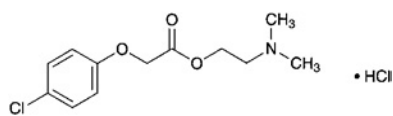
It contains live attenuated measles virus.

It conforms to the requirements of Freeze-dried Live Attenuated Measles Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Measles Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Meclofenoxate Hydrochloride

メクロフェノキサート塩酸塩



$C_{12}H_{16}ClNO_3.HCl$: 294.17

2-(Dimethylamino)ethyl (4-chlorophenoxy)acetate monohydrochloride [3685-84-5]

Meclofenoxate Hydrochloride contains not less than 98.0% of $C_{12}H_{16}ClNO_3.HCl$, calculated on the anhydrous basis.

Description Meclofenoxate Hydrochloride occurs as white crystals or crystalline powder. It has a faint, characteristic odor and a bitter taste.

It is freely soluble in water and in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Meclofenoxate Hydrochloride (1 in 20) is between 3.5 and 4.5.

Identification (1) To 0.01 g of Meclofenoxate Hydrochloride add 2 mL of ethanol (95), dissolve by warming if necessary, cool, add 2 drops of a saturated solution of hydroxylammonium chloride in ethanol (95) and 2 drops of a saturated solution of potassium hydroxide in ethanol (95), and heat in a water bath for 2 minutes. After cooling, render the solution slightly acidic with dilute hydrochloric acid, and add 3 drops of iron (III) chloride TS: a red-purple to dark purple color develops.

(2) Dissolve 0.05 g of Meclofenoxate Hydrochloride in 5 mL of water, and add 2 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Meclofenoxate Hydrochloride (1 in 10,000) as directed under

Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Meclofenoxate Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 139 – 143°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Meclofenoxate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 1.0 g of Meclofenoxate Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Meclofenoxate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Meclofenoxate Hydrochloride according to method 3, and perform the test (not more than 2 ppm).

(5) Organic acids—To 2.0 g of Meclofenoxate Hydrochloride add 50 mL of diethyl ether, shake for 10 minutes, filter through a glass filter (G3), wash the residue with two 5-mL portions of diethyl ether, and combine the washings with the filtrate. To this solution add 50 mL of neutralized ethanol and 5 drops of phenolphthalein TS, and neutralize with 0.1 mol/L sodium hydroxide VS: the volume of 0.1 mol/L sodium hydroxide VS consumed is not more than 0.54 mL.

Water <2.48> Not more than 0.50% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

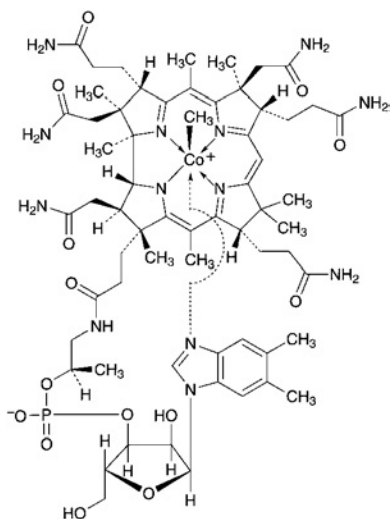
Assay Weigh accurately about 0.4 g of Meclofenoxate Hydrochloride, dissolve in 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-green through yellow-green to pale greenish yellow [indicator: 3 drops of a solution of malachite green oxalate in acetic acid (100) (1 in 100)]. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.42 mg of $C_{12}H_{16}ClNO_3.HCl$

Containers and storage Containers—Tight containers.

Mecobalamin

メコバラミン



$C_{63}H_{91}CoN_{13}O_{14}P$: 1344.38

$Co\alpha$ -[α -(5,6-Dimethyl-1*H*-benzimidazol-1-yl)]- $Co\beta$ -methylcobamide [13422-55-4]

Mecobalamin contains not less than 98.0% of $C_{63}H_{91}CoN_{13}O_{14}P$, calculated on the anhydrous basis.

Description Mecobalamin occurs as dark red crystals or crystalline powder.

It is sparingly soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

It is affected by light.

Identification (1) Conduct this procedure without exposure to light, using light-resistant vessels. Determine the absorption spectrum of a solution of Mecobalamin in hydrochloric acid-potassium chloride buffer solution, pH 2.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Mecobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Mecobalamin in phosphate buffer solution, pH 7.0 (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Mecobalamin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Mix 1 mg of Mecobalamin with 0.05 g of potassium bisulfate, and fuse by igniting. Cool, break up the mass with a glass rod, add 3 mL of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, then add dropwise sodium hydroxide TS until a light red color just develops. Add 0.5 g of sodium acetate, 0.5 mL of dilute acetic acid and 0.5 mL of a solution of disodium 1-nitroso-2-naphthol-3,6-disulfonate (1 in 500): a red to orange-red color is immediately produced.

Then add 0.5 mL of hydrochloric acid, and boil for 1 minute: the red color does not disappear.

Purity (1) Clarity and color of solution—Dissolve 20 mg of Mecobalamin in 10 mL of water: the solution is clear and red color.

(2) Related substances—Perform the test with 10 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak area of mecobalamin and others of the sample solution by the automatic integration method: each area of the peaks other than mecobalamin is not larger than 0.5% of the peak area of mecobalamin, and the total area of the peaks other than mecobalamin is not larger than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of mecobalamin.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add the mobile phase to make exactly 10 mL. Confirm that the peak area of mecobalamin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of mecobalamin obtained from 10 μ L of the test solution for system suitability.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the test solution for system suitability under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 3.0%.

Water <2.48> Not more than 12% (0.1 g, direct titration).

Assay Conduct this procedure without exposure to light, using light-resistant vessels. Weigh accurately about 50 mg of Mecobalamin and Mecobalamin Reference Standard (separately, determine the water <2.48> in the same manner as mecobalamin), dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μ L of each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of mecobalamin in each solution.

$$\begin{aligned} \text{Amount (mg) of } C_{63}H_{91}CoN_{13}O_{14}P \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Mecobalamin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 266 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 200 mL of acetonitrile add 800 mL of 0.02 mol/L phosphate buffer solution, pH 3.5, then add 3.76 g of sodium 1-hexane sulfonate to dissolve.

Flow rate: Adjust the flow rate so that the retention time of mecobalamin is about 12 minutes.

System suitability—

System performance: Dissolve 5 mg each of cyanocobalamin and hydroxocobalamin acetate in the mobile phase to make 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, cyanocobalamin and hydroxocobalamin are eluted in this order with the resolution between these peaks being not less than 3. And when the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates of the peak of mecobalamin is not less than 6000.

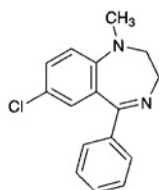
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mecobalamin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Medazepam

メダゼパム



$C_{16}H_{15}ClN_2$: 270.76

7-Chloro-1-methyl-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepine [2898-12-6]

Medazepam, when dried, contains not less than 98.5% of $C_{16}H_{15}ClN_2$.

Description Medazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in methanol, in ethanol (95), in acetic acid (100) and in diethyl ether, and practically insoluble in water.

It gradually changes in color by light.

Identification (1) Dissolve 0.01 g of Medazepam in 3 mL of citric acid-acetic acid TS: a deep orange color develops. Heat in a water bath for 3 minutes: the color changes to dark red.

(2) Determine the absorption spectrum of a solution of Medazepam in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with Medazepam as directed under Flame Coloration Test <1.04> (2): a green color is produced.

Melting point <2.60> 101 – 104°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Medazepam in 10 mL of methanol: the solution is clear and light yellow to yellow in color.

(2) Chloride <1.03>—Dissolve 1.5 g of Medazepam in 50 mL of diethyl ether, add 46 mL of water and 4 mL of sodium carbonate TS, shake, and collect the water layer. Wash the water layer with two 20-mL portions of diethyl ether, and filter. To 20 mL of the filtrate add dilute nitric acid to neutralize, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Medazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Medazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.25 g of Medazepam in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, acetone and ammonia solution (28) (60:40:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Medazepam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 27.08 mg of $C_{16}H_{15}ClN_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Medicinal Carbon

薬用炭

Description Medicinal Carbon occurs as a black, odorless and tasteless powder.

Identification Place 0.5 g of Medicinal Carbon in a test tube, and heat by direct application of flame with the aid of a current of air: it burns without any flame. Pass the evolved gas through calcium hydroxide TS: a white turbidity is produced.

Purity (1) Acidity or alkalinity—Boil 3.0 g of Medicinal

Carbon with 60 mL of water for 5 minutes, allow to cool, dilute to 60 mL with water, and filter: the filtrate is colorless and neutral.

(2) Chloride <1.03>—Take 4.0 mL of the filtrate obtained in (1) in a Nessler tube, add 6 mL of dilute nitric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.80 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.142%).

(3) Sulfate <1.14>—Take 5 mL of the filtrate obtained in (1) in a Nessler tube, add 1 mL of dilute hydrochloric acid and sufficient water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.192%).

(4) Sulfide—Boil 0.5 g of Medicinal Carbon with a mixture of 15 mL of dilute hydrochloric acid and 10 mL of water: lead (II) acetate paper does not become brown when held in the evolved gas within 5 minutes.

(5) Cyanogen compounds—Place a mixture of 5 g of Medicinal Carbon, 2 g of L-tartaric acid and 50 mL of water in a distilling flask connected to a condenser provided with a tightly fitting adapter, the end of which dips below the surface of a mixture of 2 mL of sodium hydroxide TS and 10 mL of water, contained in a small flask surrounded by ice. Heat the mixture in the distilling flask to boiling, and distil to 25 mL. Dilute the distillate with water to 50 mL. To 25 mL of the diluted distillate add 1 mL of a solution of iron (II) sulfate heptahydrate (1 in 20), heat the mixture almost to boiling, cool, and filter. To the filtrate add 1 mL of hydrochloric acid and 0.5 mL of dilute iron (III) chloride TS: no blue color is produced.

(6) Acid soluble substances—To about 1 g of Medicinal Carbon, accurately weighed, add 20 mL of water and 5 mL of hydrochloric acid, boil for 5 minutes, filter, wash the residue with 10 mL of hot water, and add the washings to the filtrate. Add 5 drops of sulfuric acid to the filtrate, evaporate to dryness, and ignite the residue strongly: the mass of the residue is not more than 3.0%.

(7) Heavy metals <1.07>—Proceed with 0.5 g of Medicinal Carbon according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(8) Zinc—Ignite 0.5 g of Medicinal Carbon to ash, add 5 mL of dilute nitric acid to the residue, boil gently for 5 minutes, filter, wash with 10 mL of water, and combine the washings and the filtrate. Add 3 mL of ammonia TS to the solution, filter again, wash with water, combine the washings and the filtrate, add another washing to make 25 mL, add 1 drop of sodium sulfide TS, and allow to stand for 3 minutes: the liquid produces no turbidity.

(9) Arsenic <1.11>—Prepare the test solution with 1.0 g of Medicinal Carbon according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 15.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 4.0% (1 g).

Adsorptive power (1) Add 1.0 g of Medicinal Carbon, previously dried, to 100 mL of water containing 120 mg of quinine sulfate, shake the mixture vigorously for 5 minutes, filter immediately, and reject the first 20 mL of the filtrate.

Add 5 drops of iodine TS to 10 mL of the subsequent filtrate: no turbidity is produced.

(2) Dissolve 250 mg of methylene blue trihydrate, exactly weighed, in water to make exactly 250 mL. Measure two 50-mL portions of this solution into each of two glass-stoppered flasks. To one flask add exactly 250 mg of Medicinal Carbon, previously dried, and shake vigorously for 5 minutes. Filter the contents of each flask, rejecting the first 20 mL of each filtrate. Pipet 25-mL portions of the remaining filtrate into two 250-mL volumetric flasks. To each volumetric flask add 50 mL of a solution of sodium acetate trihydrate (1 in 10), then add exactly 35 mL of 0.05 mol/L iodine VS with swirling. Allow them to stand for 50 minutes, shaking vigorously from time to time. Dilute each mixture to exactly 250 mL with water, allow to stand for 10 minutes, and filter each solution at a temperature not exceeding 20°C, rejecting the first 30 mL of each filtrate. Titrate <2.50> the excess iodine in a 100-mL aliquot of each filtrate with 0.1 mol/L sodium thiosulfate VS. The difference between the two titrations is not less than 1.2 mL.

Containers and storage Containers—Well-closed containers.

Medicinal Soap

薬用石ケン

Medicinal Soap is sodium salts of fatty acids.

Description Medicinal Soap occurs as white to light yellow powder or granules. It has a characteristic odor free from rancidity.

Medicinal Soap is sparingly soluble in water, and slightly soluble in ethanol (95).

A solution of Medicinal Soap (1 in 100) is alkaline.

Fatty acid Dissolve 25 g of Medicinal Soap in 300 mL of hot water, add 60 mL of dilute sulfuric acid slowly, and warm in a water bath for 20 minutes. After cooling, filter off the precipitate, and wash with warm water until the washing no longer shows acidity to methyl orange TS. Transfer the precipitate to a small beaker, and heat on a water bath to complete separation of water and transparent fatty acids. Filter the fatty acid into a small beaker while warm, dry at 100°C for 20 minutes, and perform the test with this material as directed under Fats and Fatty Oils <1.13>. The congealing point of the fatty acid is between 18°C and 28°C. The acid value is 185 – 205. The iodine value is 82 – 92.

Purity (1) Acidity or alkalinity—Dissolve 5.0 g of Medicinal Soap in 85 mL of neutralized ethanol by warming on a water bath, filter while hot through absorbent cotton, and wash the filter and the residue with three 5-mL portions of hot neutralized ethanol. Combine the filtrate and the washings, add hot neutralized ethanol to make exactly 100 mL, and perform the following tests quickly using this as the sample solution at 70°C.

(i) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS to 40 mL of the sample solution: a red color develops.

(ii) Add 3 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS to 40 mL of the sample solution:

no red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Medicinal Soap according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Ethanol-insoluble substances—Weigh accurately about 2 g of Medicinal Soap, dissolve by warming in 100 mL of neutralized ethanol, filter the solution through a glass filter (G4), wash the residue with hot neutralized ethanol, and dry at 105°C for 4 hours: the mass of the residue is not more than 1.0%.

(4) Water-insoluble substances—Wash thoroughly the dried substances obtained in (3) with 200 mL of water, and dry at 105°C for 4 hours: the mass of the residue is not more than 0.15%.

(5) Alkali carbonates—To the washings obtained in (4) add 3 drops of methyl orange TS and 2 mL of 0.05 mol/L sulfuric acid VS: a red color develops.

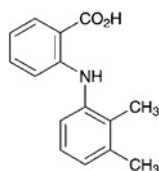
Loss on drying Not more than 5.0% in the case of the powder, and not more than 10.0% in the case of the granules.

Weigh accurately about 0.5 g of Medicinal Soap in a tared beaker, add 10 g of sea sand (No. 1), previously dried at 105°C for 1 hour, and again weigh the beaker. Add 10 mL of ethanol (95), evaporate on a water bath to dryness with thorough stirring, and dry at 105°C for 3 hours.

Containers and storage Containers—Well-closed containers.

Mefenamic Acid

メフェナム酸



$C_{15}H_{15}NO_2$: 241.29

2-(2,3-Dimethylphenylamino)benzoic acid [61-68-7]

Mefenamic Acid, when dried, contains not less than 99.0% of $C_{15}H_{15}NO_2$.

Description Mefenamic Acid occurs as a white to light yellow powder. It is odorless and tasteless at first, but leaves a slightly bitter aftertaste.

It is sparingly soluble in diethyl ether, slightly soluble in methanol, in ethanol (95) and in chloroform, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 225°C (with decomposition).

Identification (1) Dissolve 0.01 g of Mefenamic Acid in 1 mL of methanol by warming, cool, add 1 mL of a solution of *p*-nitrobenzene diazonium fluoroborate (1 in 1000) and 1 mL of sodium hydroxide TS, and mix thoroughly: an orange-red color is produced.

(2) Dissolve 0.01 g of Mefenamic Acid in 2 mL of sulfuric acid, and heat: the solution shows a yellow color and a green fluorescence.

(3) Dissolve 7 mg of Mefenamic Acid in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Chloride <1.03>—To 1.0 g of Mefenamic Acid add 20 mL of sodium hydroxide TS, and dissolve by warming. Cool, add 2 mL of acetic acid (100) and water to make 100 mL, and mix well. Remove the produced precipitate by filtration, discard the first 10 mL of the filtrate, and to subsequent 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 0.5 mL of acetic acid (100), 6 mL of nitric acid and water to make 50 mL (not more than 0.071%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Mefenamic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mefenamic Acid according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Mefenamic Acid, in 5 mL of a mixture of chloroform and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of chloroform and methanol (3:1) to make exactly 200 mL, pipet 10 mL of this solution, add a mixture of chloroform and methanol (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-methyl-1-propanol and ammonia solution (28) (3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

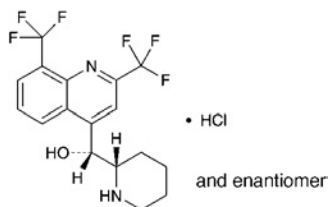
Assay Weigh accurately about 0.5 g of Mefenamic Acid, previously dried, and dissolve in 100 mL of ethanol (95), previously neutralized to phenol red TS with 0.1 mol/L sodium hydroxide VS, by warming gently. Cool, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from yellow through yellow-red to red-purple (indicator: 2 to 3 drops of phenol red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.13 mg of $C_{15}H_{15}NO_2$

Containers and storage Containers—Well-closed containers.

Mefloquine Hydrochloride

メフロキン塩酸塩



$C_{17}H_{16}F_6N_2O \cdot HCl$: 414.77
 (1*RS*)-[2,8-Bis(trifluoromethyl)quinolin-4-yl][(2*SR*)-piperidin-2-yl]methanol monohydrochloride
 [51773-92-3]

Mefloquine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{16}F_6N_2O \cdot HCl$.

Description Mefloquine Hydrochloride occurs as white crystals or a white crystalline powder.

It is freely soluble in methanol, soluble in ethanol (99.5), and slightly soluble in water.

It dissolves in sulfuric acid.

A solution of Mefloquine Hydrochloride in methanol (1 in 20) shows no optical rotation.

Melting point: about 260°C (with decomposition).

Identification (1) Dissolve 2 mg of Mefloquine Hydrochloride in 1 mL of sulfuric acid: the solution shows a blue fluorescence under ultraviolet light (main wavelength: 365 nm).

(2) Determine the absorption spectrum of a solution of Mefloquine Hydrochloride in methanol (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mefloquine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Mefloquine Hydrochloride (1 in 1000) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is formed, and the separated precipitate dissolves on the addition of an excess amount of ammonia TS.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mefloquine Hydrochloride according to Method 2 using a quartz crucible, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—To 1.0 g of Mefloquine Hydrochloride add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), burn the ethanol, gradually heat, and incinerate by ignition at 800°C. If a carbonized residue still retains, moisten the residue with a little amount

of nitric acid, and ignite again to incinerate. After cooling, to the residue add 3 mL of hydrochloric acid, warm on a water bath to dissolve, and perform the test using this solution as the test solution (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Mefloquine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than mefloquine and the peak eluted first from the sample solution is not larger than the peak area of mefloquine from the standard solution, and the total area of the peaks other than the peak of mefloquine and the peak eluted first from the sample solution is not larger than 2.5 times the peak area of mefloquine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 282 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with aminopropylsilylanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of acetonitrile and diluted phosphoric acid (1 in 14) (24:1).

Flow rate: Adjust the flow rate so that the retention time of mefloquine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of mefloquine.

System suitability—

Test for required detectability: To exactly 10 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of mefloquine obtained with 10 μ L of this solution is equivalent to 40 to 60% of that obtained with 10 μ L of the standard solution.

System performance: Dissolve 10 mg of mefloquine hydrochloride and 5 mg of diprophylline in 50 mL of the mobile phase. To 2 mL of this solution add the mobile phase to make 20 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, diprophylline and mefloquine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mefloquine is not more than 2.0%.

(4) Residual solvent—The test is specified separately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.5 g of Mefloquine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and ti-

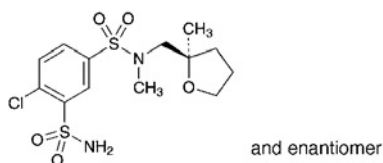
trate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.48 mg of $C_{17}H_{16}F_6N_2O.HCl$

Containers and storage Containers—Well-closed containers.

Mefruside

メフルシド



$C_{13}H_{19}ClN_2O_5S_2$: 382.88

4-Chloro-*N*-methyl-*N*-[(2*RS*)-2-methyltetrahydrofuran-2-ylmethyl]-3-sulfamoylbenzenesulfonamide [7195-27-9]

Mefruside, when dried, contains not less than 98.5% of $C_{13}H_{19}ClN_2O_5S_2$.

Description Mefruside occurs as a white crystalline powder.

It is very soluble in dimethylformamide, freely soluble in acetone, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

A solution of Mefruside in dimethylformamide (1 in 10) has no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mefruside in methanol (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mefruside, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Mefruside as directed under Flame Coloration Test (2): a green color appears.

Melting point <2.60> 149 – 152°C

Purity (1) Heavy metals <1.07>—Dissolve 1.0 g of Mefruside in 30 mL of acetone, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 30 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mefruside according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Mefruside in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make

exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (5:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Mefruside, previously dried, dissolve in 80 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Separately, perform a blank determination with a solution prepared by adding 13 mL of water to 80 mL of *N,N*-dimethylformamide, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 38.29 mg of $C_{13}H_{19}ClN_2O_5S_2$

Containers and storage Containers—Well-closed containers.

Mefruside Tablets

メフルシド錠

Mefruside Tablets contain not less than 95% and not more than 105% of the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$: 382.88).

Method of preparation Prepare as directed under Tablets, with Mefruside.

Identification (1) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.3 g of Mefruside according to the labeled amount, shake with 15 mL of heated methanol for 20 minutes, and filter. Add 25 mL of water to the filtrate, and allow to stand while ice-cooling for 30 minutes. Filter the white precipitate formed, wash with water, and dry at 105°C for 2 hours: the precipitate melts <2.60> between 149°C and 152°C.

(2) Weigh a quantity of powdered Mefruside Tablets, equivalent to 0.01 g of Mefruside according to the labeled amount, shake with 70 mL of methanol strongly for 15 minutes, add methanol to make 100 mL, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm, and between 283 nm and 287 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Mefruside Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 20 mL or more of

the dissolved solution 45 minutes after starting the test, and filter through a filter paper for quantitative analysis (5C). Discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 70 mg of mefruside for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 285 nm in a layer of 5 cm in length as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Mefruside Tablets in 45 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)

$$= W_S \times (A_T/A_S) \times (1/C) \times 36$$

W_S : Amount (mg) of mefruside for assay.

C : Labeled amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$) in 1 tablet.

Assay Weigh accurately not less than 20 Mefruside Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 65 mg of mefruside ($C_{13}H_{19}ClN_2O_5S_2$), shake with 70 mL of methanol for 15 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, take exactly 10 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 65 mg of mefruside for assay, previously dried at 105°C for 2 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 285 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of mefruside ($C_{13}H_{19}ClN_2O_5S_2$)

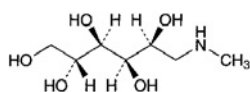
$$= W_S \times (A_T/A_S)$$

W_S : Amount (mg) of mefruside for assay

Containers and storage Containers—Tight containers.

Meglumine

メグルミン



$C_7H_{17}NO_5$: 195.21

1-Deoxy-1-methylamino-D-glucitol [6284-40-8]

Meglumine, when dried, contains not less than 99.0% of $C_7H_{17}NO_5$.

Description Meglumine occurs as a white, crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in water, and slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Meglumine (1 in 10) is between 11.0 and 12.0.

Identification (1) To 1 mL of a solution of Meglumine (1 in 10) add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS: a deep red color develops.

(2) To 2 mL of a solution of Meglumine (1 in 10) add 1 drop of methyl red TS, and add 0.5 mL of dilute sodium hydroxide TS and 0.5 g of boric acid after neutralizing with 0.5 mol/L sulfuric acid TS: a deep red color develops.

(3) Dissolve 0.5 g of Meglumine in 1 mL of diluted hydrochloric acid (1 in 3), and add 10 mL of ethanol (99.5): a white precipitate is produced. Then, rubbing the inside wall of the container with a glass rod, cool with ice and produce more precipitate. Filter the precipitate by suction through a glass filter (G3), wash the precipitate with a small volume of ethanol (99.5), and dry at 105°C for 1 hour: the residue thus obtained melts <2.60> between 149°C and 152°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-16.0 - -17.0^\circ$ (after drying, 1 g, water, 10 mL, 100 mm).

Melting point <2.60> 128 – 131°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Meglumine in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 10 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.009%).

(3) Sulfate <1.14>—Dissolve 1.0 g of Meglumine in 30 mL of water, and add 5 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Meglumine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 2.0 g of Meglumine according to Method 3, and perform the test (not more than 1 ppm).

(6) Reducing substances—To 5 mL of a solution of Meglumine (1 in 20) add 5 mL of Fehling's TS, and boil for 2 minutes: no red-brown precipitate is produced.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Meglumine, previously dried, dissolve in 25 mL of water, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (indicator: 2 drops of methyl red TS).

Each mL of 0.1 mol/L hydrochloric acid VS
= 19.52 mg of $C_7H_{17}NO_5$

Containers and storage Containers—Tight containers.

Meglumine Amidotrizoate Injection

アミドトリゾ酸メグルミン注射液

Meglumine Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 46.9 w/v% and not more than 51.8 w/v% of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$; 613.91).

Method of preparation

Amidotrizoic Acid (anhydrous)	493.2 g
Meglumine	156.8 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Meglumine Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification (1) To 2 mL of Meglumine Amidotrizoate Injection add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

Optical rotation <2.49> α_D^{20} : $-3.63 - -4.20^\circ$ (100 mm).

pH <2.54> 6.0 – 7.7

Purity (1) Primary aromatic amines—Mix 0.40 mL of Meglumine Amidotrizoate Injection with 6 mL of water, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

(2) Iodine and iodide—To 0.50 mL of Meglumine Amidotrizoate Injection add water to make 20 mL, shake with 5 mL of dilute nitric acid, filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Prepare a solution with isotonic sodium chloride solution so as to contain 0.40 mL of Meglumine

Amidotrizoate Injection per 1 mL, and perform the test: it meets the requirements.

Assay To an exactly measured 1 mL of Meglumine Amidotrizoate Injection add water to make exactly 200 mL, pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying <2.41> in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of amidotrizoic acid to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of amidotrizoic acid (C}_{11}\text{H}_9\text{I}_3\text{N}_2\text{O}_4) \\ & = W_S \times (Q_T/Q_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

Internal standard solution—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, amidotrizoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Meglumine Iotalamate Injection

イオタラム酸メグルミン注射液

Meglumine Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of iotalamic acid ($C_{11}H_9I_3N_2O_4$; 613.91).

Method of preparation

(1)	
Iotalamic Acid	227.59 g
Meglumine	72.41 g
Water for Injection	a sufficient quantity
To make 1000 mL	
(2)	
Iotalamic Acid	455 g
Meglumine	145 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients (1) or (2).

Description Meglumine Iotalamate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification (1) To 1 mL of Meglumine Iotalamate Injection add 1 mL of potassium naphthoquinone sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(2) To a volume of Meglumine Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid while shaking: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 4 hours. Proceed with the precipitate so obtained as directed in the Identification (2) under Iotalamic Acid.

Optical rotation <2.49>

Method of preparation (1) α_D^{20} : $-1.67 - -1.93^\circ$ (100 mm).

Method of preparation (2) α_D^{20} : $-3.35 - -3.86^\circ$ (100 mm).

pH <2.54> 6.5 – 7.7

Purity (1) Primary aromatic amines—To a volume of Meglumine Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—Take a volume of Meglumine Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid

according to the labeled amount, and proceed as directed in the Purity (2) under Sodium Iotalamate Injection.

Bacterial endotoxins <4.01> Less than 0.90 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Meglumine Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of iotalamic acid to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of iotalamic acid (C}_{11}\text{H}_9\text{I}_3\text{N}_2\text{O}_4) \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of iotalamic acid for assay

Internal standard solution—A solution of L-tryptophan in the mobile phase (3 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 3.9 g of phosphoric acid and 2.8 mL of triethylamine in water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of iotalamic acid is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, iotalamic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Meglumine Sodium Amidotrizoate Injection

アミドトリゾ酸ナトリウムメグルミン注射液

Meglumine Sodium Amidotrizoate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$; 613.91).

Method of preparation

(1)

Amidotrizoic Acid (anhydrous)	522.77 g
Sodium Hydroxide	25.16 g
Meglumine	43.43 g
Water for Injection	a sufficient quantity
To make 1000 mL	

(2)

Amidotrizoic Acid (anhydrous)	471.78 g
Sodium Hydroxide	5.03 g
Meglumine	125.46 g
Water for Injection	a sufficient quantity
To make 1000 mL	

(3)

Amidotrizoic Acid (anhydrous)	597.30 g
Sodium Hydroxide	6.29 g
Meglumine	159.24 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients (1), (2) or (3).

Description Meglumine Sodium Amidotrizoate Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification (1) To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 1 g of Amidotrizoic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate so obtained as directed in the Identification (2) under Amidotrizoic Acid.

(2) To 1 mL of Meglumine Sodium Amidotrizoate Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color develops.

(3) Meglumine Sodium Amidotrizoate Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49>

Method of preparation (1) α_D^{20} : $-1.01 - -1.17^\circ$ (100 mm).

Method of preparation (2) α_D^{20} : $-2.91 - -3.36^\circ$ (100 mm).

Method of preparation (3) α_D^{20} : $-3.69 - -4.27^\circ$ (100 mm).

pH <2.54> 6.0 – 7.7

Purity (1) Primary aromatic amines—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.20 g of Amidotrizoic Acid according to the labeled amount, add 6 mL of water, mix, add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, and shake. Proceed as directed in the Purity (2) under Amidotrizoic Acid: the absorbance is not more than 0.19.

(2) Iodine and iodide—To a volume of Meglumine Sodium Amidotrizoate Injection, equivalent to 0.25 g of Amidotrizoic Acid according to the labeled amount, add water to make 20 mL, add 5 mL of dilute nitric acid, shake well, and filter by suction through a glass filter (G4). Add 5 mL of chloroform to the filtrate, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of hydrogen peroxide (30), and shake vigorously: the chloroform layer has no more color than the following control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. Add 20 mL of water to 0.10 mL of this solution, add 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of hydrogen peroxide (30), and shake vigorously.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Dilute Meglumine Sodium Amidotrizoate Injection with isotonic sodium chloride solution so as to contain 0.20 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$) per mL according to the labeled amount, and perform the test: it meets the requirements.

Assay To an exactly measured volume of Meglumine Sodium Amidotrizoate Injection, equivalent to about 0.5 g of amidotrizoic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of amidotrizoic acid for assay (previously determine the loss on drying <2.41> in the same manner as Amidotrizoic Acid), dissolve in a solution of meglumine (3 in 1000) to make exactly 100 mL, then pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 100 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of amidotrizoic acid to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of amidotrizoic acid (C}_{11}\text{H}_9\text{I}_3\text{N}_2\text{O}_4) \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of amidotrizoic acid for assay, calculated on the dried basis

Internal standard solution—Dissolve 0.06 g of acetrizoic acid in a solution of meglumine (3 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.7 g of tetrabutylammonium phosphate and 7.0 g of dipotassium hydrogenphosphate in 750 mL of water, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), add water to make 800 mL, then add 210 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of amidotrizoic acid is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, amidotrizoic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of amidotrizoic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Meglumine Sodium Iodamide Injection

ヨードミドナトリウムメグルミン注射液

Meglumine Sodium Iodamide Injection is an aqueous solution for injection.

It contains not less than 59.7 w/v% and not more than 65.9 w/v% of iodamide (C₁₂H₁₁I₃N₂O₄; 627.94).

Method of preparation

Iodamide	627.9 g
Sodium Hydroxide	6.0 g
Meglumine	165.9 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Meglumine Sodium Iodamide Injection is a clear, colorless to pale yellow, slightly viscous liquid.

It gradually changes in color by light.

Identification (1) To 2 mL of Meglumine Sodium Iodamide Injection add 25 mL of water, and add 3 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is formed. Filter the precipitate by suction through a glass filter (G3), and wash with two 10-mL portions of water. Transfer the precipitate to a suitable flask, add 100 mL of water, dissolve by heating, and gently boil until the volume becomes about 30 mL. After cooling, collect the separated crystals by filtration, dry at 105°C for 1 hour, and proceed as directed in the Identification (1) and (2) under Iodamide.

(2) Determine the infrared absorption spectrum of the dried crystals obtained in (1) as directed in the potassium bromide disk method under Infrared Spectrophotometry

<2.25>: it exhibits absorption at the wave numbers of about 3390 cm⁻¹, 1369 cm⁻¹, 1296 cm⁻¹, 1210 cm⁻¹ and 1194 cm⁻¹.

(3) To 1 mL of Meglumine Sodium Iodamide Injection add 1 mL of potassium 1,2-naphthoquinone-4-sulfonate TS and 0.2 mL of sodium hydroxide TS: a deep red color is produced.

(4) Meglumine Sodium Iodamide Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> α_D^{20} : -3.84 - -4.42° (100 mm).

pH <2.54> 6.5 - 7.5

Purity (1) Primary aromatic amines—Mix 0.30 mL of Meglumine Sodium Iodamide Injection and 6 mL of water, then add 4 mL of a solution of sodium nitrite (1 in 100) and 10 mL of 1 mol/L hydrochloric acid TS, shake well, and proceed as directed in the Purity (2) under Iodamide: the absorbance is not more than 0.22.

(2) Iodine and iodide—To 0.40 mL of Meglumine Sodium Iodamide Injection add water to make 20 mL, then add 5 mL of dilute nitric acid, shake well, filter by suction through a glass filter (G3). To the filtrate add 5 mL of chloroform, and shake vigorously: no color develops in the chloroform layer. Then add 1 mL of a strong hydrogen peroxide solution, and shake vigorously: the chloroform layer has no more color than the control solution.

Control solution: Dissolve 0.10 g of potassium iodide in water to make 100 mL. To a 0.10-mL portion of this solution add 20 mL of water, 5 mL of dilute nitric acid, 5 mL of chloroform and 1 mL of strong hydrogen peroxide solution, and shake vigorously.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Dilute Meglumine Sodium Iodamide Injection with isotonic sodium chloride solution so as to contain 0.30 mL of Meglumine Sodium Iodamide Injection per mL according to the labeled amount, and perform the test: it meets the requirements.

Assay To an exactly measured 8 mL of Meglumine Sodium Iodamide Injection add sodium hydroxide TS to make exactly 100 mL, and use this solution as the sample solution. Pipet 10 mL of the sample solution into a saponification flask, add 30 mL of sodium hydroxide TS and 1 g of zinc powder, and proceed as directed in the Assay under Iodamide.

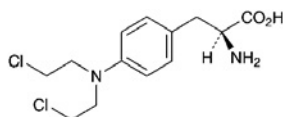
Each mL of 0.1 mol/L silver nitrate VS
= 20.93 mg of C₁₂H₁₁I₃N₂O₄

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Melphalan

メルファラン

C₁₃H₁₈Cl₂N₂O₂: 305.20

4-Bis(2-chloroethyl)amino-L-phenylalanine [148-82-3]

Melphalan contains not less than 93.0% of C₁₃H₁₈Cl₂N₂O₂, calculated on the dried basis.

Description Melphalan occurs as a white, to light yellowish white, crystalline powder.

It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute sodium hydroxide TS.

It is gradually colored by light.

Optical rotation [α]_D²⁰: about -32° (0.5 g, calculated on the dried basis, methanol, 100 mL, 100 mm).

Identification (1) To 0.02 g of Melphalan add 50 mL of methanol, dissolve by warming, add 1 mL of a solution of 4-(4-nitrobenzyl)pyridine in acetone (1 in 20), and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of warmed methanol and add 2 drops of ammonia solution (28): a purple color develops.

(2) Dissolve 0.1 g of Melphalan in 10 mL of dilute sodium hydroxide TS, and heat on a water bath for 10 minutes. After cooling, add dilute nitric acid to acidify, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

(3) Determine the absorption spectrum of a solution of Melphalan in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Ionisable chloride—Weigh accurately about 0.5 g of Melphalan, dissolve in 80 mL of diluted nitric acid (1 in 40), stir for 2 minutes, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration): the consumed volume is not more than 1.0 mL to 0.50 g of Melphalan.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Melphalan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Melphalan according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.25 g of Melphalan, add 20 mL of a solution of potassium hydroxide (1 in 5), and heat under a reflux condenser on a water bath for 2 hours. After cooling, add 75 mL of water and 5 mL of nitric acid, cool,

and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration). Make any necessary correction by using the results obtained in the Purity (1).

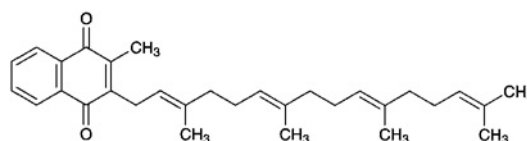
Each mL of 0.1 mol/L silver nitrate VS
= 15.26 mg of C₁₃H₁₈Cl₂N₂O₂

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Menatetrenone

メナテトレノン

C₃₁H₄₀O₂: 444.65

2-Methyl-3-[(2E,6E,10E)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl]-1,4-naphthoquinone [863-61-6]

Menatetrenone contains not less than 98.0% of C₃₁H₄₀O₂, calculated on the dehydrated basis.

Description Menatetrenone occurs as yellow, crystals, crystalline powder, waxy mass or oily material.

It is very soluble in hexane, soluble in ethanol (99.5), sparingly soluble in 2-propanol, slightly soluble in methanol, and practically insoluble in water.

It decomposes and the color becomes more intense by light.

Melting point: about 37°C

Identification (1) Dissolve 0.1 g of Menatetrenone in 5 mL of ethanol (99.5) by warming, cool, and add 1 mL of a solution of potassium hydroxide in ethanol (95) (1 in 10): a blue color develops, and upon standing it changes from blue-purple to red-brown through red-purple.

(2) Determine the infrared absorption spectrum of Menatetrenone, after melting by warming if necessary, as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Menatetrenone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Menatetrenone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Menadione—To 0.20 g of Menatetrenone add 5 mL of diluted ethanol (99.5) (1 in 2), shake well, and filter. To 0.5 mL of the filtrate add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazorone in ethanol (99.5) (1 in 20) and 1 drop of ammonia water (28), and allow to stand for 2 hours: no blue-purple color develops.

(3) cis Isomer—Dissolve 0.10 g of Menatetrenone in 10 mL of hexane, and use this solution as the sample solution. Pipet 1 mL of this solution, add hexane to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer

Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the chromatogram with a mixture of hexane and dibutyl ether (17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot corresponding to relative *R_f* value 1.1 regarding to the principal spot from the sample solution is not more intense than the spot from the standard solution.

(4) Related substances—Conduct this procedure without exposure to daylight, using a light-resistant vessel. Dissolve 0.10 g of Menatetrenone in 100 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of peaks other than the peak of menatetrenone from the sample solution is not larger than the peak area of menatetrenone from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 6 times as long as the retention time of menatetrenone beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 50 mL. Confirm that the peak area of menatetrenone obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of menatetrenone obtained from 20 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of menatetrenone is not more than 1.0%.

Water <2.48> Not more than 0.5% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using a light-resistant vessel. Weigh accurately about 0.1 g each of Menatetrenone and Menatetrenone Reference Standard (separately, determine the water <2.48> in the same manner as Menatetrenone), dissolve each in 50 mL of 2-propanol, and add ethanol (99.5) to make exactly 100 mL. Pipet 10 mL of these solutions, and add ethanol (99.5) to make exactly 100 mL. Pipet 2 mL each of these solutions, add exactly 4 mL each of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of menatetrenone to that of the internal standard.

Amount (mg) of $C_{31}H_{40}O_2$

$$= W_S \times (Q_T / Q_S)$$

W_S : Amount (mg) of Menatetrenone Reference Standard, calculated on the dehydrated basis

Internal standard solution—A solution of phytonadione in 2-propanol (1 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of menatetrenone is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, menatetrenone and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

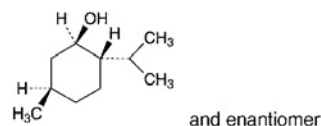
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of menatetrenone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

dl-Menthol

dl-メントール



$C_{10}H_{20}O$: 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol
[89-78-1]

dl-Menthol contains not less than 98.0% of $C_{10}H_{20}O$.

Description *dl*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

It is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

It sublimes gradually at room temperature.

Identification (1) Triturate *dl*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture liquefies.

(2) Shake 1 g of *dl*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer possesses no aroma of menthol is separated.

Congealing point <2.42> 27 – 28°C

Optical rotation <2.49> $[\alpha]_D^{20}$: –2.0 – +2.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Purity (1) Non-volatile residue—Volatilize 2.0 g of *dl*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of *dl*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of *dl*-Menthol placed in a flask add 2 mL of a solution of sodium hydroxide (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, then add 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay Weigh accurately about 2 g of *dl*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash down the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

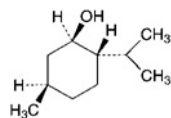
Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of C₁₀H₂₀O

Containers and storage Containers—Tight containers.

Storage—In a cold place.

l-Menthol

l-メントール



C₁₀H₂₀O: 156.27
(1*R*,2*S*,5*R*)-5-Methyl-2-(1-methylethyl)cyclohexanol
[2216-51-5]

l-Menthol contains not less than 98.0% of C₁₀H₂₀O.

Description *l*-Menthol occurs as colorless crystals. It has a characteristic and refreshing odor and a burning taste, followed by a cool taste.

l-Menthol is very soluble in ethanol (95) and in diethyl ether, and very slightly soluble in water.

l-Menthol sublimates gradually at room temperature.

Identification (1) Triturate *l*-Menthol with an equal amount of camphor, chloral hydrate or thymol: the mixture

liquefies.

(2) Shake 1 g of *l*-Menthol with 20 mL of sulfuric acid: the mixture becomes turbid with a yellow-red color. Allow to stand for 3 hours: a clear, oily layer which possesses no aroma of menthol is separated.

Optical rotation <2.49> $[\alpha]_D^{20}$: –45.0 – –51.0° (2.5 g, ethanol (95), 25 mL, 100 mm).

Melting point <2.60> 42 – 44°C

Purity (1) Non-volatile residue—Volatilize 2.0 g of *l*-Menthol on a water bath, and dry the residue at 105°C for 2 hours: the residue weighs not more than 1.0 mg.

(2) Thymol—Add 0.20 g of *l*-Menthol to a cold mixture of 2 mL of acetic acid (100), 6 drops of sulfuric acid and 2 drops of nitric acid: no green to blue-green color immediately develops.

(3) Nitromethane or nitroethane—To 0.5 g of *l*-Menthol placed in a flask add 2 mL of sodium hydroxide solution (1 in 2) and 1 mL of strong hydrogen peroxide, connect a reflux condenser to the flask, and boil the mixture gently for 10 minutes. After cooling, add water to make exactly 20 mL, and filter. Take 1 mL of the filtrate in a Nessler tube, add water to make 10 mL, neutralize with dilute hydrochloric acid, add another 1 mL of dilute hydrochloric acid, and cool. To the mixture add 1 mL of a solution of sulfanilic acid (1 in 100), allow to stand for 2 minutes, and then add 1 mL of a solution of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate (1 in 1000) and water to make 25 mL: no red-purple color immediately develops.

Assay Weigh accurately about 2 g of *l*-Menthol, add exactly 20 mL of a mixture of dehydrated pyridine and acetic anhydride (8:1), connect a reflux condenser, and heat on a water bath for 2 hours. Wash the condenser with 20 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination and make any necessary correction.

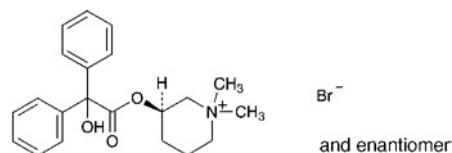
Each mL of 1 mol/L sodium hydroxide VS
= 156.3 mg of C₁₀H₂₀O

Containers and storage Containers—Tight containers.

Storage—In a cold place.

Mepenzolate Bromide

メペンゾラート臭化物



C₂₁H₂₆BrNO₃: 420.34
(3*R*,5*S*)-3-[(Hydroxy)(diphenyl)acetoxy]-1,1-dimethylpiperidinium bromide [76-90-4]

Mepenzolate Bromide, when dried, contains not less than 98.5% of mepenzolate bromide (C₂₁H₂₆BrNO₃).

Description Mepenzolate Bromide is white to pale yellow crystals or crystalline powder. It is odorless, and has a bitter

taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in hot water, slightly soluble in water and in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 230°C (with decomposition).

Identification (1) To 0.03 g of Mepenzolate Bromide add 10 drops of sulfuric acid: a red color develops.

(2) Dissolve 0.01 g of Mepenzolate Bromide in 20 mL of water and 5 mL of dilute hydrochloric acid, and to 5 mL of this solution add 1 mL of Dragendorff's TS: an orange precipitate is produced.

(3) Determine the absorption spectrum of a solution of Mepenzolate Bromide in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Dissolve 0.5 g of Mepenzolate Bromide in 50 mL of water and 3 mL of nitric acid by heating. This solution responds to the Qualitative Tests <1.09> for Bromide.

Purity (1) Heavy Metals <1.07>—Proceed with 1.0 g of Mepenzolate Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not less than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mepenzolate Bromide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.40 g of Mepenzolate Bromide in exactly measured 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 40 mg of benzophenone in methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution, standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, methanol, water and acetic acid (100) (3:3:2:1) to a distance of about 10 cm, and air-dry the plate and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than either the principal spot or the spot corresponding to benzophenone from the sample solution are not more intense than the spot from standard solution (1), and the spot corresponding to benzophenone from the sample solution is not more intense than the spot from standard solution (2). Spray evenly Dragendorff's TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Mepenzolate Bromide, previously dried, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform

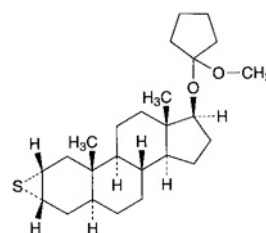
a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 42.03 mg of C₂₁H₂₆BrNO₃

Containers and storage Containers—Tight containers.

Mepitiostane

メピチオスタン



C₂₅H₄₀O₂S: 404.65

2α,3α-Epithio-17β-(1-methoxycyclopentyl)-5α-androstane [21362-69-6]

Mepitiostane contains not less than 96.0% and not more than 102.0% of C₂₅H₄₀O₂S, calculated on the anhydrous basis.

Description Mepitiostane occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in triethylamine, in chloroform, in diethyl ether and in cyclohexane, soluble in diethylene glycol dimethyl ether and in petroleum ether, sparingly soluble in acetone, slightly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is hydrolyzed in moist air.

Identification (1) Dissolve 1 mg of Mepitiostane in 1 mL of methanol, and add 0.5 mL of palladium (II) chloride TS: an orange precipitate is formed. To this suspension add 1 mL of water and 2 mL of chloroform, shake well, and allow to stand: an orange color develops in the chloroform layer.

(2) Dissolve 0.1 g of Mepitiostane in 2 mL of diethylene glycol dimethyl ether, shake with 1 mL of 1 mol/L hydrochloric acid TS, and filter. To the filtrate add 1.5 mL of 2,4-dinitrophenylhydrazine-diethylene glycol dimethyl ether TS and 1.5 mL of diluted ethanol (95) (2 in 3): an orange-yellow precipitate is formed. Filter the precipitate, recrystallize from ethanol (99.5), and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the crystals melt <2.60> between 144°C and 149°C.

(3) Determine the infrared absorption spectrum of Mepitiostane as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +20 – +23° (0.1 g, chloroform, 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Mepitiostane in 4 mL of petroleum ether: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of

Mepitiostane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Mepitiostane in exactly 5 mL of a mixture of acetone and triethylamine (1000:1), and use this solution as the sample solution. Separately, dissolve 10 mg of Epitiostanol Reference Standard in a mixture of acetone and triethylamine (1000:1) to make exactly 10 mL. Pipet 1 mL and 3 mL of this solution, to each add a mixture of acetone and triethylamine (1000:1) to make exactly 25 mL, and use these solutions as the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, heat between 120°C and 130°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution showing the same *R_f* value as the standard solutions are not more intense than the spot from the standard solution (2), and the remaining spots other than the principal spot are not more intense than the spot from the standard solution (1).

Water <2.48> Not more than 0.7% (0.3 g, back titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.3 g of Mepitiostane, and dissolve in cyclohexane to make exactly 10 mL. Pipet 2 mL of this solution, add 10 mL of ethanol (99.5), mix with exactly 2 mL each of 0.01 mol/L hydrochloric acid TS and the internal standard solution, add ethanol (99.5) to make 20 mL, allow to stand at ordinary temperature for 30 minutes, and use this solution as the sample solution. Separately, weigh accurately about 45 mg of Epitiostanol Reference Standard, dissolve in exactly 2 mL of the internal standard solution, add ethanol (99.5) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of epitiostanol to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{25}H_{40}O_2S = W_S \times (Q_T/Q_S) \times 5 \times 1.3202$$

W_S : Amount (mg) of Epitiostanol Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of *n*-octylbenzene in ethanol (99.5) (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (20:3).

Flow rate: Adjust the flow rate so that the retention time of

epitiostanol is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, epitiostanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

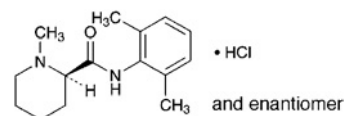
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of epitiostanol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, under Nitrogen atmosphere, and in a cold place.

Mepivacaine Hydrochloride

メピバカイン塩酸塩



$C_{15}H_{22}N_2O \cdot HCl$: 282.81

(2*R*S)-*N*-(2,6-Dimethylphenyl)-1-methylpiperidine-2-carboxamide monohydrochloride [1722-62-9]

Mepivacaine Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{22}N_2O \cdot HCl$.

Description Mepivacaine Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water and in methanol, soluble in acetic acid (100), sparingly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

A solution of Mepivacaine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 256°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Mepivacaine Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mepivacaine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Mepivacaine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.2 g of Mepivacaine Hydrochloride in 10 mL of water: the pH of this solution is between 4.0 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Mepivacaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Mepivacaine Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Mepivacaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 0.10 g of Mepivacaine Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 4 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether, methanol and ammonia solution (28) (100:5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly bismuth nitrate-potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Mepivacaine Hydrochloride, previously dried, dissolve in 10 mL of acetic acid (100) and add 70 mL of acetic anhydride. Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 28.28 \text{ mg of } C_{15}H_{22}N_2O.HCl \end{aligned}$$

Containers and storage Containers—Tight containers.

Mepivacaine Hydrochloride Injection

メピバカイン塩酸塩注射液

Mepivacaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of mepivacaine hydrochloride ($C_{15}H_{22}N_2O.HCl$: 282.81).

Method of preparation Prepare as directed under Injections, with Mepivacaine Hydrochloride.

Description Mepivacaine Hydrochloride Injection is a clear, colorless liquid.

pH: 4.5 – 6.8

Identification To a volume of Mepivacaine Hydrochloride Injection, equivalent to 0.02 g of Mepivacaine Hydrochloride according to the labeled amount, add 1 mL of sodium hydrochloride TS, and extract with 20 mL of hexane. To 8 mL of the hexane extract add 20 mL of 1 mol/L hydrochloric

acid TS, shake vigorously, and determine the absorption spectrum of the water layer separated as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 261 nm and 265 nm, and between 270 nm and 273 nm.

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Mepivacaine Hydrochloride Injection, equivalent to about 40 mg of Mepivacaine Hydrochloride according to the labeled amount, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloric acid TS to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of mepivacaine hydrochloride for assay, previously dried at 105°C for 3 hours, dissolve in 0.001 mol/L hydrochloric acid TS, add exactly 4 mL of the internal standard solution and 0.001 mol/L hydrochloride TS to make 20 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of mepivacaine to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{15}H_{22}N_2O.HCl \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of mepivacaine hydrochloride for assay

Internal standard solution—A solution of benzophenone in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.88 g of sodium lauryl sulfate in 1000 mL of a mixture of 0.02 mol/L phosphate buffer solution, pH 3.0 and acetonitrile (11:9).

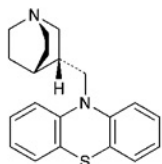
Flow rate: Adjust the flow rate so that the retention time of mepivacaine is about 6 minutes.

Selection of column: Proceed with 5 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of mepivacaine and benzophenone in this order with the resolution between these peaks being not less than 6.

Containers and storage Containers—Hermetic containers.

Mequitazine

メキタジン



and enantiomer

C₂₀H₂₂N₂S: 322.4710-[(3*RS*)-1-Azabicyclo[2.2.2]oct-3-ylmethyl]-10*H*-phenothiazine [29216-28-2]

Mequitazine, when dried, contains not less than 98.5% of C₂₀H₂₂N₂S.

Description Mequitazine occurs as white crystals or crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and practically insoluble in water.

It is gradually colored by light.

A solution of Mequitazine in methanol (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mequitazine in ethanol (95) (1 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mequitazine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 146 – 150°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mequitazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.05 g of Mequitazine in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, then pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop with a mixture of ethyl acetate, methanol and diethylamine (7:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spot other than the principal spot from the sample solution is not more than 3 and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

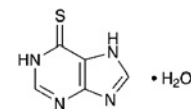
Assay Weigh accurately about 0.25 g of Mequitazine, dissolve in 50 mL of acetic acid (100), titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.25 mg of C₂₀H₂₂N₂S

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Mercaptopurine Hydrate

メルカプトプリン水和物

C₅H₄N₄S·H₂O: 170.191,7-Dihydro-6*H*-purine-6-thione monohydrate [6112-76-1]

Mercaptopurine Hydrate contains not less than 98.0% of mercaptopurine (C₅H₄N₄S: 152.18), calculated on the anhydrous basis.

Description Mercaptopurine Hydrate occurs as light yellow to yellow crystals or crystalline powder. It is odorless.

It is practically insoluble in water, in acetone and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Dissolve 0.6 g of Mercaptopurine Hydrate in 6 mL of sodium hydroxide solution (3 in 100), and add slowly 0.5 mL of iodomethane with vigorous stirring. Stir well for 10 minutes, cool in an ice bath, and adjust the pH with acetic acid (31) to about 5. Collect the separated crystals by filtration, recrystallize from water, and dry at 120°C for 30 minutes: the crystals melt <2.60> between 218°C and 222°C (with decomposition).

(2) Determine the absorption spectrum of a solution of Mercaptopurine Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity of solution—Dissolve 0.20 g of Mercaptopurine Hydrate in 10 mL of ammonia TS: the solution is clear.

(2) Sulfate <1.14>—Dissolve 0.05 g of Mercaptopurine Hydrate in 10 mL of dilute hydrochloric acid, add 5 drops of barium chloride TS, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Mercaptopurine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Hypoxanthine—Dissolve 50 mg of Mercaptopurine Hydrate in exactly 10 mL of a solution of ammonia solution (28) in methanol (1 in 10), and use this solution as the sample solution. Separately, dissolve 5.0 mg of hypoxanthine in a solution of ammonia solution (28) in methanol (1 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, *n*-butyl formate and ammonia solution (28) (8:6:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution is not observed at the same place as that from the standard solution, or if a spot is observed at the same place, it is not larger than that from the standard solution.

(5) Phosphorus—Take 0.20 g of Mercaptopurine Hydrate in a crucible, add 2 mL of diluted sulfuric acid (3 in 7), then heat gently, slowly adding dropwise several 0.5-mL portions of nitric acid, until the liquid becomes colorless. Continue to heat until most of the liquid has evaporated, cool, and dissolve the residue in 10 mL of water. Transfer the solution to a 25-mL volumetric flask, wash the crucible with two 4-mL portions of water, combine the washings with the solution in the volumetric flask, and use this solution as the sample solution. Separately, dissolve 0.4396 g of potassium dihydrogenphosphate in water to make exactly 200 mL. To 2.0 mL of this solution add water to make exactly 100 mL. Transfer 2.0 mL of this solution to a 25-mL volumetric flask, add 16 mL of water, and use this solution as the standard solution. To the sample solution and the standard solution add 1 mL of diluted sulfuric acid (3 in 7), 0.5 mL of nitric acid, 0.75 mL of hexaammonium heptamolybdate TS, 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS and water to make 25 mL, and allow to stand for 5 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance of the subsequent solution of the sample solution at 750 nm is not larger than that of the subsequent solution of the standard solution.

Water <2.48> 10.0 – 12.0% (0.2 g, back titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Mercaptopurine Hydrate, dissolve in 90 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination with a mixture of 90 mL of *N,N*-dimethylformamide and 15 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L tetramethylammonium hydroxide VS
= 15.22 mg of $C_5H_4N_4S$

Containers and storage Containers—Well-closed containers.

Mercurochrome

Merbromin

マーキュロクロム

Mercurochrome is a sodium salt of a mixture of brominated and mercurized fluoresceins.

When dried, it contains not less than 18.0% and not more than 22.4% of bromine (Br: 79.90), and not less than 22.4% and not more than 26.7% of mercury (Hg: 200.59).

Description Mercurochrome occurs as blue-green to greenish red-brown scales or granules. It is odorless.

It is freely soluble in water, but sometimes leaves a small amount of insoluble matter. It is practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) A solution of Mercurochrome (1 in 2000) shows a red color and a yellow-green fluorescence.

(2) To 5 mL of a solution of Mercurochrome (1 in 250) add 3 drops of dilute sulfuric acid: a reddish orange precipitate is produced.

(3) Heat 0.1 g of Mercurochrome with small crystals of iodine in a test tube: red crystals are sublimed on the upper part of the tube. If yellow crystals are produced, scratch with a glass rod: the color of the crystals changes to red.

(4) Place 0.1 g of Mercurochrome in a porcelain crucible, add 1 mL of a solution of sodium hydroxide (1 in 6), evaporate to dryness with stirring, and ignite. Dissolve the residue in 5 mL of water, acidify with hydrochloric acid, and shake with 3 drops of chlorine TS and 2 mL of chloroform: a yellowish brown color develops in the chloroform layer.

Purity (1) Dyestuff—Dissolve 0.40 g of Mercurochrome in 20 mL of water, add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

(2) Soluble halides—Dissolve 5.0 g of Mercurochrome in 80 mL of water, add 10 mL of dilute nitric acid and water to make 100 mL, shake, and filter. Transfer 40 mL of the filtrate to a Nessler tube, add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, mix well, and allow to stand for 5 minutes protected from direct sunlight: no turbidity is produced, or even if produced, it is not more than that of the following control solution.

Control solution: To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, then add 1 mL of silver nitrate TS, and proceed as directed above.

(3) Soluble mercury salts—To 5 mL of the filtrate obtained in (1) add 5 mL of water, and use this solution as the sample solution. Dissolve 40 mg of mercury (II) chloride, weighed accurately, in water to make 1000 mL, and add 3 mL of dilute sulfuric acid to 20 mL of this solution. To 5 mL of the solution add 5 mL of water, and use this as the control solution. Add 1 drop each of sodium sulfide TS to these solutions, and compare: the sample solution has no more color than the control solution.

(4) Insoluble mercury compounds—Dissolve 2.5 g of Mercurochrome in 50 mL of water, allow to stand for 24

hours, centrifuge, and wash the precipitate with small portions of water until the last washing becomes colorless. Transfer the precipitate to a glass-stoppered flask, add exactly 5 mL of 0.05 mol/L iodine VS, allow to stand for 1 hour with frequent agitation, add 4.3 mL of 0.1 mol/L sodium thiosulfate VS dropwise with shaking, and add 1 mL of starch TS: a blue color develops.

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 5 hours).

Assay (1) Mercury—Weigh accurately about 0.6 g of Mercurochrome, previously powdered and dried, transfer to an iodine flask, dissolve in 50 mL of water, add 8 mL of acetic acid (31), 20 mL of chloroform and exactly 30 mL of 0.05 mol/L iodine VS, stopper tightly, and allow to stand for 1 hour with frequent, vigorous shaking. Titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS with vigorous shaking (indicator: 1 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

(2) Bromine—Weigh accurately about 0.5 g of Mercurochrome, previously powdered and dried, in a porcelain crucible, add 2 g of potassium nitrate, 3 g of potassium carbonate and 3 g of anhydrous sodium carbonate, mix well, cover the surface of the mixture with 3 g of a mixture of equal amounts of potassium carbonate and anhydrous sodium carbonate, and ignite almost to fusion. Cool, dissolve the ignited mixture in 80 mL of warm water, acidify with nitric acid, and add exactly 25 mL of 0.1 mol/L silver nitrate VS. Shake well, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS = 7.990 mg of Br

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mercurochrome Solution

Merbromin Solution

マーキュロクロム液

Mercurochrome Solution contains not less than 0.42 w/v% and not more than 0.56 w/v% of mercury (Hg: 200.59).

Method of preparation

Mercurochrome	20 g
Purified Water	a sufficient quantity
To make 1000 mL	

Prepare by mixing the above ingredients.

Description Mercurochrome Solution is a dark red liquid.

Identification (1) To 1 mL of Mercurochrome Solution add 40 mL of water: the resulting solution shows a red color and a yellow-green fluorescence.

(2) Dilute 1 mL of Mercurochrome Solution with 4 mL

of water, and add 3 drops of dilute sulfuric acid: a red-orange precipitate is produced.

(3) Evaporate 5 mL of Mercurochrome Solution to dryness, and proceed with the residue as directed in the Identification (3) under Mercurochrome.

(4) To 5 mL of Mercurochrome Solution add 1 mL of a solution of sodium hydroxide (1 in 6), and proceed as directed in the Identification (4) under Mercurochrome.

Purity Dyestuff—To 20 mL of Mercurochrome Solution add 3 mL of dilute sulfuric acid, and filter: the filtrate has no more color than Matching Fluid C.

Assay Transfer exactly measured 30 mL of Mercurochrome Solution to an iodine flask, dilute with 20 mL of water, add 8 mL of acetic acid (31) and 20 mL of chloroform, and proceed as directed in the Assay (1) under Mercurochrome.

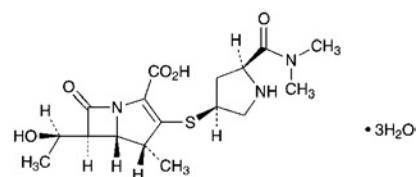
Each mL of 0.05 mol/L iodine VS = 10.03 mg of Hg

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Meropenem Hydrate

メロペネム水和物



$C_{17}H_{25}N_3O_5S \cdot 3H_2O$: 437.51

(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-(Dimethylcarbamoyl)pyrrolidin-3-ylsulfanyl]-6-[(1*R*)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid trihydrate [119478-56-7]

Meropenem Hydrate contains not less than 980 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Meropenem Hydrate is expressed as mass (potency) of meropenem ($C_{17}H_{25}N_3O_5S$: 383.46).

Description Meropenem Hydrate occurs as a white to light yellow crystalline powder.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 0.01 g of Meropenem Hydrate in 2 mL of water, add 3 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 5 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectra of solutions of Meropenem Hydrate and Meropenem Reference Standard (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectra of Meropenem Hydrate and Meropenem Reference Standard as directed in the potassium bromide disk method under In-

frared Spectrophotometry <2.25>, and compare the spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-17 - -21^\circ$ (0.22 g calculated as the anhydrous basis, water, 50 mL, 100 mm).

pH <2.54> Dissolve 0.2 g of Meropenem Hydrate in 20 mL of water: the pH of the solution is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Meropenem Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Being specified separately.

Water <2.48> Not less than 11.4% and not more than 13.4% (0.35 g, volumetric titration, direct titration).

Residue on ignition <2.44> Being specified separately.

Bacterial endotoxins <4.01> Less than 0.12 EU/mg (potency).

Assay Weigh accurately an amount of Meropenem Hydrate and Meropenem Reference Standard, equivalent to about 50 mg (potency), add exactly 10 mL of the internal standard solution to dissolve, add triethylamine-phosphate buffer solution, pH 5.0 to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of meropenem to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of meropenem (C}_{17}\text{H}_{25}\text{N}_3\text{O}_5\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Meropenem Reference Standard

Internal standard solution—A solution of benzyl alcohol in triethylamine-phosphate buffer solution, pH 5.0 (1 in 300).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of triethylamine-phosphate buffer solution, pH 5.0 and methanol (5:1).

Flow rate: Adjust the flow rate so that the retention time of meropenem is about 7 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, meropenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 20.

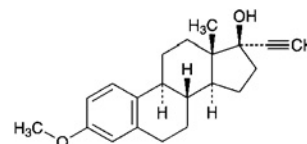
System repeatability: When the test is repeated 5 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the

peak area of meropenem to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Mestranol

メストラノール



$\text{C}_{21}\text{H}_{26}\text{O}_2$: 310.43

3-Methoxy-19-nor-17 α -pregna-1,3,5(10)-trien-20-yn-17-ol [72-33-3]

Mestranol, when dried, contains not less than 97.0% and not more than 102.0% of $\text{C}_{21}\text{H}_{26}\text{O}_2$.

Description Mestranol occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 2 mg of Mestranol in 1 mL of a mixture of sulfuric acid and ethanol (99.5) (2:1): a red-purple color develops with a yellow-green fluorescence.

(2) Determine the absorption spectrum of a solution of Mestranol in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mestranol Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Mestranol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Mestranol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+2 - +8^\circ$ (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 148 – 154°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Mestranol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Mestranol according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Mestranol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica

gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (99.5) (29:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 5) on the plate, and heat at 105°C for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg each of Mestranol and Mestranol Reference Standard, previously dried, dissolve in ethanol (99.5) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 279 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

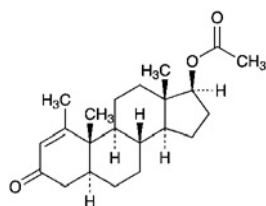
$$\begin{aligned} &\text{Amount (mg) of } C_{21}H_{26}O_2 \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Mestranol Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Acetate

メテノロン酢酸エステル



$C_{22}H_{32}O_3$: 344.49

1-Methyl-3-oxo-5 α -androst-1-en-17 β -yl acetate [434-05-9]

Metenolone Acetate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

Description Metenolone Acetate occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetone, in 1,4-dioxane and in chloroform, soluble in ethanol (95) and in methanol, sparingly soluble in diethyl ether and in sesame oil, slightly soluble in hexane and in petroleum ether, and practically insoluble in water.

Identification (1) Dissolve 1 mg of Metenolone Acetate in 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1), and heat for 30 minutes in a water bath: a red-brown color develops.

(2) To 0.01 g of Metenolone Acetate add 0.5 mL of dilute sodium hydroxide-ethanol TS, and heat for 1 minute on a water bath. After cooling, add 0.5 mL of diluted sulfuric acid (1 in 2), and boil gently for 1 minute: the odor of ethyl acetate is perceptible.

(3) Dissolve 0.05 g of Metenolone Acetate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate

(1 in 6), and boil for 2 hours under a reflux condenser. After cooling, add this solution gradually to 50 mL of cold water, and stir for 15 minutes. Filter the precipitate so obtained by suction through a glass filter (G4), wash with 10 mL of water, and dry at 105°C for 1 hour: it melts <2.60> between 157°C and 161°C.

(4) Determine the infrared absorption spectrum of Metenolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +39 – +42° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 141 – 144°C

Purity (1) Clarity and color of solution—Dissolve 0.50 g of Metenolone Acetate in 10 mL of 1,4-dioxane: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 35 mg of Metenolone Acetate in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, dilute with chloroform to exactly 250 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

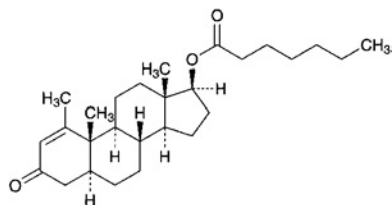
Assay Weigh accurately about 10 mg of Metenolone Acetate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, and dilute with methanol to exactly 50 mL. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_{22}H_{32}O_3 = (A/391) \times 10,000$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Enanthate

メテノロンエナント酸エステル



$C_{27}H_{42}O_3$: 414.62

1-Methyl-3-oxo-5 α -androst-1-en-17 β -yl heptanoate
[303-42-4]

Metenolone Enanthate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{27}H_{42}O_3$.

Description Metenolone Enanthate occurs as white crystals or crystalline powder. It is odorless.

It is very soluble in ethanol (95), in acetone, in 1,4-dioxane and in chloroform, freely soluble in methanol, in ethyl acetate, in diethyl ether, in cyclohexane, in petroleum ether and in toluene, soluble in sesame oil, and practically insoluble in water.

Identification (1) Heat 1 mg of Metenolone Enanthate with 5 mL of a mixture of ethanol (95) and sulfuric acid (1:1) on a water bath for 30 minutes: a red-brown color develops.

(2) Dissolve 0.05 g of Metenolone Enanthate in 3 mL of methanol, add 0.3 mL of a solution of potassium carbonate (1 in 6), boil under a reflux condenser for 2 hours, cool, add slowly this solution to 50 mL of cold water, and stir for 15 minutes. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the washings become neutral, and dry at 105°C for 1 hour: it melts <2.60> between 156°C and 162°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: +39 – +43° (after drying, 0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 67 – 72°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Metenolone Enanthate in 10 mL of 1,4-dioxane: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metenolone Enanthate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Metenolone Enanthate in exactly 10 mL of chloroform, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot does not appear.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacu-

um, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.1 g of Metenolone Enanthate, previously dried, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute with methanol to make exactly 100 mL. Pipet 10 mL of this solution, and dilute again with methanol to make exactly 100 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

Amount (mg) of $C_{27}H_{42}O_3$ = $(A/325) \times 100,000$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metenolone Enanthate Injection

メテノロンエナント酸エステル注射液

Metenolone Enanthate Injection is an oily solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of metenolone enanthate ($C_{27}H_{42}O_3$: 414.62).

Method of preparation Prepare as directed under Injections, with Metenolone Enanthate.

Description Metenolone Enanthate Injection is a clear, pale yellow, oily liquid.

Identification (1) Measure a volume of Metenolone Enanthate Injection, equivalent to 0.1 g of Metenolone Enanthate according to the labeled amount, add 20 mL of petroleum ether, and extract with three 20-mL portions of diluted acetic acid (100) (5 in 7). Combine the extracts, wash with 20 mL of petroleum ether, add 300 mL of cold water while cooling in an ice bath, and stir sufficiently. Filter the produced precipitate by suction through a glass filter (G4), wash with water until the last washing becomes neutral, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 6 hours. With this sample, proceed as directed in the Identification (1) under Metenolone Enanthate.

(2) Measure a volume of Metenolone Enanthate Injection, equivalent to 0.01 g of Metenolone Enanthate according to the labeled amount, dissolve in 10 mL of chloroform, and use this solution as the sample solution. Separately dissolve 0.01 g of metenolone enanthate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 15 cm, and air-dry the plate. Again develop this plate with a mixture of cyclohexane and ethyl acetate (1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spot from the sample solution and the spot from the standard solution show the same *R_f* value.

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Metenolone Enanthate Injection, equivalent to about 0.1 g of metenolone enanthate ($C_{27}H_{42}O_3$), add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 0.1 g of metenolone enanthate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 3 mL each of the sample solution and standard solution, and treat each solution as follows: add 10 mL of isoniazid TS, exactly measured, add methanol to make exactly 20 mL, and allow to stand for 60 minutes. Determine the absorbances, A_T and A_S , of the solutions from the sample solution and standard solution, respectively, at 384 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 3 mL of chloroform as the blank.

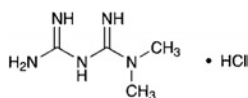
$$\text{Amount (mg) of metenolone enanthate (C}_{27}\text{H}_{42}\text{O}_3) \\ = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of metenolone enanthate for assay

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Metformin Hydrochloride

メトホルミン塩酸塩



$C_4H_{11}N_5 \cdot HCl$: 165.62

1,1-Dimethylbiguanide monohydrochloride
[1115-70-4]

Metformin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_4H_{11}N_5 \cdot HCl$.

Description Metformin Hydrochloride occurs as white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and slightly soluble in ethanol (99.5).

Melting point: about 221°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Metformin Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metformin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Metformin Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of

Metformin Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 2.5 g of Metformin Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add water to make exactly 10 mL, and use this solution as the standard solution (2). Separately, to 0.10 g of 1-cyanoguanidine add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 4-methyl-2-pentanone, 2-methoxyethanol, water and acetic acid (100) (30:20:5:3) to a distance of about 10 cm, air-dry the plate, then dry at 105°C for 10 minutes. Spray evenly sodium pentacyanonitrosylferrate (III)-potassium hexacyanoferrate (III) TS on the plate: the spot other than the principal spot is not more intense than the spot with the standard solution (1), the number of them showing more intense than the spot with the standard solution (2) is not more than two, and the spot appeared at the position corresponding to the spot with the standard solution (3) is not more intense than the spot with the standard solution (3).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Metformin Hydrochloride, previously dried, dissolve in 40 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.05 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\text{Each mL of 0.05 mol/L perchloric acid VS} \\ = 4.141 \text{ mg of } C_4H_{11}N_5 \cdot HCl$$

Containers and storage Containers—Tight containers.

Metformin Hydrochloride Tablets

メトホルミン塩酸塩錠

Metformin Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$: 165.62).

Method of preparation Prepare as directed under Tablets, with Metformin Hydrochloride.

Identification Shake an amount of powdered Metformin Hydrochloride Tablets, equivalent to 250 mg of Metformin Hydrochloride according to the labeled amount, with 25 mL of 2-propanol, and filter. Evaporate the filtrate under reduced pressure in a water bath at 40°C, and determine the infrared absorption spectrum of the residue as directed in the

potassium chloride disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 3370 cm^{-1} , 3160 cm^{-1} , 1627 cm^{-1} , 1569 cm^{-1} and 1419 cm^{-1} .

Uniformity of dosage unit <6.02> It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Metformin Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.15 g of metformin hydrochloride ($\text{C}_4\text{H}_{11}\text{N}_5\cdot\text{HCl}$), add 70 mL of a mixture of water and acetonitrile (3:2), shake for 10 minutes, add the mixture of water and acetonitrile (3:2) to make exactly 100 mL, and filter through a membrane filter with a pore size of not more than $0.45\ \mu\text{m}$. Discard the first 10 mL of the filtrate, pipet 3 mL of the subsequent filtrate, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of metformin hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in the mixture of water and acetonitrile (3:2) to make exactly 100 mL. Pipet 3 mL of this solution, add exactly 3 mL of the internal standard solution and the mixture of water and acetonitrile (3:2) to make 50 mL, and use this solution as the standard solution. Perform the test with $5\ \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of metformin to that of the internal standard.

$$\text{Amount (mg) of metformin hydrochloride (C}_4\text{H}_{11}\text{N}_5\cdot\text{HCl)} \\ = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of metformin hydrochloride for assay

Internal standard solution—Dissolve 0.3 g of isobutyl parahydroxybenzoate in 100 mL of the mixture of water and acetonitrile (3:2).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5\ \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 40°C .

Mobile phase: Dissolve 0.8 g of sodium lauryl sulfate in 620 mL of diluted phosphoric acid (1 in 2500), and add 380 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of metformin is about 10 minutes.

System suitability—

System performance: When the procedure is run with $5\ \mu\text{L}$ of the standard solution under the above operating conditions, metformin and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

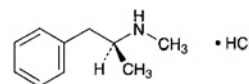
System repeatability: When the test is repeated 6 times with $5\ \mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metformin to that of the internal standard is not

more than 1.0%.

Containers and storage Containers—Well-closed containers.

Methamphetamine Hydrochloride

メタンフェタミン塩酸塩



$\text{C}_{10}\text{H}_{15}\text{N}\cdot\text{HCl}$: 185.69
(2*S*)-*N*-Methyl-1-phenylpropan-2-amine
monohydrochloride [51-57-0]

Methamphetamine Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_{10}\text{H}_{15}\text{N}\cdot\text{HCl}$.

Description Methamphetamine Hydrochloride occurs as colorless crystals or a white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

The pH of a solution of Methamphetamine Hydrochloride (1 in 10) is between 5.0 and 6.0.

Identification (1) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of hydrogen hexachloroplatinate (IV) TS: an orange-yellow, crystalline precipitate is produced.

(2) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of iodine TS: a brown precipitate is produced.

(3) To 5 mL of a solution of Methamphetamine Hydrochloride (1 in 100) add 0.5 mL of 2,4,6-trinitrophenol TS: a yellow, crystalline precipitate is produced.

(4) A solution of Methamphetamine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+16 - +19^\circ$ (after drying, 0.2 g, water, 10 mL, 100 mm).

Melting point <2.60> $171 - 175^\circ\text{C}$

Purity (1) Acidity or alkalinity—Dissolve 2.0 g of Methamphetamine Hydrochloride in 40 mL of freshly boiled and cooled water, add 2 drops of methyl red TS, and use this solution as the sample solution.

(i) To 20 mL of the sample solution add 0.20 mL of 0.01 mol/L sulfuric acid VS: a red color develops.

(ii) To 20 mL of the sample solution add 0.20 mL of 0.02 mol/L sodium hydroxide VS: a yellow color develops.

(2) Sulfate <1.14>—Dissolve 0.05 g of Methamphetamine Hydrochloride in 40 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS, and allow to stand for 10 minutes: the solution remains unchanged.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C , 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Methamphetamine Hydrochloride, previously dried, and dissolve in 50 mL of a

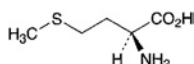
mixture of acetic anhydride and acetic acid (100) (7:3). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 18.57 mg of C₁₀H₁₅N.HCl

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

L-Methionine

L-メチオニン



C₅H₁₁NO₂S: 149.21
(2*S*)-2-Amino-4-(methylsulfanyl)butanoic acid
[63-68-3]

L-Methionine, when dried, contains not less than 98.5% of C₅H₁₁NO₂S.

Description L-Methionine occurs as white crystals or crystalline powder. It has a characteristic odor.

It is freely soluble in formic acid, soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Methionine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +21.0 – +25.0° (after drying, 0.5 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of L-Methionine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Methionine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Methionine in 20 mL of water, and add 6 mL of dilute nitric acid and water to make 40 mL. Perform the test using this solution as the test solution.

Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 6 mL of dilute nitric acid and water to make 40 mL. In this test, to the test solution and the control solution add 10 mL each of silver nitrate TS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Methionine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Methionine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Methionine in 40 mL of water and 2 mL of dilute acetic acid, dissolve by

warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Transfer 1.0 g of L-Methionine to a 100-mL decomposition flask, add 5 mL of nitric acid and 2 mL of sulfuric acid, put a small funnel on the mouth of the flask, and heat carefully until white fumes are evolved. After cooling, add two 2-mL portions of nitric acid, heat, add 2-mL portions of hydrogen peroxide (30) several times, and heat until the solution becomes colorless or pale yellow. After cooling, add 2 mL of saturated ammonium oxalate monohydrate solution, and heat again until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Methionine in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

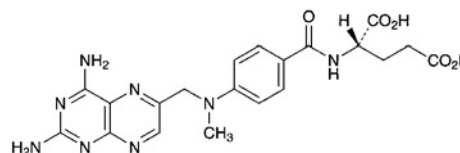
Assay Weigh accurately about 0.15 g of L-Methionine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 14.92 mg of C₅H₁₁NO₂S

Containers and storage Containers—Tight containers.

Methotrexate

メトトレキサート



C₂₀H₂₂N₈O₅: 454.44
N-{4-[(2,4-Diaminopteridin-6-ylmethyl)(methyl)amino]benzoyl}-L-glutamic acid
[59-05-2]

Methotrexate is a mixture of 4-amino-10-methylfolic

acid and closely related compounds.

It contains not less than 94.0% and not more than 102.0% of $C_{20}H_{22}N_8O_5$, calculated on the anhydrous basis.

Description Methotrexate occurs as a yellow-brown, crystalline powder.

It is slightly soluble in pyridine, and practically insoluble in water, in acetonitrile, in ethanol (95) and in diethyl ether.

It dissolves in dilute sodium hydroxide TS and in dilute sodium carbonate TS.

It is gradually affected by light.

Identification (1) Dissolve 1 mg of Methotrexate in 100 mL of 0.1 mol/L hydrochloric acid TS. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methotrexate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methotrexate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Methotrexate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Water <2.48> Take 5 mL of pyridine for water determination and 20 mL of methanol for Karl Fischer method in a dried titration flask, and titrate with water determination TS until the end point. Weigh accurately about 0.2 g of Methotrexate, immediately place in the titration flask, and add a known excess volume of Karl Fischer TS. Mix well for 30 minutes, and perform the test: the water content is not more than 12.0%.

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 25 mg each of Methotrexate and Methotrexate Reference Standard, dissolve in the mobile phase to make exactly 250 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the peak areas, A_T and A_S , of methotrexate in each solution.

$$\text{Amount (mg) of } C_{20}H_{22}N_8O_5 = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Methotrexate Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 302 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of disodium hydrogen phosphate-citric acid buffer solution, pH 6.0 and acetonitrile (89:11).

Flow rate: Adjust the flow rate so that the retention time of

methotrexate is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of Methotrexate and folic acid in 100 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, folic acid and methotrexate are eluted in this order with the resolution between these peaks being not less than 8.

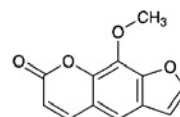
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methotrexate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methoxsalen

メトキサレン



$C_{12}H_8O_4$: 216.19

9-Methoxy-7H-furo[3,2-g]chromen-7-one
[298-81-7]

Methoxsalen contains not less than 98.0% and not more than 102.0% of $C_{12}H_8O_4$, calculated on the anhydrous basis.

Description Methoxsalen occurs as white to pale yellow crystals or crystalline powder. It is odorless and tasteless.

It is freely soluble in chloroform, slightly soluble in methanol, in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) To 0.01 g of Methoxsalen add 5 mL of dilute nitric acid, and heat: a yellow color develops. Make this solution alkaline with a solution of sodium hydroxide (2 in 5): the color changes to red-brown.

(2) To 0.01 g of Methoxsalen add 5 mL of sulfuric acid, and shake: a yellow color develops.

(3) Determine the absorption spectrum of a solution of Methoxsalen in ethanol (95) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methoxsalen Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 145 – 149°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Methoxsalen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Methoxsalen according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Methoxsalen in 10 mL of chloroform, and use this solution as the sample

solution. Pipet 2 mL of the sample solution, add chloroform to make exactly 50 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, hexane and ethyl acetate (40:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> Not more than 0.5% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Methoxsalen and Methoxsalen Reference Standard, and dissolve each in ethanol (95) to make exactly 100 mL. Pipet 2 mL each of these solutions, and dilute each with ethanol (95) to make exactly 25 mL. Pipet 10 mL each of these solutions, and dilute each again with ethanol (95) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 300 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} &\text{Amount (mg) of } C_{12}H_8O_4 \\ &= W_S \times (A_T/A_S) \end{aligned}$$

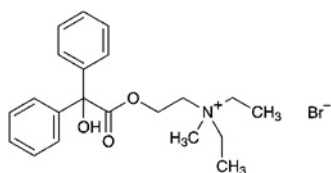
W_S : Amount (mg) of Methoxsalen Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylbenactyzium Bromide

メチルベナクチジウム臭化物



$C_{21}H_{28}BrNO_3$: 422.36

N,N-Diethyl-2-[(hydroxyl)(diphenyl)acetoxy]-*N*-methylethylaminium bromide [3166-62-9]

Methylbenactyzium Bromide, when dried, contains not less than 99.0% of $C_{21}H_{28}BrNO_3$.

Description Methylbenactyzium Bromide occurs as white crystals or crystalline powder. It is odorless, and has an extremely bitter taste.

It is freely soluble in water and in acetic acid (100), soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Methylbenactyzium Bromide (1 in 50) is between 5.0 and 6.0.

Identification (1) Shake 0.5 mL of a solution of Methylbenactyzium Bromide (1 in 100) with 5 mL of phosphate buffer solution, pH 7.0, 2 to 3 drops of bromothymol blue TS and 5 mL of chloroform: a yellow color develops in the chloroform layer.

(2) To about 1 g of Methylbenactyzium Bromide add 5 mL of water and 10 mL of sodium hydroxide TS, allow to stand for 5 minutes, add 5 mL of dilute hydrochloric acid, collect the precipitate, wash well with water, recrystallize from a mixture of water and ethanol (95) (10:3), and dry at 105°C for 1 hour: the crystals melt <2.60> between 145°C and 150°C. Continue the heating up to about 200°C: a red color develops.

(3) Add 2 mL of dilute nitric acid to 5 mL of a solution of Methylbenactyzium Bromide (1 in 10): the solution responds to the Qualitative Tests <1.09> (1) for bromide.

Melting point <2.60> 168 – 172°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Methylbenactyzium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Methylbenactyzium Bromide. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Methylbenactyzium Bromide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Methylbenactyzium Bromide, previously dried, and dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (4:1). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} &\text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 42.24 \text{ mg of } C_{21}H_{28}BrNO_3 \end{aligned}$$

Containers and storage Containers—Tight containers.

Methylcellulose

メチルセルロース

Cellulose, methyl ether
[9004-67-5]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Methylcellulose is a methyl ether of cellulose.

It contains not less than 26.0% and not more than 33.0% of methoxy group (-OCH₃: 31.03), calculated on the dried basis.

The viscosity of Methylcellulose is shown in millipas-

cal second (mPa·s).

♦**Description** Methylcellulose occurs as a white to yellowish white, powder or granules.

It is practically insoluble in ethanol (99.5).

Methylcellulose swells, when water is added, and forms a clear or slightly turbid, viscous liquid.♦

Identification (1) Disperse evenly 1.0 g of Methylcellulose over the surface of 100 mL of water in a beaker, while gently tapping the top of the container, if necessary, and allow the beaker to stand: it aggregates on the surface of water.

(2) Add 1.0 g of Methylcellulose to 100 mL of hot water, and stir: it becomes a suspension. Cool the suspension to 5°C, and stir: the resulting liquid is a clear or a slightly cloudy, viscous fluid.

(3) To 0.1 mL of the viscous fluid obtained in (2) add 9 mL of diluted sulfuric acid (9 in 10), stir, heat in a water bath for exactly 3 minutes, and immediately cool in ice water. Add carefully 0.6 mL of ninhydrin TS, stir, and allow to stand at 25°C: the solution shows a light red color first, then changes to purple color within 100 minutes.

(4) Pour and spread out 2 to 3 mL of the viscous fluid obtained in (2) onto a glass plate, and allow the water to evaporate: a transparent film results.

(5) Pipet 50 mL of water, add exactly 50 mL of the viscous fluid obtained in (2), and warm to rise the temperature at a rate of 2 to 5°C per minute while stirring: the temperature, when a white turbidity of the solution starts to increase, is not less than 50°C.

Viscosity <2.53> Method I: Apply to Methylcellulose having a labeled viscosity of less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 4.000 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 200.0 g, stopper the bottle, stir by mechanical means at 350- to 450-revolutions per minute for 10 to 20 minutes to get a homogeneous dispersion. If necessary, take off the sample attached on the walls of the bottle, put them in the dispersed solution, and dissolve by continuing the stirring in a water bath not exceeding 5°C for 20 to 40 minutes. Add cooled water, if necessary, to make 200.0 g, and use this solution as the sample solution. Centrifuge the solution if necessary to expel any entrapped air bubbles. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method I under Viscosity Determination: not less than 80% and not more than 120% of the labeled viscosity.

Method II: Apply to Methylcellulose having a labeled viscosity of not less than 600 mPa·s. Put exactly an amount of Methylcellulose, equivalent to 10.00 g on the dried basis, in a tared, wide-mouth bottle, add hot water to make 500.0 g, stopper the bottle, and prepare the sample solution in the same manner as directed in Method I. Perform the test with the sample solution at 20 ± 0.1°C as directed in Method II (2) under Viscosity Determination, using a single cylinder-type rotational viscometer, according to the following operating conditions: not less than 75% and not more than 140% of the labeled viscosity.

Operating conditions—

Apparatus: Brookfield type viscometer LV model

Rotor No., rotation frequency, and conversion factor: According to the following table, depending on the labeled viscosity.

Labeled viscosity (mPa·s)	Rotor No.	Rotation frequency /min	Conversion factor
Not less than 600 and less than 1400	3	60	20
" 1400 " 3500	3	12	100
" 3500 " 9500	4	60	100
" 9500 " 99,500	4	6	1000
" 99,500	4	3	2000

Procedure of apparatus: Read value after 2 minutes of rotation, and stop the rotation for 2 minutes. Repeat this procedure more two times, and average three observed values.

pH <2.54> Allow the sample solution obtained in the Viscosity to stand at 20 ± 2°C for 5 minutes: the pH of the solution thus obtained is between 5.0 and 8.0.

Purity Heavy metals—Put 1.0 g of Methylcellulose in a 100-mL Kjeldahl flask, add a sufficient amount of a mixture of nitric acid and sulfuric acid (5:4) to wet the sample, and heat gently. Repeat this procedure until to use totally 18 mL of the mixture of nitric acid and sulfuric acid. Then boil gently until the solution changes to black. After cooling, add 2 mL of nitric acid, and heat until the solution changes to black. Repeat this procedure until the solution no longer changes to black, and heat strongly until dense white fumes are evolved. After cooling, add 5 mL of water, boil gently until dense white fumes are evolved, then heat until the volume of the solution becomes to 2 to 3 mL. After cooling, if the solution reveals yellow color by addition of 5 mL of water, add 1 mL of hydrogen peroxide (30), and heat until the volume of the solution becomes to 2 to 3 mL. After cooling, dilute the solution with 2 to 3 mL of water, transfer to a Nessler tube, add water to make 25 mL, and use this solution as the test solution. Separately, put 2.0 mL of Standard Lead Solution in a 100-mL kjeldahl flask, add 18 mL of the mixture of nitric acid and sulfuric acid (5:4) and an amount of nitric acid equal to that used for preparation of the test solution, and heat until white fumes are evolved. After cooling, add 10 mL of water. In the case where hydrogen peroxide (30) is added for the preparation of the test solution, add the same amount of hydrogen peroxide (30), then proceed in the same manner for preparation of the test solution, and use so obtained solution as the control solution. Adjust the test solution and the control solution to pH 3.0 to 4.0 with ammonia solution (28), and add water to make 40 mL, respectively. To these solutions add 1.2 mL of thioacetamide-alkaline glycerin TS, 2 mL of acetate buffer solution, pH 3.5 and water to make 50 mL, separately. After allowing to stand for 5 minutes, observe vertically both tubes on a white background: the color obtained with the test solution is not more intense than that with the control solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 5.0% (1 g, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 1.5% (1 g).

Assay (i) Apparatus—Reaction bottle: A 5-mL pressure-tight glass vial, having 20 mm in outside diameter and 50 mm in height, the neck 20 mm in outside diameter and 13 mm in inside diameter, equipped with a septum of butyl-rubber processed the surface with fluoroplastics, which can be fixed tightly to vial with aluminum cap, or equivalent.

Heater: A square-shaped aluminum block, having holes 20

mm in diameter and 32 mm in depth, adopted to the reaction bottle. Capable of stirring the content of the reaction bottle by means of magnetic stirrer or of reciprocal shaker about 100 times per minute.

(ii) Procedure—Weigh accurately about 65 mg of Methylcellulose, transfer to the reaction bottle, add 0.06 to 0.10 g of adipic acid, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Stir or shake for 60 minutes while heating so that the temperature of the bottle content is $130 \pm 2^\circ\text{C}$. In the case when the stirrer or shaker is not available, heat for 30 minutes with repeated shaking at 5-minute intervals by hand, and continue heating for an additional 30 minutes. Allow the bottle to cool, and again weigh accurately. If the mass loss is less than 0.50% or there is no evidence of a leak, use the upper layer of the mixture as the sample solution. Separately, put 0.06 to 0.10 g of adipic acid in a reaction bottle, 2.0 mL of the internal standard solution and 2.0 mL of hydroiodic acid, stopper the bottle immediately, and weigh accurately. Add 45 μL of iodomethane for assay through the septum using micro-syringe, weigh accurately, stir thoroughly, and use the upper layer of the mixture as the standard solution. Perform the test with 1 to 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of iodomethane to that of the internal standard.

$$\begin{aligned} \text{Content (\%)} & \text{ of methoxy group } (-\text{CH}_3\text{O}) \\ & = (Q_T/Q_S) \times (W_S/W) \times 21.86 \end{aligned}$$

W_S : Amount (mg) of iodomethane in the standard solution

W : Amount (mg) of sample, calculated on the dried basis

Internal standard solution—A solution of *n*-octane in *o*-xylylene (3 in 100).

Operating conditions—

Detector: A thermal conductivity detector or hydrogen flame-ionization detector.

Column: A glass column 3 – 4 mm in inside diameter and 1.8 – 3 m in length, packed with siliceous earth for gas chromatography, 125 to 150 μm in diameter, coated with methyl silicone polymer at the ratio of 10 – 20%.

Column temperature: A constant temperature of about 100°C .

Carrier gas: Helium for thermal conductivity detector, or Helium or Nitrogen for hydrogen, flame-ionization detector.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

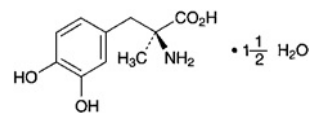
System suitability—

System performance: When the procedure is run with 1 – 2 μL of the standard solution under the above operating conditions, iodomethane, isopropyl iodide and the internal standard are eluted in this order, with complete separation of these peaks.

♦**Containers and storage** Containers—Well-closed containers. ♦

Methyldopa Hydrate

メチルドパ水和物



$\text{C}_{10}\text{H}_{13}\text{NO}_4 \cdot 1\frac{1}{2}\text{H}_2\text{O}$: 238.24

(2*S*)-2-Amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid sesquihydrate [41372-08-1]

Methyldopa Hydrate contains not less than 98.0% of methyldopa ($\text{C}_{10}\text{H}_{13}\text{NO}_4$: 211.21), calculated on the anhydrous basis.

Description Methyldopa Hydrate occurs as a white to pale grayish white, crystalline powder.

It is slightly soluble in water, in methanol and in acetic acid (100), very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It dissolves in dilute hydrochloric acid.

Identification (1) To 0.01 g of Methyldopa Hydrate add 3 drops of ninhydrin TS, and heat in a water bath for 3 minutes: a purple color develops.

(2) Determine the absorption spectrum of a solution of Methyldopa Hydrate in 0.1 mol/L hydrochloric acid TS (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyldopa Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Methyldopa Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Methyldopa Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-25 - -28^\circ$ (calculated on the anhydrous basis, 1 g, aluminum (III) chloride TS, 20 mL, 100 mm).

Purity (1) Acidity—Shake 1.0 g of Methyldopa Hydrate with 100 mL of freshly boiled and cooled water, and add 0.20 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of methyl red TS: a yellow color develops.

(2) Chloride <1.03>—Perform the test with 0.5 g of Methyldopa Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Methyldopa Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Methyldopa Hydrate in 5 mL of dilute hydrochloric acid, and perform the test (not more than 2 ppm).

(5) 3-*O*-Methylmethyldopa—Dissolve 0.10 g of Methyl-

dopa Hydrate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 5 mg of 3-*O*-methylmethyldopa for thin-layer chromatography in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (13:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-nitroaniline-sodium nitrite TS on the plate, and air-dry the plate, then spray evenly a solution of sodium carbonate decahydrate (1 in 4) on the plate: the spot from the sample solution corresponding to that from the standard solution is not more intense than the spot from the standard solution.

Water <2.48> 10.0 – 13.0% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Methyldopa Hydrate, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 to 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of $C_{10}H_{13}NO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methyldopa Tablets

メチルドパ錠

Methyldopa Tablets contain not less than 90% and not more than 110% of the labeled amount of methyldopa ($C_{10}H_{13}NO_4$; 211.21).

Method of preparation Prepare as directed under Tablets, with Methyldopa Hydrate.

Identification (1) To a quantity of powdered Methyldopa Tablets, equivalent to 0.1 g of Methyldopa Hydrate according to the labeled amount, add 10 mL of water, and heat in a water bath for 5 minutes with occasional shaking. After cooling, centrifuge for 5 minutes at 2000 rotations per minute, apply 1 drop of the supernatant solution to a filter paper, and dry with warm air. Place 1 drop of ninhydrin TS over the spot, and heat for 5 minutes at 100°C: a purple color develops.

(2) To 0.5 mL of the supernatant liquid obtained in the Identification (1) add 2 mL of 0.05 mol/L sulfuric acid TS, 2 mL of iron (II) tartrate TS and 4 drops of ammonia TS, and shake well: a deep purple color develops.

(3) To 0.7 mL of the supernatant liquid obtained in the Identification (1) add 0.1 mol/L hydrochloric acid TS to make 20 mL. To 10 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 283 nm.

iolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 277 nm and 283 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Methyldopa Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 30 mL or more of the dissolved solution 60 minutes after start of the test, and filter through a membrane filter with a pore size not exceeding 0.8 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 25 μ g of methyldopa ($C_{10}H_{13}NO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of methyldopa for assay (its loss on drying <2.41> is determined, separately, at 125°C for 2 hours), and dissolve in water to make exactly 200 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Methyldopa Tablets in 60 minutes is not less than 75%.

Dissolution rate (%) with respect to
the labeled amount of methyldopa ($C_{10}H_{13}NO_4$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 45$$

W_S : Amount (mg) of methyldopa for assay, calculated on the dried basis

C : Labeled amount (mg) of methyldopa ($C_{10}H_{13}NO_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methyldopa Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of methyldopa ($C_{10}H_{13}NO_4$), add 50 mL of 0.05 mol/L sulfuric acid TS, shake thoroughly for 15 minutes, add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 0.11 g of Methyldopa Reference Standard (previously dry at 125°C for 2 hours, and determine the loss on drying <2.41>), dissolve in 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution, add exactly 5 mL of iron (II) tartrate TS, and add ammonia-ammonium acetate buffer solution, pH 8.5, to make exactly 100 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of 0.05 mol/L sulfuric acid TS in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 520 nm, respectively.

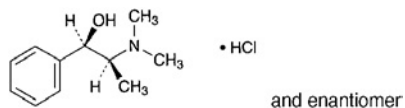
$$\text{Amount (mg) of methyldopa } (C_{10}H_{13}NO_4) \\ = W_S \times (A_T/A_S)$$

W_S : amount (mg) of Methyldopa Reference Standard, calculated on the dried basis

Containers and storage Containers—Well-closed containers.

dl-Methylephedrine Hydrochloride

dl-メチルエフェドリン塩酸塩



$C_{11}H_{17}NO \cdot HCl$: 215.72

(1*RS*,2*SR*)-2-Dimethylamino-1-phenylpropan-1-ol monohydrochloride [18760-80-0]

dl-Methylephedrine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{11}H_{17}NO \cdot HCl$.

Description *dl*-Methylephedrine Hydrochloride occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in water, sparingly soluble in ethanol (99.5), slightly soluble in acetic acid (100), and practically insoluble in acetic anhydride.

A solution of *dl*-Methylephedrine Hydrochloride (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of *dl*-Methylephedrine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of *dl*-Methylephedrine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of *dl*-Methylephedrine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of *dl*-Methylephedrine Hydrochloride in 20 mL of water is between 4.5 and 6.0.

Melting point <2.60> 207 – 211°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of *dl*-Methylephedrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of *dl*-Methylephedrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride in 20 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than the peak of methylephedrine is not more than the peak area of methylephedrine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

Time span of measurement: About 2 times as long as the retention time of methylephedrine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add water to make exactly 20 mL. Confirm that the peak area of methylephedrine obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of methylephedrine obtained from 20 μ L of the standard solution.

System performance: Dissolve 50 mg of *dl*-Methylephedrine Hydrochloride and 0.4 mg of methyl parahydroxybenzoate in 50 mL of water. When the procedure is run with 20 μ L of this solution under the above operating conditions, methylephedrine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylephedrine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of *dl*-Methylephedrine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 21.57 mg of $C_{11}H_{17}NO \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

10% *dl*-Methylephedrine Hydrochloride Powder

dl-Methylephedrine Hydrochloride Powder

dl-メチルエフェドリン塩酸塩散 10%

10% *dl*-Methylephedrine Hydrochloride Powder contains not less than 9.3% and not more than 10.7% of *dl*-methylephedrine hydrochloride (C₁₁H₁₇NO.HCl: 215.72).

Method of preparation

<i>dl</i> -Methylephedrine Hydrochloride	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification Determine the absorption spectrum of a solution of 10% *dl*-Methylephedrine Hydrochloride Powder (1 in 200) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 253 nm, between 255 nm and 259 nm, and between 261 nm and 264 nm.

Assay Weigh accurately about 0.5 g of 10% *dl*-Methylephedrine Hydrochloride Powder, add exactly 4 mL of the internal standard solution and 25 mL of water, shake vigorously for 20 minutes to dissolve, add water to make 50 mL, filter through a membrane filter with pore size of 0.45 μm, if necessary, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of *dl*-methylephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 4 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios of the peak area, Q_T and Q_S , of methylephedrine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } dl\text{-methylephedrine hydrochloride} \\ & \text{(C}_{11}\text{H}_{17}\text{NO.HCl)} \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of *dl*-methylephedrine hydrochloride for assay

Internal standard solution—A solution of methyl parahydroxybenzoate in acetonitrile (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 13.6 g of potassium dihydrogen phosphate and 3 g of sodium 1-heptane sulfonate in 1000 mL of water, and adjust the pH to 2.5 with phosphoric acid. To 900 mL of this solution add 200 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylephedrine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, methylephedrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

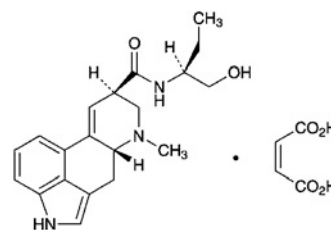
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylephedrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methylergometrine Maleate

メチルエルゴメトリンマレイン酸塩



C₂₀H₂₅N₃O₂·C₄H₄O₄: 455.50

(8*S*)-*N*-[(1*S*)-1-(Hydroxymethyl)propyl]-6-methyl-9,10-dihydroergoline-8-carboxamide monomaleate [7054-07-1]

Methylergometrine Maleate, when dried, contains not less than 95.0% and not more than 105.0% of C₂₀H₂₅N₃O₂·C₄H₄O₄.

Description Methylergometrine Maleate occurs as a white to pale yellow, crystalline powder. It is odorless.

It is slightly soluble in water, in methanol and in ethanol (95), and practically insoluble in diethyl ether.

It gradually changes to yellow by light.

Melting point: about 190°C (with decomposition).

Identification (1) A solution of Methylergometrine Maleate (1 in 200) shows a blue fluorescence.

(2) The solution obtained in the Assay develops a deep blue in color. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylergometrine Maleate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) To 5 mL of a solution of Methylergometrine Maleate

(1 in 500) add 1 drop of potassium permanganate TS: the red color of the test solution fades immediately.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: +44 – +50° (after drying, 0.1 g, water, 20 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 8 mg of Methylergometrine Maleate in 2 mL of a mixture of ethanol (95) and ammonia solution (28) (9:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of ethanol (95) and ammonia solution (28) (9:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, and immediately develop the plate with a mixture of chloroform, methanol and water (75:25:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% (0.2 g, in vacuum, phosphorus (V) oxide, 4 hours).

Assay Weigh accurately about 10 mg each of Methylergometrine Maleate and Methylergometrine Maleate Reference Standard, previously dried, add water to make exactly 250 mL, and use these solutions as the sample solution and the standard solution. Pipet 2 mL each of the sample solution and the standard solution separately into brown glass-stoppered test tubes, add exactly 4 mL each of 4-dimethylaminobenzaldehyde-iron (III) chloride TS while ice cooling, warm for 10 minutes at 45°C, and allow to stand for 20 minutes at room temperature. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2.0 mL of water in the same manner, as the blank. Determine the absorbances, A_{T} and A_{S} , of the subsequent solutions of the sample solution and the standard solution at 545 nm, respectively.

$$\begin{aligned} &\text{Amount (mg) of } \text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4 \\ &= W_{\text{S}} \times (A_{\text{T}}/A_{\text{S}}) \end{aligned}$$

W_{S} : Amount (mg) of Methylergometrine Maleate Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Methylergometrine Maleate Tablets

メチルエルゴメトリンマレイン酸塩錠

Methylergometrine Maleate Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methylergometrine maleate ($\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$; 455.50).

Method of preparation Prepare as directed under Tablets, with Methylergometrine maleate.

Identification (1) The sample solution obtained in the As-

say shows a blue fluorescence.

(2) The colored solution obtained in the Assay shows a deep blue color. Determine the absorption spectrum of the colored solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 543 nm and 547 nm and between 620 nm and 630 nm.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Transfer 1 tablet of Methylergometrine Maleate Tablets to a brown glass-stoppered centrifuge tube, add 10 mL of water, shake for 10 minutes vigorously, and disintegrate the tablet. Add 3 g of sodium chloride and 2 mL of ammonia solution (28), add exactly 25 mL of chloroform, and after vigorous shaking for 10 minutes, centrifuge for 5 minutes. Discard the water layer, take the chloroform extracts, add chloroform to make exactly V mL of a solution containing about 5 μg of methylergometrine maleate ($\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 1.25 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into a brown glass-stoppered centrifuge tube, and add 3 g of sodium chloride and 2 mL of ammonia solution (28). Add exactly 25 mL of chloroform, shake vigorously for 10 minutes, and centrifuge for 5 minutes. Discard the water layer, and use the chloroform layer as the standard solution. Pipet 20 mL each of the sample solution and the standard solution separately into brown glass-stoppered centrifuge tubes, add immediately exactly 10 mL of dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS, and shake for 5 minutes vigorously. Centrifuge these solutions for 5 minutes, take the water layers, and allow them to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute 4-dimethylaminobenzaldehyde-iron (III) chloride TS as the blank. Determine the absorbances, A_{T} and A_{S} , of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

$$\begin{aligned} &\text{Amount (mg) of methylergometrine maleate} \\ &(\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4) \\ &= W_{\text{S}} \times (A_{\text{T}}/A_{\text{S}}) \times (V/250) \end{aligned}$$

W_{S} : Amount (mg) of Methylergometrine Maleate Reference Standard

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Methylergometrine Maleate Tablets at 100 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with a pore size of not more than 0.8 μm . Discard the first 10 mL of the filtrate, to exactly V mL of the subsequent filtrate add water to make exactly V' mL so that each mL contains about 0.13 μg of methylergometrine maleate ($\text{C}_{20}\text{H}_{25}\text{N}_3\text{O}_2 \cdot \text{C}_4\text{H}_4\text{O}_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator for 4 hours (in vacuum, phosphorus (V) oxide), and dissolve in water to make exactly 100 mL. Pipet 5

mL of this solution, add water to make exactly 100 mL, then pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine immediately the intensities of the fluorescence, F_T and F_S , of the sample solution and the standard solution at 338 nm as the excitation wavelength and at 427 nm as the fluorescence wavelength as directed under Fluorometry <2.22>.

The dissolution rate of Methylergometrine Maleate Tablets in 30 minutes should be not less than 70%.

Dissolution rate (%) with respect to the labeled amount of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$)

$$= W_S \times (F_T/F_S) \times (V'/V) \times (1/C) \times (9/20)$$

W_S : Amount (mg) of Methylergometrine Maleate Reference Standard

C: Labeled amount (mg) of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Methylergometrine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 mg of methylergometrine maleate ($C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4$), transfer to a brown separator, add 15 mL of sodium hydrogen carbonate solution (1 in 20), and extract with four 20-mL portions of chloroform. Filter each portion of the chloroform extracts through a pledget of absorbent cotton, previously moistened with chloroform, into another dried, brown separator, combine all the extracts, and use this extract as the sample solution. Separately, weigh accurately about 10 mg of Methylergometrine Maleate Reference Standard, previously dried in a desiccator (silica gel) for 4 hours, dissolve in water, and add water to make exactly 100 mL. Pipet 3 mL of this solution, and transfer to a brown separator, proceed in the same manner as the preparation of the sample solution, and use this extract as the standard solution. To each total volume of the sample solution and the standard solution add exactly 25 mL each of dilute *p*-dimethylaminobenzaldehyde-ferric chloride TS, and after vigorous shaking for 5 minutes, allow to stand for 30 minutes. Draw off the water layer, centrifuge, and allow to stand for 1 hour. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dilute 4-dimethylaminobenzaldehyde-ferric chloride TS as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution at 545 nm, respectively.

$$\begin{aligned} &\text{Amount (mg) of methylergometrine maleate} \\ & (C_{20}H_{25}N_3O_2 \cdot C_4H_4O_4) \\ &= W_S \times (A_T/A_S) \times (3/100) \end{aligned}$$

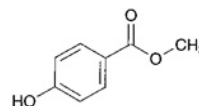
W_S : Amount (mg) of Methylergometrine Maleate Reference Standard

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Methyl Parahydroxybenzoate

パラオキシ安息香酸メチル



$C_8H_8O_3$: 152.15

Methyl 4-hydroxybenzoate [98-76-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Methyl Parahydroxybenzoate, when dried, contains not less than 98.0% and not more than 102.0% of $C_8H_8O_3$.

♦**Description** Methyl Parahydroxybenzoate, occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and slightly soluble in water.◆

Identification (1) The melting point <2.60> of Methyl Parahydroxybenzoate is between 125°C and 128°C.

◆(2) Determine the infrared absorption spectrum of Methyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 10 mL of ethanol (95): the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Methyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆(3) Heavy metals <1.07>—Dissolve 1.0 g of Methyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 0.10 g of Methyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of

methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.0 g of Methyl Parahydroxybenzoate, add exactly 20 mL of 1 mol/L sodium hydroxide VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

Each mL of 1 mol/L sodium hydroxide VS
= 152.1 mg of C₈H₈O₃

♦ **Containers and storage** Containers—Well-closed containers. ♦

Compound Methyl Salicylate Spirit

複方サリチル酸メチル精

Method of preparation

Methyl Salicylate	40 mL
Capsicum Tincture	100 mL
<i>d</i> - or <i>dl</i> -Camphor	50 g
Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Compound Methyl Salicylate Spirit is a reddish yellow liquid, having a characteristic odor and a burning taste.

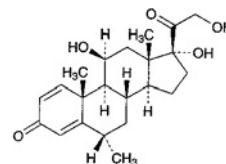
Identification (1) Shake 1 mL of Compound Methyl Salicylate Spirit with 5 mL of dilute ethanol, and add 1 drop of iron (III) chloride TS: a purple color is produced (methyl salicylate).

(2) Shake thoroughly 0.5 mL of Compound Methyl Salicylate Spirit with 10 mL of chloroform, and use this solution as the sample solution. Dissolve 0.04 g of methyl salicylate in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and chloroform (4:1) to a distance of about 10 cm, air-dry the plate, and examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution and the standard solution show the same *R_f* value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Methylprednisolone

メチルプレドニゾロン



C₂₂H₃₀O₅: 374.47

11β,17,21-Trihydroxy-6α-methylpregna-1,4-diene-3,20-dione [83-43-2]

Methylprednisolone, when dried, contains not less than 96.0% and not more than 104.0% of C₂₂H₃₀O₅.

Description Methylprednisolone occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in methanol and in 1,4-dioxane, slightly soluble in ethanol (95) and in chloroform, and practically insoluble in water and in diethyl ether.

Melting point: 232 – 240°C (with decomposition).

Identification (1) Add 2 mL of sulfuric acid to 2 mg of Methylprednisolone: a deep red color develops with no fluorescence. Then add 10 mL of water to this solution: the color fades, and a gray, flocculent precipitate is produced.

(2) Dissolve 0.01 g of Methylprednisolone in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: a red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Methylprednisolone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> [α]_D²⁰: +79 – +86° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Related substances—Dissolve 50 mg of Methylprednisolone in 5 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 12 cm, and air-dry the plate. Then heat at 105°C for 10 minutes, cool, and spray evenly alkaline blue tetrazolium TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.2 g).

Assay Weigh accurately about 10 mg of Methylprednisolone

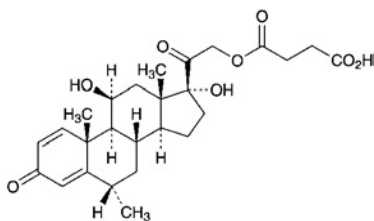
lone, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of this solution add methanol to make exactly 50 mL, and determine the absorbance A at the wavelength of maximum absorption at about 243 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{22}H_{30}O_5 \\ = (A/400) \times 1000 \end{aligned}$$

Containers and storage Containers—Tight containers.

Methylprednisolone Succinate

メチルプレドニゾロンコハク酸エステル



$C_{26}H_{34}O_8$: 474.54

11 β ,17,21-Trihydroxy-6 α -methylpregna-1,4-diene-3,20-dione 21-(hydrogen succinate) [2921-57-5]

Methylprednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{26}H_{34}O_8$.

Description Methylprednisolone Succinate occurs as a white, crystals or crystalline powder.

It is soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 235°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Methylprednisolone Succinate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methylprednisolone Succinate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methylprednisolone Succinate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Methylprednisolone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in ethanol (95), evaporating to dryness, and drying.

Optical rotation <2.49> $[\alpha]_D^{25}$: +99 – +103° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Methylprednisolone Succinate according to Method 4, and perform the test. Prepare the control solution with 1.0 mL of

Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Methylprednisolone Succinate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 15 mg of Methylprednisolone Succinate in 5 mL of methanol, add a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peaks other than the peak of methylprednisolone succinate is not more than 1/2 of the peak area of methylprednisolone succinate from the standard solution, and the total area of the peaks other than the peak of methylprednisolone succinate is not more than the peak area of methylprednisolone succinate from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of methylprednisolone succinate.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 10 mL. Confirm that the peak area of methylprednisolone succinate obtained from 5 μ L of this solution is equivalent to 7 to 13% of that obtained from 5 μ L of the standard solution.

System performance: Proceed as directed in the System suitability in the Assay.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of methylprednisolone succinate is not more than 1.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 15 mg each of Methylprednisolone Succinate and Methylprednisolone Succinate Reference Standard, previously dried, dissolve separately in 5 mL of methanol, and add the mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of methylprednisolone succinate to that of the internal standard.

$$\text{Amount (mg) of } C_{26}H_{34}O_8 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Methylprednisolone Succinate

Reference Standard

Internal standard solution—A solution of ethyl parahydroxybenzoate in a mixture of 0.05 mol/L phosphate buffer solution, pH 3.5 and acetonitrile (1:1) (3 in 20,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 1000 mL of 0.05 mol/L potassium dihydrogen phosphate TS add a suitable amount of 0.05 mol/L disodium hydrogen phosphate TS to make a solution having pH 5.5. To 640 mL of this solution add 360 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of methylprednisolone succinate is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, methylprednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methylprednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Methylrosanilinium Chloride

Crystal Violet

メチルロザニリン塩化物

C₂₅H₃₀ClN₃: 407.98

Methylrosanilinium Chloride is hexamethylpararosaniline chloride, and is usually admixed with pentamethylpararosaniline chloride and tetramethylpararosaniline chloride.

It contains not less than 96.0% of methylrosanilinium chloride [as hexamethylpararosaniline chloride (C₂₅H₃₀ClN₃)], calculated on the dried basis.

Description Methylrosanilinium Chloride occurs as green fragments having a metallic luster or a dark green powder. It is odorless or has a slight odor.

It is soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

Identification (1) To 1 mL of sulfuric acid add 1 mg of Methylrosanilinium Chloride: it dissolves, and shows an orange to red-brown color. To this solution add water dropwise: the color of the solution changes from brown through green to blue.

(2) Dissolve 0.02 g of Methylrosanilinium Chloride in 10

mL of water, add 5 drops of hydrochloric acid, and use this solution as the sample solution. To 5 mL of the sample solution add tannic acid TS dropwise: an intense blue precipitate is formed.

(3) To 5 mL of the sample solution obtained in (2) add 0.5 g of zinc powder, and shake: the solution is decolorized. Place 1 drop of this solution on filter paper, and apply 1 drop of ammonia TS adjacent to it: a blue color is produced at the zone of contact of the both solutions.

Purity (1) Ethanol-insoluble substances—Weigh accurately about 1 g of Methylrosanilinium Chloride, previously dried at 105°C for 4 hours, heat with 50 mL of ethanol (95) under a reflux condenser for 15 minutes in a water bath, and filter the mixture through a tared glass filter (G4). Wash the residue on the filter with warm ethanol (95) until the last washing does not show a purple color, and dry at 105°C for 2 hours: the mass of the residue is not more than 1.0%.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Methylrosanilinium Chloride according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Zinc—To 0.10 g of Methylrosanilinium Chloride add 0.1 mL of sulfuric acid, and incinerate by ignition. After cooling, boil with 5 mL of dilute hydrochloric acid, 0.5 mL of dilute nitric acid and 4 mL of water, add 5 mL of ammonia TS, boil again, and filter. To the filtrate add 2 to 3 drops of sodium sulfide TS: no turbidity is produced.

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of Methylrosanilinium Chloride, according to Method 3, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 7.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 1.5% (0.5 g).

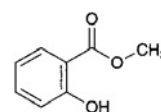
Assay Transfer about 0.4 g of Methylrosanilinium Chloride, accurately weighed, to a wide-mouthed, conical flask, add 25 mL of water and 10 mL of hydrochloric acid, dissolve, and add exactly 50 mL of 0.1 mol/L titanium (III) chloride VS while passing a stream of carbon dioxide through the flask. Heat to boil, and boil gently for 15 minutes, swirling the liquid frequently. Cool while passing a stream of carbon dioxide through the flask, titrate <2.50> the excess titanium (III) chloride with 0.05 mol/L ammonium iron (III) sulfate VS until a faint, red color is produced (indicator: 5 mL of ammonium thiocyanate TS). Perform a blank determination.

Each mL of 0.1 mol/L titanium (III) chloride VS
= 20.40 mg of C₂₅H₃₀ClN₃

Containers and storage Containers—Tight containers.

Methyl Salicylate

サリチル酸メチル



C₈H₈O₃: 152.15

Methyl 2-hydroxybenzoate [119-36-8]

Methyl Salicylate contains not less than 98.0% of $C_8H_8O_3$.

Description Methyl Salicylate is a colorless to pale yellow liquid. It has a strong, characteristic odor.

It is miscible with ethanol (95) and with diethyl ether.

It is very slightly soluble in water.

Specific gravity d_{20}^{20} : 1.182 – 1.192

Boiling point: 219 – 224°C

Identification Shake 1 drop of Methyl Salicylate thoroughly with 5 mL of water for 1 minute, and add 1 drop of iron (III) chloride TS: a purple color develops.

Purity (1) Acidity—Shake 5.0 mL of Methyl Salicylate thoroughly with 25 mL of freshly boiled and cooled water and 1.0 mL of 0.1 mol/L sodium hydroxide VS for 1 minute, add 2 drops of phenol red TS, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS until the red color disappears: not more than 0.45 mL of 0.1 mol/L sodium hydroxide VS is consumed.

(2) Heavy metals <1.07>—Shake 10.0 mL of Methyl Salicylate thoroughly with 10 mL of water, add 1 drop of hydrochloric acid, and saturate with hydrogen sulfide by passing it through the mixture: neither the oily layer nor the aqueous layer shows a dark color.

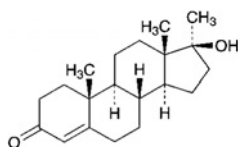
Assay Weigh accurately about 2 g of Methyl Salicylate, add an exactly measured 50 mL of 0.5 mol/L potassium hydroxide-ethanol VS, and heat on a water bath for 2 hours under a reflux condenser. Cool, and titrate <2.50> the excess potassium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.5 mol/L potassium hydroxide-ethanol VS = 76.07 mg of $C_8H_8O_3$

Containers and storage Containers—Tight containers.

Methyltestosterone

メチルテストステロン



$C_{20}H_{30}O_2$: 302.45

17 β -Hydroxy-17 α -methylandro-4-en-3-one [58-18-4]

Methyltestosterone, when dried, contains not less than 98.0% and not more than 102.0% of $C_{20}H_{30}O_2$.

Description Methyltestosterone occurs as white to pale yellow, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Methyltestosterone in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry

<2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Methyltestosterone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Methyltestosterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.55>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Methyltestosterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +79 – +85° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point <2.60> 163 – 168°C

Purity Related substances—Dissolve 40 mg of Methyltestosterone in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 10 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 20 mg each of Methyltestosterone and Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve each in methanol to make exactly 200 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of methyltestosterone to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of } C_{20}H_{30}O_2 \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Methyltestosterone Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about

35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Methyltestosterone Tablets

メチルテストステロン錠

Methyltestosterone Tablets contain not less than 90.0% and not more than 110.0% of the labeled amount of methyltestosterone ($C_{20}H_{30}O_2$; 302.45).

Method of preparation Prepare as directed under Tablets, with Methyltestosterone.

Identification To a portion of powdered Methyltestosterone Tablets, equivalent to 10 mg of Methyltestosterone according to the labeled amount, add 50 mL of acetone, shake for 30 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 10 mL of acetone, and use this solution as the sample solution. Separately, dissolve 10 mg of Methyltestosterone Reference Standard in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (9:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spot from the sample solution and the standard solution show the same *R_f* value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Methyltestosterone Tablets add 5 mL of water to disintegrate, add 50 mL of methanol, and shake for 30 minutes. Add methanol to make exactly 100 mL, and centrifuge. Measure exactly *V* mL of the supernatant liquid, add methanol to make exactly *V'* mL of a solution containing about 10 μ g of methyltestosterone ($C_{20}H_{30}O_2$) per mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, and dissolve in 5 mL of water and 50 mL of methanol, then add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make ex-

actly 50 mL, and use this solution as the standard solution. Determine the absorbances, *A_T* and *A_S*, of the sample solution and the standard solution at the wavelength of maximum absorption at about 241 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.25>.

$$\begin{aligned} &\text{Amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_2\text{)} \\ &= W_S \times (A_T/A_S) \times (V'/V) \times (1/10) \end{aligned}$$

W_S: Amount (mg) of Methyltestosterone Reference Standard

Assay Weigh accurately the mass of not less than 20 Methyltestosterone Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 25 mg of methyltestosterone ($C_{20}H_{30}O_2$), add about 70 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, filter through a membrane filter (not exceeding 0.45 μ m in pore size), and use the filtrate as the sample solution. Separately, weigh accurately about 20 mg of Methyltestosterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 10 hours, dissolve in methanol to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, *Q_T* and *Q_S*, of the peak area of methyltestosterone to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of methyltestosterone (C}_{20}\text{H}_{30}\text{O}_2\text{)} \\ &= W_S \times (Q_T/Q_S) \times (5/4) \end{aligned}$$

W_S: Amount (mg) of Methyltestosterone Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in methanol (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of methyltestosterone is about 10 minutes.

System suitability—

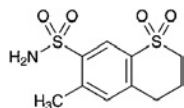
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and methyltestosterone are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of methyltestosterone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Meticrane

メチクラン



$C_{10}H_{13}NO_4S_2$: 275.34
6-Methylthiochromane-7-sulfonamide 1,1-dioxide
[1084-65-7]

Meticrane, when dried, contains not less than 98.0% of $C_{10}H_{13}NO_4S_2$.

Description Meticrane occurs as white, crystals or crystalline powder. It is odorless and has a slight bitter taste.

It is freely soluble in dimethylformamide, slightly soluble in acetonitrile and in methanol, very slightly soluble in ethanol (95), and practically insoluble in water.

Melting point: about 234°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Meticrane in methanol (3 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Meticrane, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Ammonium <1.02>—Perform the test with 0.10 g of Meticrane. Prepare the control solution with 3.0 mL of Standard Ammonium Solution (not more than 0.03%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Meticrane according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Meticrane according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Meticrane in 50 mL of acetonitrile. To 5 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than meticrane from the sample solution is not larger than the peak area of meticrane from the standard solution.

Operating conditions 1—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (17:3).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 7 minutes.

Time span of measurement: About 4 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 1—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 μ L of the standard solution.

System performance: Dissolve 0.01 g each of Meticrane and caffeine in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions 1, caffeine and meticrane are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions 1, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Operating conditions 2—

Detector, column, and column temperature: Proceed as directed in the operating conditions 1.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of meticrane is about 2 minutes.

Time span of measurement: About 10 times as long as the retention time of meticrane beginning after the solvent peak.

System suitability 2—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of meticrane obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of meticrane obtained from 10 μ L of the standard solution.

System performance: Dissolve 0.02 g each of Meticrane and methyl parahydroxybenzoate in 100 mL of acetonitrile. To exactly 2 mL of this solution add the mobile phase to make exactly 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions 2, meticrane and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions 2, the relative standard deviation of the peak area of meticrane is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

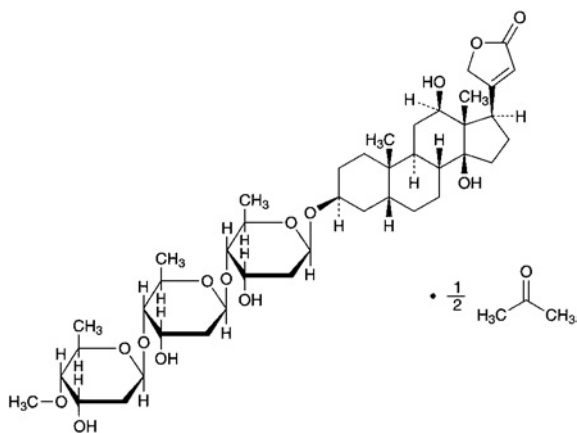
Assay Weigh accurately about 0.5 g of Meticrane, previously dried, dissolve in 50 mL of dimethylformamide, add 5 mL of water, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 27.54 mg of $C_{10}H_{13}NO_4S_2$

Containers and storage Containers—Well-closed containers.

Metildigoxin

メチルジゴキシン



$C_{42}H_{66}O_{14} \cdot \frac{1}{2}C_3H_6O$: 824.00

3β-[2,6-Dideoxy-4-O-methyl-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyl-(1→4)-2,6-dideoxy-β-D-ribo-hexopyranosyloxy]-12β,14-dihydroxy-5β-card-20(22)-enolide—acetone (2/1)
[30685-43-9, acetone-free]

Metildigoxin contains not less than 96.0% and not more than 103.0% of $C_{42}H_{66}O_{14} \cdot \frac{1}{2}C_3H_6O$, calculated on the anhydrous basis.

Description Metildigoxin occurs as a white to light yellowish white, crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, in pyridine and in acetic acid (100), soluble in chloroform, sparingly soluble in methanol, slightly soluble in ethanol (95) and in acetone, very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 2 mg of Metildigoxin in 2 mL of acetic acid (100), shake well with 1 drop of iron (III) chloride TS, and add gently 2 mL of sulfuric acid to divide into two layers: a brown color develops at the interface, and a deep blue color gradually develops in the acetic acid layer.

(2) Dissolve 2 mg of Metildigoxin in 2 mL of 1,3-dinitrobenzene TS, add 2 mL of a solution of tetramethylammonium hydroxide in ethanol (95) (1 in 200), and shake: a purple color gradually develops, and changes to blue-purple.

(3) Determine the absorption spectrum of a solution of Metildigoxin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Metildigoxin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Metildigoxin as directed in the potassium bromide disk method un-

der Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Metildigoxin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Metildigoxin and Metildigoxin Reference Standard in acetone, respectively, then evaporate the acetone to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_{546.1}^{20}$: +22.0 – +25.5° (1 g, calculated on the anhydrous basis, pyridine, 10 mL, 100 mm).

Purity (1) Arsenic <1.11>—Prepare the test solution with 0.5 g of Metildigoxin according to Method 3, and perform the test (not more than 4 ppm).

(2) Related substances—Dissolve 10 mg of Metildigoxin in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-butanone and chloroform (3:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Acetone Weigh accurately about 0.1 g of Metildigoxin, dissolve in exactly 2 mL of the internal standard solution, add *N,N*-dimethylformamide to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of acetone in a 50-mL volumetric flask containing about 10 mL of *N,N*-dimethylformamide, and add *N,N*-dimethylformamide to make 50 mL. Pipet 5 mL of this solution, add exactly 20 mL of the internal standard solution, then add *N,N*-dimethylformamide to make 100 mL, and use this solution as the standard solution. Perform the test with 1 μL each of the sample solution and the standard solution as directed under Gas Chromatography <2.02>, and calculate the ratios, Q_T and Q_S , of the peak area of acetone to that of the internal standard: the amount of acetone is between 2.0% and 5.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of acetone} \\ = (W_S/W_T) \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (g) of acetone

W_T : amount (g) of the sample

Internal standard solution—A solution of *t*-butanol in *N,N*-dimethylformamide (1 in 2000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 2 mm in inside diameter and 1 to 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature between 170°C and 230°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of acetone is about 2 minutes.

Selection of column: Proceed with 1 μL of the standard so-

lution under the above operating conditions, and calculate the resolution. Use a column giving elution of acetone and *t*-butanol in this order with the resolution between these peaks being not less than 2.0.

Water <2.48> Not more than 3.0% (0.3 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately 0.1 g each of Metildigoxin and Metildigoxin Reference Standard, and dissolve each in methanol to make exactly 50 mL. Pipet 5 mL each of the solutions, add methanol to each to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Pipet 5 mL each of the sample solution and the standard solution, add 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS to each, shake well, add methanol to make exactly 25 mL, and allow to stand at $20 \pm 0.5^\circ\text{C}$ for 20 minutes. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution prepared by mixing 15 mL of 2,4,6-trinitrophenol-ethanol TS and 2 mL of sodium hydroxide TS and adding methanol to make exactly 25 mL as the blank. Determine the maximum absorbances, A_T and A_S , of the subsequent solutions obtained from the sample solution and the standard solution, respectively, by measuring every 5 minutes, at 495 nm.

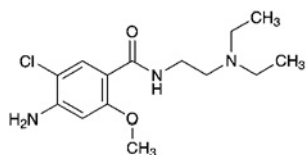
$$\begin{aligned} &\text{Amount (mg) of } \text{C}_{42}\text{H}_{66}\text{O}_{14} \cdot \frac{1}{2}\text{C}_3\text{H}_6\text{O} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Metildigoxin Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Metoclopramide

メトクロプラミド



$\text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2$: 299.80

4-Amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide [364-62-5]

Metoclopramide, when dried, contains not less than 99.0% of $\text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2$.

Description Metoclopramide occurs as white crystals or a crystalline powder, and is odorless.

It is freely soluble in acetic acid (100), soluble in methanol and in chloroform, sparingly soluble in acetic anhydride, in ethanol (95) and in acetone, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Dissolve 0.01 g of Metoclopramide in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for Primary Aromatic Amines.

(2) Dissolve 0.01 g of Metoclopramide in 5 mL of dilute

hydrochloric acid and 20 mL of water, and to 5 mL of this solution add 1 mL of Dragendorff's TS: a reddish orange precipitate is produced.

(3) Dissolve 0.1 g of Metoclopramide in 1 mL of 1 mol/L hydrochloric acid TS, and dilute with water to make 100 mL. To 1 mL of the solution add water to make 100 mL, determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> $146 - 149^\circ\text{C}$

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Metoclopramide in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Metoclopramide as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 1.0 g of Metoclopramide in 5 mL of 1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Metoclopramide in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of this solution, exactly measured, with methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28) (19:1) to a distance of about 10 cm. Dry the plate, first in air and then at 80°C for 30 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C , 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Metoclopramide, previously dried and accurately weighed, in 50 mL of acetic acid (100), add 5 mL of acetic anhydride, and warm for 5 minutes. Allow to cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform the blank determination, and make any necessary correction.

$$\begin{aligned} &\text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 29.98 \text{ mg of } \text{C}_{14}\text{H}_{22}\text{ClN}_3\text{O}_2 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Metoclopramide Tablets

メトクロプラミド錠

Metoclopramide Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of

metoclopramide ($C_{14}H_{22}ClN_3O_2$; 299.80).

Method of preparation Prepare as directed under Tablets, with Metoclopramide.

Identification (1) To a quantity of powdered Metoclopramide Tablets, equivalent to 50 mg of Metoclopramide according to the labeled amount, add 15 mL of 0.5 mol/L hydrochloric acid TS, and heat in a water bath at 70°C for 15 minutes while frequent shaking. After cooling, centrifuge for 10 minutes, and to 5 mL of the supernatant liquid add 1 mL of 4-dimethylaminobenzaldehyde-hydrochloric acid TS: a yellow color develops.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 270 nm and 274 nm, and between 306 nm and 310 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Metoclopramide Tablets add 10 mL of 0.1 mol/L hydrochloric acid TS, disperse the particles with the aid of ultrasonic waves, then add 0.1 mol/L hydrochloric acid TS to make exactly 25 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly V mL of a solution so that each mL contains about 12 μ g of metoclopramide ($C_{14}H_{22}ClN_3O_2$), and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} & \text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2) \\ & = W_S \times (A_T/A_S) \times (V/1000) \end{aligned}$$

W_S : Amount (mg) of metoclopramide for assay

Dissolution <6.10> Being specified separately.

Assay Weigh accurately not less than 20 Metoclopramide Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of metoclopramide ($C_{14}H_{22}ClN_3O_2$), add 300 mL of 0.1 mol/L hydrochloric acid TS, shake for 1 hour, and add 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Centrifuge for 10 minutes, pipet 4 mL of the supernatant liquid, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of metoclopramide for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 500 mL. Pipet 4 mL of this solution, add 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 308 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

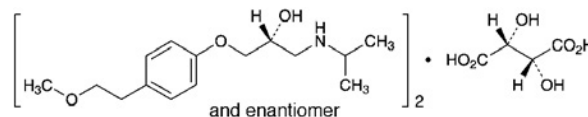
$$\begin{aligned} & \text{Amount (mg) of metoclopramide } (C_{14}H_{22}ClN_3O_2) \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of metoclopramide for assay

Containers and storage Containers—Tight containers.

Metoprolol Tartrate

メトプロロール酒石酸塩



$(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$; 684.81
(2*RS*)-1-[4-(2-Methoxyethyl)phenoxy]-
3-[(1-methylethyl)amino]propan-2-ol
hemi-(2*R*,3*R*)-tartrate
[56392-17-7]

Metoprolol Tartrate, when dried, contains not less than 99.0% and not more than 101.0% of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$.

Description Metoprolol Tartrate occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in methanol, in ethanol (95) and in acetic acid (100).

Optical rotation $[\alpha]_D^{20}$: +7.0 – +10.0° (after drying, 1 g, water, 50 mL, 100 mm).

Identification (1) Determine the absorption spectrum of a solution of Metoprolol Tartrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metoprolol Tartrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample from a solution in acetone (23 in 1000), filter and dry the crystals, and perform the test with the crystals.

(3) A solution of Metoprolol Tartrate (1 in 5) responds to the Qualitative Tests <1.09> (1) for tartrate.

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Metoprolol Tartrate in 10 mL of water is between 6.0 and 7.0.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Metoprolol Tartrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Metoprolol Tartrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution

on a plate of silica gel for thin-layer chromatography. After saturating the plate with the atmosphere by allowing to stand in a developing vessel, which contains the developing solvent and a glass vessel containing ammonia water (28), develop with the developing solvent, a mixture of ethyl acetate and methanol (4:1), to a distance of about 12 cm, and air-dry the plate. Allow to stand the plate in an iodine vapors until the spot with the standard solution appears obviously: the spot other than the principal spot and other than the spot on the original point is not more than three spots, and they are not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Metoprolol Tartrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.24 mg of $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Metoprolol Tartrate Tablets

メトプロロール酒石酸塩錠

Metoprolol Tartrate Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$; 684.81).

Method of preparation Prepare as directed under Tablets, with Metoprolol Tartrate.

Identification To an amount of powdered Metoprolol Tartrate Tablets, equivalent to 10 mg of Metoprolol Tartrate according to the labeled amount, add 100 mL of ethanol (95), shake for 15 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 274 nm and 278 nm and between 281 nm and 285 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Metoprolol Tartrate Tablets add 1 mL of water for every 10 mg of Metoprolol Tartrate, shake for 20 minutes, then add 75 mL of ethanol (95), shake for 15 minutes, add ethanol (95) to make exactly 100 mL, and centrifuge. Pipet V mL of the supernatant liquid, add ethanol (95) to make exactly V' so that each mL contains about 0.1 mg of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 5 mL of water, and add ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine

the absorbances, A_T and A_S , of the sample solution and the standard solution at 276 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.

Amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/5)$

W_S : Amount (mg) of metoprolol tartrate for assay

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Metoprolol Tartrate Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 22 μ g of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$ according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 56 mg of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, and dissolve in water to make exactly 200 mL. Pipet 8 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of metoprolol. The dissolution rate in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 36$

W_S : Amount (mg) of metoprolol tartrate for assay

C : Labeled amount (mg) of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$ in 1 tablet

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metoprolol are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metoprolol is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Metoprolol Tartrate Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.12 g of metoprolol tartrate $((C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6)$, add 60 mL of a mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) and exactly 10 mL of the internal standard solution, shake for 15 minutes, and add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.12 g of metoprolol tartrate for assay, previously dried in vacuum at 60°C for 4 hours, dissolve in 60 mL of the mixture of ethanol (99.5) and

1 mol/L hydrochloric acid TS (100:1), add exactly 10 mL of the internal standard solution, then add the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of metoprolol to that of the internal standard.

Amount (mg) of metoprolol tartrate ((C₁₅H₂₅NO₃)₂·C₄H₆O₆)
= $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of metoprolol tartrate for assay

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of ethanol (99.5) and 1 mol/L hydrochloric acid TS (100:1) (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 14.0 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust to pH 3.2 with diluted perchloric acid (17 in 2000). To 750 mL of this solution add 250 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of metoprolol is about 8 minutes.

System suitability—

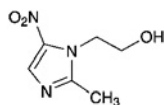
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, metoprolol and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of metoprolol to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Metronidazole

メトロニダゾール



C₆H₉N₃O₃: 171.15

2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethanol [443-48-1]

Metronidazole, when dried, contains not less than 99.0% and not more than 101.0% of C₆H₉N₃O₃.

Description Metronidazole occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (99.5) and in acetone, and slightly soluble in water.

It dissolves in dilute hydrochloric acid.

It is colored to yellow-brown by light.

Identification (1) Determine the absorption spectrum of a solution of Metronidazole in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Metronidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 159 – 163°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Metronidazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) 2-Methyl-5-nitroimidazol—Dissolve 0.10 g of Metronidazole in acetone to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 2-methyl-5-nitroimidazole for thin-layer chromatography in acetone to make exactly 20 mL, then pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately develop the plate with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Metronidazole, previously dried, and dissolve in 30 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 0.5 mL of *p*-naphtholbenzein TS) until the color of the solution changes from orange-yellow to green. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 17.12 mg of C₆H₉N₃O₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Metronidazole Tablets

メトロニダゾール錠

Metronidazole Tablets contain not less than 93.0%

and not more than 107.0% of the labeled amount of metronidazole ($C_6H_9N_3O_3$; 171.15).

Method of preparation Prepare as directed under Tablets, with Metronidazole.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 275 nm and 279 nm.

(2) Shake vigorously a quantity of powdered Metronidazole Tablets, equivalent to 0.20 g of Metronidazole according to the labeled amount, with 20 mL of acetone for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.10 g of metronidazole in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate immediately with a mixture of acetone, water and ethyl acetate (8:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f value of the principal spots obtained from the sample solution and the standard solution is the same.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Metronidazole Tablets add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 25 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter the solution through a membrane filter with pore size of 0.45 μ m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

$$\begin{aligned} & \text{Amount (mg) of metronidazole (C}_6\text{H}_9\text{N}_3\text{O}_3\text{)} \\ & = W_S \times (A_T/A_S) \times 10 \end{aligned}$$

W_S : Amount (mg) of metronidazole for assay

Assay Weigh accurately the mass of not less than 20 Metronidazole Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 0.25 g of metronidazole ($C_6H_9N_3O_3$), add 25 mL of a mixture of water and methanol (1:1), shake vigorously for 10 minutes, and add the mixture of water and methanol (1:1) to make exactly 50 mL. Pipet 5 mL of this solution, and add a mixture of water and methanol (4:1) to make exactly 100 mL. Filter this solution through a membrane filter with pore size of 0.45 μ m, discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of metronidazole for assay, previously dried in vacuum on silica gel for 24 hours, dissolve in the mixture of water and methanol (4:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of metronidazole.

$$\begin{aligned} & \text{Amount (mg) of metronidazole (C}_6\text{H}_9\text{N}_3\text{O}_3\text{)} \\ & = W_S \times (A_T/A_S) \times 10 \end{aligned}$$

W_S : Amount (mg) of metronidazole for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 320 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of metronidazole is about 5 minutes.

System suitability—

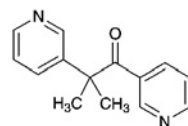
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of metronidazole are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of metronidazole is not more than 1.0%.

Containers and storage Containers—Tight containers.

Metyrapone

メチラポン



$C_{14}H_{14}N_2O$; 226.27

2-Methyl-1,2-di(pyridin-3-yl)propan-1-one [54-36-4]

Metyrapone, when dried, contains not less than 98.0% of $C_{14}H_{14}N_2O$.

Description Metyrapone occurs as a white to pale yellow, crystalline powder. It has a characteristic odor and a bitter taste.

It is very soluble in methanol, in ethanol (95), in acetic anhydride, in chloroform, in diethyl ether and in nitrobenzene, and sparingly soluble in water.

It dissolves in 0.5 mol/L sulfuric acid TS.

Identification (1) Mix 5 mg of Metyrapone with 0.01 g of 1-chloro-2,4-dinitrobenzene, melt by gently heating for 5 to 6 seconds, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Determine the absorption spectrum of a solution of Metyrapone in 0.5 mol/L sulfuric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 50 – 54°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of

Metyrapone in 5 mL of methanol: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Metyrapone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Metyrapone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Metyrapone in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 50 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (15:1) to a distance of about 10 cm, and air-dry the plate for about 15 minutes. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

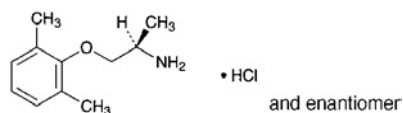
Assay Weigh accurately about 0.2 g of Metyrapone, previously dried, dissolve in 10 mL of nitrobenzene and 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 11.31 \text{ mg of } C_{14}H_{14}N_2O \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Mexiletine Hydrochloride

メキシレチン塩酸塩



$C_{11}H_{17}NO \cdot HCl$: 215.72
(1*S*)-2-(2,6-Dimethylphenoxy)-1-methylethylamine
monohydrochloride [5370-01-4]

Mexiletine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{11}H_{17}NO \cdot HCl$.

Description Mexiletine Hydrochloride occurs as a white powder.

It is freely soluble in water and in ethanol (95), slightly soluble in acetonitrile, and practically insoluble in diethyl ether.

A solution of Mexiletine Hydrochloride (1 in 20) shows no

optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Mexiletine Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mexiletine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mexiletine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Mexiletine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, recrystallize the sample from ethanol (95), filter, dry the crystals, and repeat the test on the crystals.

(3) A solution of Mexiletine Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the pH of this solution is between 3.8 and 5.8.

Melting point <2.60> 200 – 204°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Mexiletine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy Metals <1.07>—Proceed with 2.0 g of Mexiletine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Mexiletine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: each peak area of the peaks other than the peak of mexiletine from the sample solution is not larger than the peak area of mexiletine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of mexiletine obtained from 20 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 3 times as long as the retention time of mexiletine beginning after peaks of the solvent.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg each of Mexiletine Hydrochloride and Mexiletine Hydrochloride Reference

Standard, each previously dried, and dissolve each in the mobile phase to make exactly 20 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of mexiletine to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{11}H_{17}NO.HCl \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Mexiletine Hydrochloride Reference Standard

Internal standard solution—A solution of phenethylamine hydrochloride in the mobile phase (3 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (about 7 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2.5 g of sodium lauryl sulfate and 3 g of sodium dihydrogenphosphate dihydrate in 600 mL of water, and add 420 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of mexiletine is about 6 minutes.

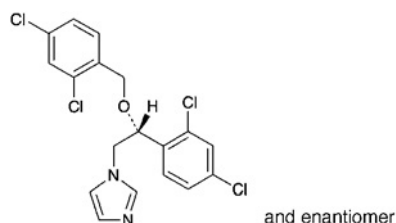
Selection of column: Proceed with 20 μ L of the standard solution under the above conditions, and calculate the resolution. Use a column giving elution of the internal standard and mexiletine in this order with the resolution between these peaks being not less than 9.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Miconazole

ミコナゾール



$C_{18}H_{14}Cl_4N_2O$: 416.13

1-[(2*RS*)-2-(2,4-Dichlorobenzoyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole [22916-47-8]

Miconazole, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O$.

Description Miconazole occurs as a white to pale yellowish white, crystalline powder.

It is freely soluble in methanol, in ethanol (95) and in acetic

acid (100), soluble in diethyl ether, and practically insoluble in water.

A solution of Miconazole in methanol (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Miconazole in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Miconazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 84 – 87°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Miconazole according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Miconazole in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 60%, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

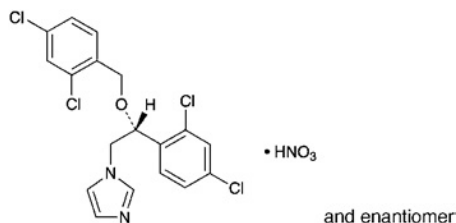
Assay Weigh accurately about 0.3 g of Miconazole, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of *p*-naphtholbenzein TS) until the color of the solution changes from light yellow-brown to light yellow-green. Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 41.61 \text{ mg of } C_{18}H_{14}Cl_4N_2O \end{aligned}$$

Containers and storage Containers—Tight containers.

Miconazole Nitrate

ミコナゾール硝酸塩



$C_{18}H_{14}Cl_4N_2O \cdot HNO_3$; 479.14

1-[(2*RS*)-2-(2,4-Dichlorobenzoyloxy)-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole mononitrate
[22832-87-7]

Miconazole Nitrate, when dried, contains not less than 98.5% of $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$.

Description Miconazole Nitrate occurs as a white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, sparingly soluble in methanol, slightly soluble in ethanol (95), in acetone and in acetic acid (100), and very slightly soluble in water and in diethyl ether.

Melting point: about 180°C (with decomposition).

Identification (1) To 2 mL of a solution of Miconazole Nitrate in methanol (1 in 100) add 2 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the absorption spectrum of a solution of Miconazole Nitrate in methanol (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Perform the test with a solution of Miconazole Nitrate in methanol (1 in 100) as directed under Flame Coloration Test <1.04> (2): a green color appears.

(4) A solution of Miconazole Nitrate in methanol (1 in 100) responds to the Qualitative Tests <1.09> for nitrate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Miconazole Nitrate in 100 mL of methanol: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.10 g of Miconazole Nitrate in 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and *N,N*-dimethylformamide to make 50 mL (not more than 0.09%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Miconazole Nitrate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Miconazole Nitrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Miconazole Nitrate in 10 mL of methanol, and use this solution as the

sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 20 mL, pipet 1 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 50 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of *n*-hexane, chloroform, methanol and ammonia solution (28) (60:30:10:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate in iodine vapor for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Miconazole Nitrate, previously dried, dissolve in 50 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

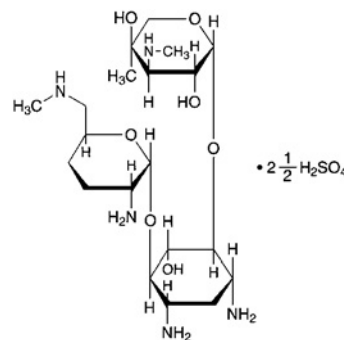
Each mL of 0.1 mol/L perchloric acid VS
= 47.91 mg of $C_{18}H_{14}Cl_4N_2O \cdot HNO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Micronomicin Sulfate

ミクロノマイシン硫酸塩



$C_{20}H_{41}N_5O_7 \cdot 2\frac{1}{2}H_2SO_4$; 708.77

2-Amino-2,3,4,6-tetrahydro-6-methylamino- α -D-erythro-hexopyranosyl-(1 \rightarrow 4)-[3-deoxy-4-C-methyl-3-methylamino- β -L-arabinopyranosyl-(1 \rightarrow 6)]-2-deoxy-D-streptamine hemipentasulfate
[52093-21-7, Micronomicin]

Micronomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora sagamiensis*.

It contains not less than 590 μ g (potency) and not more than 660 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Micronomicin Sulfate is expressed as mass (potency) of micronomicin ($C_{20}H_{41}N_5O_7$; 463.57).

Description Micronomicin Sulfate occurs as a white to light

yellowish white powder.

It is very soluble in water, sparingly soluble in ethylene glycol, and practically insoluble in methanol and in ethanol (99.5).

It is hygroscopic.

Identification (1) Dissolve 50 mg each of Micronomicin Sulfate and Micronomicin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spots obtained from the sample solution and the standard solution are red-purple to red-brown and their R_f values are the same.

(2) To 5 mL of a solution of Micronomicin Sulfate (1 in 100) add 1 mL of barium chloride TS: a white precipitate is formed, and it does not dissolve by addition of dilute nitric acid.

Optical rotation <2.49> $[\alpha]_D^{20}$: +110 – +130° (0.25 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Micronomicin Sulfate in 10 mL of water is between 3.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.5 g of Micronomicin Sulfate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Micronomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.40 g of Micronomicin Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), 1-butanol and ammonia solution (28) (10:8:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in a mixture of acetone and pyridine (25:1) (1 in 500), and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Water <2.48> Not more than 10.0% (0.2 g, volumetric titration, back titration). Use a mixture of methanol for water determination and ethylene glycol for water determination (1:1) instead of methanol for water determination.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base lay-

er.

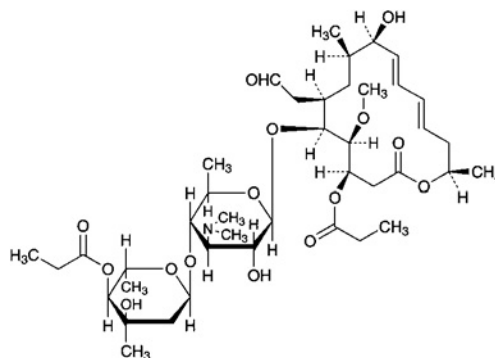
(iii) Standard solutions—Weigh accurately an amount of Micronomicin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 – 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Micronomicin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 2 μg (potency) and 0.5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Midecamycin

ミデカマイシン



$\text{C}_{41}\text{H}_{67}\text{NO}_{15}$: 813.97

(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-

5-[2,6-Dideoxy-3-*C*-methyl-4-*O*-propanoyl- α -*L*-ribohexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [35457-80-8]

Midecamycin is a macrolide substance having antibacterial activity produced by the growth of *Streptomyces mycarofaciens*.

It contains not less than 950 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Midecamycin is expressed as mass (potency) of midecamycin ($\text{C}_{41}\text{H}_{67}\text{NO}_{15}$).

Description Midecamycin occurs as a white crystalline powder.

It is very soluble in methanol, freely soluble in ethanol (95), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a

solution of Midecamycin in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Midecamycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 153 – 158°C

Purity Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

Loss on drying <2.41> Not more than 2.0% (1.0 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

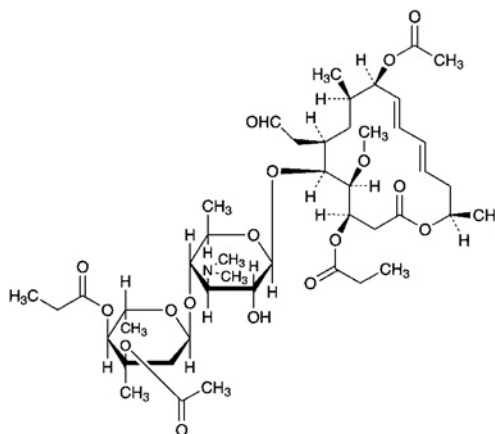
(iii) Standard solutions—Weigh accurately an amount of Midecamycin Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, add water to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin, previously dried, equivalent to about 20 mg (potency), dissolve in 10 mL of methanol, and add water to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Midecamycin Acetate

ミデカマイシン酢酸エステル



C₄₅H₇₁NO₁₇: 898.04

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-9-Acetoxy-5-[3-*O*-acetyl-2,6-dideoxy-3-*C*-methyl-4-*O*-propanoyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-4-methoxy-8-methyl-3-propanoyloxyhexadeca-10,12-dien-15-olide [55881-07-7]

Midecamycin Acetate is a derivative of midecamycin.

It contains not less than 950 µg (potency) and not more than 1010 µg (potency) per mg, calculated on the dried basis. The potency of Midecamycin Acetate is expressed as mass of midecamycin acetate (C₄₅H₇₁NO₁₇).

Description Midecamycin Acetate occurs as white, crystals or crystalline powder.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Midecamycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Midecamycin Acetate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Midecamycin Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or spectrum of dried Midecamycin Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Midecamycin Acetate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 2.0% (0.1 g, in vacuum not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Midecamycin Acetate Reference Standard, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5–15°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Midecamycin Acetate, previously dried, equivalent to about 25 mg (potency), and dissolve in methanol to make exactly 50 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 20 µg (potency) and 5 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Migrenin

ミグレニン

Migrenin is composed of 90 parts of antipyrine, 9 parts of caffeine, and 1 part of citric acid in mass.

Migrenin, when dried, contains not less than 87.0% and not more than 93.0% of antipyrine ($C_{11}H_{12}N_2O$: 188.23) and not less than 8.6% and not more than 9.5% of caffeine ($C_8H_{10}N_4O_2$: 194.19).

Description Migrenin occurs as a white powder or crystalline powder. It is odorless and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and slightly soluble in diethyl ether.

The pH of a solution of Migrenin (1 in 10) is between 3.0 and 4.0.

It is affected by moisture and light.

Identification (1) To 5 mL of a solution of Migrenin (1 in 100) add 2 drops of sodium nitrite TS and 1 mL of dilute sulfuric acid: a deep green color develops.

(2) To 5 mL of a solution of Migrenin (1 in 50) add 1 drop of hydrochloric acid and 0.2 mL of formaldehyde solution, heat in a water bath for 30 minutes, add an excess of ammonia TS, and filter. Acidify the filtrate with hydrochloric acid, shake with 3 mL of chloroform, and separate the chloroform layer. Evaporate the chloroform solution on a water bath, add 10 drops of hydrogen peroxide TS and 1 drop of hydrochloric acid to the residue, and evaporate on a water bath to dryness: the residue shows a yellow-red color. Invert

the residue over a vessel containing 3 drops of ammonia TS: a red-purple color develops, disappearing on the addition of 2 to 3 drops of sodium hydroxide TS.

(3) A solution of Migrenin (1 in 10) responds to the Qualitative Tests <1.09> for citrate.

Melting point <2.60> 104–110°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Migrenin in 40 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Migrenin according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay (1) Antipyrine—Weigh accurately about 0.25 g of Migrenin, previously dried in an iodine flask, dissolve in 25 mL of sodium acetate TS, add exactly 30 mL of 0.05 mol/L iodine VS, and allow to stand for 20 minutes with occasional shaking. Add 15 mL of chloroform to dissolve the precipitate so obtained, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.05 mol/L iodine VS} \\ = 9.411 \text{ mg of } C_{11}H_{12}N_2O \end{aligned}$$

(2) Caffeine—To about 1 g of Migrenin, previously dried and accurately weighed, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 90 mg of Caffeine Reference Standard, previously dried at 80°C for 4 hours, add exactly 5 mL of the internal standard solution, dissolve in chloroform to make 10 mL, and use this solution as the standard solution. Perform the test with 1 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of caffeine to that of the internal standard.

$$\text{Amount (mg) of caffeine (} C_8H_{10}N_4O_2 \text{)} = W_S \times (Q_T / Q_S)$$

W_S : Amount (mg) of Caffeine Reference Standard

Internal standard solution—A solution of ethenzamide in chloroform (1 in 50).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 2.6 mm in inside diameter and 210 cm in length, packed with siliceous earth for gas chromatography (180 to 250 µm in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 15%.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethenzamide is about 4 minutes.

System suitability—

System performance: Dissolve 0.9 g of antipyrine and 0.09

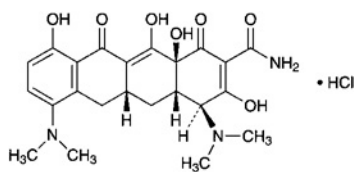
g of caffeine in 10 mL of chloroform. When the procedure is run with 1 μ L of this solution under the above operating conditions, caffeine and antipyrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 1 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of caffeine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Minocycline Hydrochloride

ミノサイクリン塩酸塩



$C_{23}H_{27}N_3O_7 \cdot HCl$: 493.94

(4*S*,4*aS*,5*aR*,12*aS*)-4,7-Bis(dimethylamino)-3,10,12,12*a*-tetrahydroxy-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide monohydrochloride
[13614-98-7]

Minocycline Hydrochloride is the hydrochloride of a derivative of tetracycline.

It contains not less than 890 μ g (potency) and not more than 950 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Minocycline Hydrochloride is expressed as mass (potency) of minocycline ($C_{23}H_{27}N_3O_7$: 457.48).

Description Minocycline Hydrochloride occurs as a yellow crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in methanol, sparingly soluble in water, and slightly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Minocycline Hydrochloride in a solution of hydrochloric acid in methanol (19 in 20,000) (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Minocycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Minocycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Minocycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Minocycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Minocycline Hydrochloride in

100 mL of water: the pH of the solution is between 3.5 and 4.5.

Purity (1) A solution of Minocycline Hydrochloride (1 in 100) is clear, and when the test is performed within 1 hour after preparation of this solution, the absorbance of the solution at 560 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.06.

(2) Heavy metals <1.07>—Proceed with 0.5 g of Minocycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(3) Related substances—Dissolve 50 mg of Minocycline Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Perform the test immediately after the preparation of the sample solution with 20 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure each peak area by the automatic integration method. Calculate the amount of each peak area by the area percentage method: the amount of epiminocycline is not more than 1.2%, the amount of each peak other than minocycline and epiminocycline is not more than 1.0%, and the total area of the peaks other than minocycline and epiminocycline is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes. The retention time of epiminocycline is about 10 minutes under this condition.

Time span of measurement: About 2.5 times as long as the retention time of minocycline beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add the mobile phase to make exactly 100 mL. Confirm that the peak area of minocycline obtained from 20 μ L of this solution is equivalent to 3.5 to 6.5% of that of minocycline obtained from 20 μ L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of minocycline is not more than 2.0%.

Water <2.48> Not less than 4.3% and not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Minocycline Hydrochloride and Minocycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of minocycline of these solutions.

Amount [μg (potency)] of minocycline ($\text{C}_{23}\text{H}_{27}\text{N}_3\text{O}_7$)
 $= W_S \times (A_T/A_S) \times 1000$

W_S : Amount [mg (potency)] of Minocycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5\ \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 25°C .

Mobile phase: Adjust the pH of a mixture of a solution of ammonium oxalate monohydrate (7 in 250), *N,N*-dimethylformamide and 0.1 mol/L disodium dihydrogen ethylenediamine tetraacetate TS (11:5:4) to 6.2 with tetrabutylammonium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of minocycline is about 12 minutes.

System suitability—

System performance: Dissolve 0.05 g (potency) of Minocycline Hydrochloride Reference Standard in 25 mL of water. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with $20\ \mu\text{L}$ of this solution under the above operating conditions, epiminocycline and minocycline are eluted in this order with the resolution between these peaks being not less than 2.0.

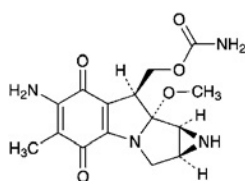
System repeatability: When the test is repeated 6 times with $20\ \mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of peak areas of minocycline is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mitomycin C

マイトマイシン C



$\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_5$; 334.33
 (1*aS*,8*S*,8*aR*,8*bS*)-6-Amino-4,7-dioxo-8*a*-methoxy-5-methyl-1,1*a*,2,8,8*a*,8*b*-hexahydroazirino[2',3':3,4]pyrrolo[1,2-*a*]indol-8-ylmethyl carbamate [50-07-7]

Mitomycin C is a substance having antitumor activity produced by the growth of *Streptomyces caespitosus*.

It contains not less than $970\ \mu\text{g}$ (potency) and not more than $1030\ \mu\text{g}$ (potency) per mg, calculated on the dried basis. The potency of Mitomycin C is expressed as mass (potency) of mitomycin C ($\text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_5$).

Description Mitomycin C occurs as blue-purple, crystals or crystalline powder.

It is freely soluble in *N,N*-dimethylacetamide, slightly soluble in water and in methanol, and very slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Mitomycin C (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Mitomycin C Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Mitomycin C as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Mitomycin C Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity Related substances—Conduct this procedure rapidly after the sample and the standard solutions are prepared. Dissolve 50 mg of Mitomycin C in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10\ \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each area of the peak other than mitomycin C obtained from the sample solution is not more than the peak area of mitomycin C from the standard solution, and the total area of the peaks other than mitomycin C from the sample solution is not more than 3 times the peak area of mitomycin C from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5\ \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 30°C .

Mobile phase A: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To 800 mL of this solution add 200 mL of methanol.

Mobile phase B: To 20 mL of 0.5 mol/L ammonium acetate TS add water to make 1000 mL. To this solution add 1000 mL of methanol.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 10	100	0
10 - 30	100 → 0	0 → 100
30 - 45	0	100

Flow rate: About 1.0 mL/min

Time span of measurement: About 2 times as long as the

retention time of mitomycin C beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 10 mL of the standard solution, and add methanol to make exactly 100 mL. Confirm that the peak area of mitomycin C obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the standard solution.

System performance: Dissolve 25 mg of Mitomycin C and 40 mg of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of methanol. When the procedure is run with 10 μ L of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 15.

System repeatability: When the test is repeated 3 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 3.0%.

Loss on drying <2.41> Not more than 1.0% (0.1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Assay Weigh accurately an amount of Mitomycin C and Mitomycin C Reference Standard, equivalent to about 25 mg (potency), dissolve each in *N,N*-dimethylacetamide to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of mitomycin C.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{15}\text{H}_{18}\text{N}_4\text{O}_5 \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Mitomycin C Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 365 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with phenylated silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 40 mL of 0.5 mol/L ammonium acetate TS add 5 mL of diluted acetic acid (100) (1 in 20) and water to make 1000 mL. To 600 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of mitomycin C is about 7 minutes.

System suitability—

System performance: Dissolve about 25 mg of Mitomycin C Reference Standard and about 0.375 g of 3-ethoxy-4-hydroxybenzaldehyde in 50 mL of *N,N*-dimethylacetamide. When the procedure is run with 10 μ L of this solution under the above operating conditions, mitomycin C and 3-ethoxy-4-hydroxybenzaldehyde are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of mitomycin C is not more than 1.0%.

Containers and storage Containers—Tight containers.

Morphine and Atropine Injection

モルヒネ・アトロピン注射液

Morphine and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.91 w/v% and not more than 1.09 w/v% of morphine hydrochloride hydrate ($\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$: 375.84), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [$(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}$: 694.83].

Method of preparation

Morphine Hydrochloride Hydrate	10 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection	a significant quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Morphine and Atropine Injection is a clear, colorless liquid.

It is gradually colored by light.

pH: 2.5 – 5.0

Identification To 2 mL of Morphine and Atropine Injection add 2 mL of ammonia TS, and extract with 10 mL of diethyl ether. Filter the extract with a filter paper, evaporate the filtrate on a water bath to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Separately, dissolve 0.1 g of morphine hydrochloride in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (1). Separately, dissolve 3 mg of atropine sulfate in 10 mL of water, perform with 2 mL of this solution the same procedure as used for preparation of the sample solution, and use the solution so obtained as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia solution (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS on the plate: the two spots obtained from the sample solution show the same color tone and the same R_f value with either spot of orange color obtained from the standard solution (1) or the standard solution (2) (morphine and atropine).

Extractable volume <6.05> It meets the requirement.

Assay (1) Morphine hydrochloride hydrate—Pipet 2 mL of Morphine and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, add exactly 10 mL of the internal standard solution to dissolve, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L of the

sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of morphine hydrochloride hydrate} \\ &(\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}) \\ &= W_S \times (Q_T/Q_S) \times 1.1679 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH with sodium hydroxide TS to 3.0. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

(2) **Atropine sulfate hydrate**—Pipet 2 mL of Morphine and Atropine Injection, add exactly 2 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Atropine Sulfate Reference Standard (separately determine its loss on drying <2.41> in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak areas of atropine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of atropine sulfate hydrate} \\ &[(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}] \\ &= W_S \times (Q_T/Q_S) \times (1/25) \times 1.027 \end{aligned}$$

W_S : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 12,500).

Operating conditions—

Column, column temperature, and mobile phase: Proceed

as directed in the operating conditions in the Assay (1).

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Flow rate: Adjust the flow rate so that the retention time of morphine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, morphine, the internal standard and atropine are eluted in this order, and the resolution between morphine and the internal standard is not less than 3.

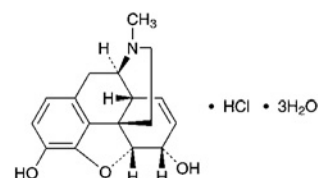
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Morphine Hydrochloride Hydrate

モルヒネ塩酸塩水和物



$\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$: 375.84
(5*R*,6*S*)-4,5-Epoxy-17-methyl-7,8-didehydromorphinan-3,6-diol monohydrochloride trihydrate [6055-06-7]

Morphine Hydrochloride Hydrate contains not less than 98.0% and not more than 102.0% of morphine hydrochloride ($\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl}$: 321.80), calculated on the anhydrous basis.

Description Morphine Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in formic acid, soluble in water, sparingly soluble in methanol, and slightly soluble in ethanol (95).

It is colored by light.

Identification (1) Determine the absorption spectrum of a solution of Morphine Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Morphine Hydrochloride in dilute sodium hydroxide TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Morphine Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at

the same wave numbers.

(3) A solution of Morphine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-111 - -116^\circ$ (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water is between 4.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Morphine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 2 to 3 drops of barium chloride TS: no turbidity is produced.

(3) Meconic acid—Dissolve 0.20 g of Morphine Hydrochloride Hydrate in 5 mL of water, and add 5 mL of dilute hydrochloric acid and 2 drops of iron (III) chloride TS: no red color develops.

(4) Related substances—Dissolve 0.1 g of Morphine Hydrochloride Hydrate in 10 mL of diluted ethanol (95) (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ethanol (95) (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethanol (99.5), toluene, acetone and ammonia solution (28) (14:14:7:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 13 – 15% (0.1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Morphine Hydrochloride Hydrate, dissolve in 3.0 mL of formic acid, add 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), mix, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.18 mg of $C_{17}H_{19}NO_3 \cdot HCl$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Morphine Hydrochloride Injection

モルヒネ塩酸塩注射液

Morphine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$: 375.84).

Method of preparation Prepare as directed under Injec-

tions, with Morphine Hydrochloride Hydrate.

Description Morphine Hydrochloride Injection is a clear, colorless liquid.

It is affected by light.

pH: 2.5 – 5.0

Identification Take a volume of Morphine Hydrochloride Injection, equivalent to 0.04 g of Morphine Hydrochloride Hydrate according to the labeled amount, add water to make 20 mL, and use this solution as the sample solution. To 5 mL of the sample solution add water to make 100 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm. And to 5 mL of the sample solution add dilute sodium hydroxide TS to make 100 mL, and determine the absorption spectrum: it exhibits a maximum between 296 nm and 300 nm.

Extractable volume <6.05> It meets the requirement.

Assay Take exactly a volume of Morphine Hydrochloride Injection, equivalent to about 80 mg of morphine hydrochloride hydrate ($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$), and add water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

Amount (mg) of morphine hydrochloride
($C_{17}H_{19}NO_3 \cdot HCl \cdot 3H_2O$)
= $W_S \times (Q_T / Q_S) \times 4 \times 1.1679$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Morphine Hydrochloride Tablets

モルヒネ塩酸塩錠

Morphine Hydrochloride Tablets contain not less than 93% and not more than 107% of the labeled amount of morphine hydrochloride hydrate ($\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$; 375.84).

Method of preparation Prepare as directed under Tablets, with Morphine Hydrochloride Hydrate.

Identification Weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of water, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 283 nm and 287 nm. And weigh a quantity of powdered Morphine Hydrochloride Tablets equivalent to 0.01 g of Morphine Hydrochloride Hydrate, add 100 mL of dilute sodium hydroxide TS, shake for 10 minutes, and filter. Determine the absorption spectrum of the filtrate: it exhibits a maximum between 296 nm and 300 nm.

Assay Take not less than 20 Morphine Hydrochloride Tablets, weigh accurately, and powder. Weigh accurately a quantity of the powder, equivalent to about 20 mg of morphine hydrochloride hydrate ($\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}$), add exactly 10 mL of the internal standard solution, extract the mixture with ultrasonic waves for 10 minutes, and add water to make 50 mL. Filter this solution, and use the filtrate as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of morphine hydrochloride} \\ & (\text{C}_{17}\text{H}_{19}\text{NO}_3 \cdot \text{HCl} \cdot 3\text{H}_2\text{O}) \\ & = W_S \times (Q_T / Q_S) \times 1.1679 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Freeze-dried Live Attenuated Mumps Vaccine

乾燥弱毒生おたふくかぜワクチン

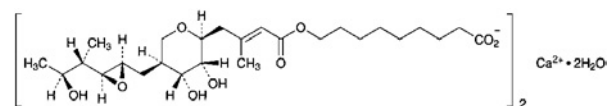
Freeze-dried Live Attenuated Mumps Vaccine is a dried preparation containing live attenuated mumps virus.

It conforms to the requirements of Freeze-dried Live Attenuated Mumps Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Live Attenuated Mumps Vaccine becomes a clear, colorless, yellowish or reddish liquid on addition of solvent.

Mupirocin Calcium Hydrate

ムピロシンカルシウム水和物



$\text{C}_{52}\text{H}_{86}\text{CaO}_{18} \cdot 2\text{H}_2\text{O}$: 1075.34

Monocalcium bis[9-((2*E*)-4-((2*S*,3*R*,4*R*,5*S*)-5-[(2*S*,3*S*,4*S*,5*S*)-2,3-epoxy-5-hydroxy-4-methylhexyl]-3,4-dihydroxy-3,4,5,6-tetrahydro-2*H*-pyran-2-yl)-3-methylbut-2-enoyloxy)nonanoate] dihydrate [115074-43-6]

Mupirocin Calcium Hydrate is the calcium salt of a substance having antibacterial activity produced by the growth of *Pseudomonas fluorescens*.

It contains not less than 895 μg (potency) and not more than 970 μg (potency) per mg, calculated on the anhydrous basis. The potency of Mupirocin Calcium Hydrate is expressed as mass (potency) of mupirocin ($\text{C}_{26}\text{H}_{44}\text{O}_9$; 500.62).

Description Mupirocin Calcium Hydrate occurs as a white powder and has a bitter taste.

It is freely soluble in methanol and slightly soluble in water and in ethanol (95).

Identification (1) To 1 mL of a solution of Mupirocin Calcium Hydrate in methanol (1 in 200) add 4 mL of hydroxylamine perchlorate-ethanol TS and 1 mL of *N,N'*-dicyclohexylcarbodiimide-ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate hexahydrate-ethanol TS to the solution, and shake: a dark purple color develops.

(2) Determine the absorption spectrum of a solution of Mupirocin Calcium Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 219 nm and 224 nm.

(3) Determine the infrared absorption spectrum of Mupirocin Calcium Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1708 cm^{-1} , 1648 cm^{-1} , 1558 cm^{-1} , 1231 cm^{-1} , 1151 cm^{-1} and 894 cm^{-1} .

(4) A solution of Mupirocin Calcium Hydrate (3 in 1000) responds to the Qualitative Tests <1.09> (3) for calcium salt.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: $-16 - -20^\circ$ (1 g calculated on the anhydrous basis, methanol, 20 mL, 100 mm).

Purity (1) Related substances—Dissolve 50 mg of Mupirocin Calcium Hydrate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make 10 mL, and use this solution as the sample solution (1). Pipet 2 mL of this solution, add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 100 mL, and use this solution as the sample solution (2). Preserve these sample solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution (1) and the sample solution (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas of each peak of the sample solution (1) and the sample solution (2) by the automatic integration method. Calculate the amount of the related substances by the following formula: the amount of principal related substance (appeared at about 0.7 of the relative retention time to mupirocin) is not more than 4.0%, and the total amount of related substances (the total area of the peaks other than of the solvent and mupirocin) is not more than 6.0%.

$$\begin{aligned} \text{Amount (\%)} & \text{ of principal related substance} \\ & = \frac{A_i}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_m}} \end{aligned}$$

$$\begin{aligned} \text{Total amount (\%)} & \text{ of related substances} \\ & = \frac{A}{A + A_m} \times 100 \times \frac{P \times 100}{100 - \frac{A \times 100}{A + A_m}} \end{aligned}$$

A: Total peak areas other than of the solvent and mupiro-

cin from the sample solution (1)

A_i: Peak area of the relative retention time of about 0.7 to mupirocin from the sample solution (1)

A_m: A value of 50 times of peak area of mupirocin from the sample solution (2)

P: Potency per mg obtained from the assay.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of mupirocin beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 1 mL of the sample solution (2), and add a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0, and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 20 mL. Confirm that the peak area of mupirocin obtained from 20 μL of this solution is equivalent to 4 to 6% of that obtained from 20 μL of the sample solution (2).

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the sample solution (2) under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 2.0%.

(2) Inorganic salt from manufacturing process—Being specified separately.

Water <2.48> Not less than 3.0% and not more than 4.5% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Mupirocin Calcium Hydrate and Mupirocin Lithium Reference Standard, equivalent to about 20 mg (potency), dissolve in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Preserve these solutions at a temperature between 4°C and 8°C. Perform the test with exactly 20 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, *A_T* and *A_S*, of mupirocin of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] & \text{ of mupirocin (C}_{26}\text{H}_{44}\text{O}_9) \\ & = W_s \times (A_T/A_S) \times 1000 \end{aligned}$$

W_s: Amount [mg (potency)] of Mupirocin Lithium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 7.71 g of ammonium acetate in 750 mL of water, adjust the pH to 5.7 with acetic acid (100), and add water to make 1000 mL. To 300 mL of this solution add 100 mL of tetrahydrofuran.

Flow rate: Adjust the flow rate so that the retention time of

mupirocin is about 12.5 minutes.

System suitability—

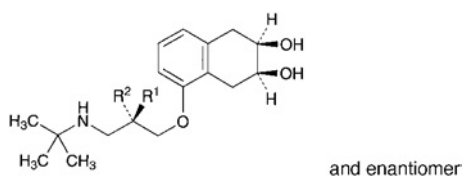
System performance: Dissolve about 20 mg of Mupirocin Lithium Reference Standard and about 5 mg of ethyl parahydroxybenzoate in a mixture of 0.1 mol/L acetic acid-sodium acetate buffer solution, pH 4.0 and a solution of tetrahydrofuran (3 in 4) (1:1) to make 200 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, mupirocin and ethyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of mupirocin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Nadolol

ナドロール



$C_{17}H_{27}NO_4$: 309.40

$R^1 = OH$, $R^2 = H$

(2*RS*,3*SR*)-5-[(2*SR*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol

$R^1 = H$, $R^2 = OH$

(2*RS*,3*SR*)-5-[(2*RS*)-3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol [42200-33-9]

Nadolol, when dried, contains not less than 98.0% of $C_{17}H_{27}NO_4$.

Description Nadolol occurs as a white to yellow-brownish white crystalline powder.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), and slightly soluble in water and in chloroform.

A solution of Nadolol in methanol (1 in 100) shows no optical rotation.

Melting point: about 137°C

Identification (1) Determine the absorption spectrum of a solution of Nadolol in methanol (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nadolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1585 cm^{-1} , 1460 cm^{-1} , 1092 cm^{-1} , 935 cm^{-1} and 770 cm^{-1} .

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of

Nadolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) **Related substances**—Dissolve 0.5 g of Nadolol in 10 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 100 μ L each of the sample solution and a mixture of methanol and chloroform (1:1) as a control solution with 25 mm each of width at an interval of about 10 mm on the starting line of a plate 0.25 mm in thickness of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform and diluted ammonia TS (1 in 3) (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm), and confirm the positions of the principal spot and the spots other than the principal spot from the sample solution. Scratch and collect the silica gel of the positions of the plate corresponding to the principal spot and the spots other than the principal spot. To the silica gel collected from the principal spot add exactly 30 mL of ethanol (95), and to the silica gel from the spots other than the principal spot add exactly 10 mL of ethanol (95). After shaking them for 60 minutes, centrifuge, and determine the absorbances of these supernatant liquids at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. Separately, proceed in the same manner with each position of the silica gel from the control solution corresponding to the principal spot and the spots other than the principal spot of the sample solution, and perform a blank determination to make correction. Amount of the related substances calculated by the following equation is not more than 2.0%.

$$\text{Amount (\%)} \text{ of related substances} = \{A_b / (A_b + 3A_a)\} \times 100$$

A_a : Corrected absorbance of the principle spot.

A_b : Corrected absorbance of the spots other than the principle spot.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Isomer ratio Prepare a paste with 0.01 g of Nadolol as directed in the paste method under Infrared Spectrophotometry <2.25> so that its transmittance at an absorption band at a wave number of about 1585 cm^{-1} is 25 to 30%, and determine the infrared absorption spectrum between 1600 cm^{-1} and 1100 cm^{-1} . Determine the absorbances, A_{1265} and A_{1250} , from the transmittances, T_{1265} and T_{1250} , at wave numbers of about 1265 cm^{-1} (racemic substance A) and 1250 cm^{-1} (racemic substance B), respectively: the ratio A_{1265}/A_{1250} is between 0.72 and 1.08.

Assay Weigh accurately about 0.28 g of Nadolol, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green-blue (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

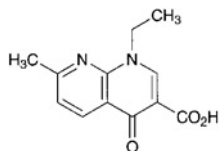
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 30.94 \text{ mg of } C_{17}H_{27}NO_4 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nalidixic Acid

ナリジクス酸



$C_{12}H_{12}N_2O_3$: 232.24

1-Ethyl-7-methyl-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid [389-08-2]

Nalidixic Acid, when dried, contains not less than 99.0% and not more than 101.0% of $C_{12}H_{12}N_2O_3$.

Description Nalidixic Acid occurs as white to light yellow crystals or crystalline powder.

It is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Nalidixic Acid in 0.01 mol/L sodium hydroxide TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nalidixic Acid, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 225 – 231°C

Purity (1) Chloride <1.03>—To 2.0 g of Nalidixic Acid add 50 mL of water, warm at 70°C for 5 minutes, cool quickly, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.012%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Nalidixic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Nalidixic Acid in 20 mL of 0.01 mol/L sodium hydroxide TS. Pipet 5 mL of this solution, add water to make exactly 10 mL, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than nalidixic acid with the sample solution is not larger than the peak area of nalidixic acid with the standard solution,

and the total area of the peaks other than the peak of nalidixic acid with the sample solution is not larger than 2.5 times the peak area of nalidixic acid with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 6.24 g of sodium dihydrogen phosphate dihydrate in 950 mL of water, adjust the pH to 2.8 with phosphoric acid, and add water to make 1000 mL. To 300 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nalidixic acid is about 19 minutes.

Time span of measurement: About 3 times as long as the retention time of nalidixic acid beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 5 mL of the standard solution, and add water to make exactly 10 mL. Confirm that the peak area of nalidixic acid obtained with 10 μ L of this solution is equivalent to 40 to 60% of that with 10 μ L of the standard solution.

System performance: Dissolve 25 mg of methyl parahydroxybenzoate in 100 mL of a mixture of water and methanol (1:1). To 1 mL of this solution add water to make 10 mL. To 5 mL of this solution add 5 mL of the standard solution. When the procedure is run with 10 μ L of this solution under the above operating conditions, methyl parahydroxybenzoate and nalidixic acid are eluted in this order with the resolution between these peaks being not less than 13.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nalidixic acid is not more than 2.0%.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

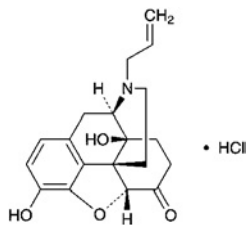
Assay Weigh accurately about 0.3 g of Nalidixic Acid, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L tetramethyl ammonium hydroxide VS (potentiometric titration). Separately, to 50 mL of *N,N*-dimethylformamide add 13 mL of a mixture of water and methanol (89:11), perform a blank determination with the solution, and make any necessary correction.

Each mL of 0.1 mol/L tetramethyl ammonium hydroxide VS = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Tight containers.

Naloxone Hydrochloride

ナロキソン塩酸塩



$C_{19}H_{21}NO_4 \cdot HCl$: 363.84
(5*R*,14*S*)-17-Allyl-4,5-epoxy-3,14-dihydroxymorphinan-6-one monohydrochloride [357-08-4]

Naloxone Hydrochloride contains not less than 98.5% of $C_{19}H_{21}NO_4 \cdot HCl$, calculated on the dried basis.

Description Naloxone Hydrochloride occurs as white to yellowish white, crystals or crystalline powder.

It is freely soluble in water, soluble in methanol, slightly soluble in ethanol (99.5) and in acetic acid (100), and very slightly soluble in acetic anhydride.

It is hygroscopic.

It is gradually colored by light.

Identification (1) Determine the absorption spectrum of a solution of Naloxone Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Naloxone Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Naloxone Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{25}$: $-170 - -181^\circ$ (0.25 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.10 g of Naloxone Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 4.5 and 5.5.

Purity Related substances—Conduct this procedure as rapidly as possible without exposure to light, using light-resistant containers. Dissolve 0.08 g of Naloxone Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of ammonia-saturated 1-butanol TS and methanol (20:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly iron (III) chloride-potassium hexacyanoferrate (III) TS on the

plate: the number of the spot other than the principal spot from the sample solution is not more than 1 and it is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% [0.1 g, 105°C, 5 hours. Use a desiccator (phosphorus (V) oxide) for cooling].

Residue on ignition <2.44> Not more than 0.2% (0.1 g).

Assay Weigh accurately about 0.3 g of Naloxone Hydrochloride, dissolve in 80 mL of acetic acid (100) by warming. After cooling, add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.38 mg of $C_{19}H_{21}NO_4 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naphazoline and Chlorpheniramine Solution

ナファゾリン・クロルフェニラミン液

Naphazoline and Chlorpheniramine Solution contains not less than 0.045 w/v% and not more than 0.055 w/v% of naphazoline nitrate ($C_{14}H_{14}N_2 \cdot HNO_3$: 273.29), and not less than 0.09 w/v% and not more than 0.11 w/v% of chlorpheniramine maleate ($C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$: 390.86).

Method of preparation

Naphazoline Nitrate	0.5 g
Chlorpheniramine Maleate	1 g
Chlorobutanol	2 g
Glycerin	50 mL
Purified Water	a sufficient quantity
To make 1000 mL	

Dissolve, and mix the above ingredients.

Description Naphazoline and Chlorpheniramine Solution is a clear, colorless liquid.

Identification (1) To 20 mL of Naphazoline and Chlorpheniramine Solution add 2 mL of a solution of potassium hydroxide (7 in 10) and 5 mL of pyridine, and heat at 100°C for 5 minutes: a red color is produced (chlorobutanol).

(2) Place 10 mL of Naphazoline and Chlorpheniramine Solution in a glass-stoppered test tube, add 10 mL of ethanol (95), 2 mL of sodium hydroxide TS and 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), and shake: a blue color is produced (glycerin).

(3) To 20 mL of Naphazoline and Chlorpheniramine Solution add 5 mL of sodium hydroxide TS, extract with 10 mL of diethyl ether, and separate the diethyl ether layer. Take 5 mL of this solution, distil off the solvent, dissolve the residue in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 0.01 g each of naphazoline nitrate and Chlorpheniramine Maleate Reference Standard in 10 mL and 5 mL of methanol, respectively, and use these solutions

as standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same *R_f* values as the spots from standard solutions (1) and (2). Spray evenly Dragendorff's TS on the plate: the spots from standard solutions (1) and (2) and the corresponding spot from the sample solutions reveal an orange color.

Assay Pipet 4 mL of Naphazoline and Chlorpheniramine Solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the sample solution. Weigh accurately about 50 mg of naphazoline nitrate for assay, dried at 105°C for 2 hours, and about 0.1 g of Chlorpheniramine Maleate Reference Standard, dried at 105°C for 3 hours, dissolve in water to make exactly 100 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, then add water to make 10 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak height of naphazoline nitrate and chlorpheniramine maleate to that of the internal standard of the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak height of naphazoline nitrate and chlorpheniramine maleate to that of the internal standard of the standard solution.

$$\begin{aligned} \text{Amount (mg) of naphazoline nitrate (C}_{14}\text{H}_{14}\text{N}_2\cdot\text{HNO}_3) \\ = W_{\text{Sa}} \times (Q_{\text{Ta}}/Q_{\text{Sa}}) \times (1/25) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of chlorpheniramine maleate} \\ (\text{C}_{16}\text{H}_{19}\text{ClN}_2\cdot\text{C}_4\text{H}_4\text{O}_4) \\ = W_{\text{Sb}} \times (Q_{\text{Tb}}/Q_{\text{Sb}}) \times (1/25) \end{aligned}$$

W_{Sa} : Amount (mg) of naphazoline nitrate for assay

W_{Sb} : Amount (mg) of Chlorpheniramine Maleate Reference Standard

Internal standard solution—A solution of ethenzamide in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column, about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of acetonitrile and a solution of sodium laurylsulfate (1 in 500) in diluted phosphoric acid (1 in 1000) (1:1).

Flow rate: Adjust the flow rate so that the retention time of chlorpheniramine maleate is about 10 minutes.

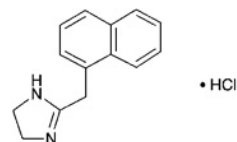
Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions. Use a column giving well-resolved peaks of the internal standard, naphazoline nitrate and chlorpheniramine maleate in this order.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Naphazoline Hydrochloride

ナファゾリン塩酸塩



$\text{C}_{14}\text{H}_{14}\text{N}_2\cdot\text{HCl}$: 246.74

2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole monohydrochloride [550-99-2]

Naphazoline Hydrochloride, when dried, contains not less than 98.5% of $\text{C}_{14}\text{H}_{14}\text{N}_2\cdot\text{HCl}$.

Description Naphazoline Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, soluble in ethanol (95) and in acetic acid (100), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: 255 – 260°C (with decomposition).

Identification (1) To 10 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 30 mL of a solution of Naphazoline Hydrochloride (1 in 100) add 2 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Evaporate the combined diethyl ether extracts to dryness with the aid of a current of air. Dry the residue at 80°C for 1 hour: the residue melts <2.60> between 117°C and 120°C.

(3) Dissolve 0.02 g of the residue obtained in (2) in 2 to 3 drops of dilute hydrochloric acid and 5 mL of water, and add 2 mL of Reinecke salt TS: a red-purple, crystalline precipitate is formed.

(4) A solution of Naphazoline Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.10 g of Naphazoline Hydrochloride in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Naphazoline Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Naphazoline Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

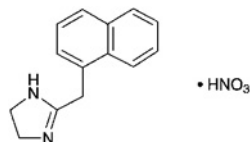
Each mL of 0.1 mol/L perchloric acid VS

= 24.67 mg of $C_{14}H_{14}N_2 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naphazoline Nitrate

ナファゾリン硝酸塩



$C_{14}H_{14}N_2 \cdot HNO_3$: 273.29
2-(Naphthalen-1-ylmethyl)-4,5-dihydro-1*H*-imidazole
mononitrate [5144-52-5]

Naphazoline Nitrate, when dried, contains not less than 98.5% of $C_{14}H_{14}N_2 \cdot HNO_3$.

Description Naphazoline Nitrate occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in water, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 10 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of bromine TS, and boil: a deep purple color develops.

(2) To 20 mL of a solution of Naphazoline Nitrate (1 in 100) add 5 mL of sodium hydroxide TS, and extract with two 25-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness with the aid of a current of air, and dry the residue at 80°C for 1 hour: the residue so obtained melts <2.60> between 117°C and 120°C.

(3) A solution of Naphazoline Nitrate (1 in 20) responds to the Qualitative Tests <1.09> for nitrate.

pH <2.54> Dissolve 0.1 g of Naphazoline Nitrate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 5.0 and 7.0.

Melting point <2.60> 167 – 170°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Naphazoline Nitrate in 50 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Naphazoline Nitrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Naphazoline Nitrate, previously dried, dissolve in 10 mL of acetic acid (100) and 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

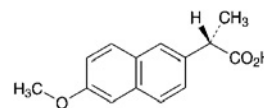
Each mL of 0.1 mol/L perchloric acid VS

= 27.33 mg of $C_{14}H_{14}N_2 \cdot HNO_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Naproxen

ナプロキセン



$C_{14}H_{14}O_3$: 230.26
(2*S*)-2-(6-Methoxynaphthalen-2-yl)propanoic acid
[22204-53-1]

Naproxen, when dried, contains not less than 98.5% of $C_{14}H_{14}O_3$.

Description Naproxen occurs as white crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in methanol, in ethanol (99.5) and in chloroform, sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.01 g of Naproxen in 5 mL of methanol, add 5 mL of water, then add 2 mL of potassium iodide TS and 5 mL of a solution of potassium iodate (1 in 100), and shake: a yellow to yellow-brown color develops. To this solution add 5 mL of chloroform, and shake: a light red-purple color develops in the chloroform layer.

(2) To 1 mL of a solution of Naproxen in ethanol (99.5) (1 in 300) add 4 mL of hydroxylamine perchlorate-dehydrated ethanol TS and 1 mL of *N,N'*-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a red-purple color develops.

(3) Determine the absorption spectrum of a solution of Naproxen in ethanol (99.5) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Naproxen, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{25}$: +63.0 – +68.5° (after drying, 0.1 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 154 – 158°C

Purity (1) Clarity of solution—Dissolve 2.0 g of Naproxen in 20 mL of acetone: the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 400 nm is not more than 0.070.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Naprox-

en according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Naproxen according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Naproxen in 10 mL of a mixture of chloroform and ethanol (99.5) (1:1), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 100 mL. Pipet 5 mL of this solution, add a mixture of chloroform and ethanol (99.5) (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane, dichloromethane, tetrahydrofuran and acetic acid (100) (50:30:17:3) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5g of Naproxen, previously dried, add 100 mL of diluted methanol (4 in 5), dissolve by gentle warming if necessary, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

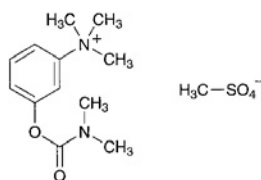
$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 23.03 \text{ mg of } C_{14}H_{14}O_3 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Neostigmine Methylsulfate

ネオスチグミンメチル硫酸塩



$C_{13}H_{22}N_2O_6S$: 334.39

3-(Dimethylcarbamoyloxy)-*N,N,N*-trimethylanilinium methyl sulfate [51-60-5]

Neostigmine Methylsulfate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{13}H_{22}N_2O_6S$.

Description Neostigmine Methylsulfate occurs as a white,

crystalline powder.

It is very soluble in water, and freely soluble in acetonitrile and in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Neostigmine Methylsulfate (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Neostigmine Methylsulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Neostigmine Methylsulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of dried Neostigmine Methylsulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of freshly boiled and cooled water: the pH of the solution is between 3.0 and 5.0.

Melting point <2.60> 145 – 149°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Neostigmine Methylsulfate in 10 mL of water: the solution is clear and colorless.

(2) Sulfate—Dissolve 0.20 g of Neostigmine Methylsulfate in 10 mL of water, add 1 mL of dilute hydrochloric acid and 1 mL of barium chloride TS: no turbidity is produced immediately.

(3) Dimethylaminophenol—Dissolve 0.10 g of Neostigmine Methylsulfate in 5 mL of water, add 1 mL of sodium hydroxide TS, and while cooling with ice, add 1 mL of diazobenzenesulfonic acid TS: no color develops.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Neostigmine Methylsulfate and Neostigmine Methylsulfate Reference Standard, previously dried, dissolve each in the mobile phase to make exactly 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of neostigmine in each solution.

$$\begin{aligned} \text{Amount (mg) of } C_{13}H_{22}N_2O_6S \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Neostigmine Methylsulfate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 259 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen-

phosphate dihydrate in 1000 mL of water, adjust to pH 3.0 with phosphoric acid, and add 0.871 g of sodium 1-pentanesulfonate to dissolve. To 890 mL of this solution add 110 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of neostigmine is about 9 minutes.

System suitability—

System performance: Dissolve 25 mg of Neostigmine Methylsulfate and 4 mg of dimethylaminophenol in 50 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, dimethylaminophenol and neostigmine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of neostigmine methylsulfate is not more than 1.0%.

Containers and storage Containers—Tight containers.

Neostigmine Methylsulfate Injection

ネオスチグミンメチル硫酸塩注射液

Neostigmine Methylsulfate Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$: 334.39).

Method of preparation Prepare as directed under Injections, with Neostigmine Methylsulfate.

Description Neostigmine Methylsulfate Injection is a clear, colorless liquid.

It is slowly affected by light.

pH: 5.0 – 6.5

Identification Take a volume of Neostigmine Methylsulfate Injection equivalent to 5 mg of neostigmine methylsulfate according to the labeled amount, add water to make 10 mL if necessary, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Extractable volume <6.05> It meets the requirement.

Bacterial endotoxins <4.01> Less than 5 EU/mg.

Assay Use Neostigmine Methylsulfate Injection as the sample solution. Separately, weigh accurately about 25 mg of Neostigmine Methylsulfate Reference Standard, previously dried at 105°C for 3 hours, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Neostigmine Methylsulfate.

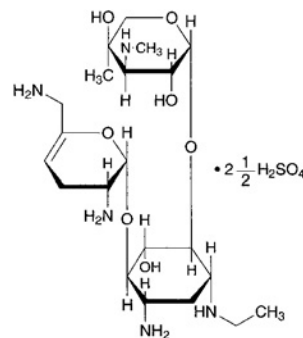
Amount (mg) of neostigmine methylsulfate ($C_{13}H_{22}N_2O_6S$)
 $= W_S \times (A_T/A_S)$

W_S : Amount (mg) of Neostigmine Methylsulfate Reference Standard

Containers and storage Containers—Hermetic containers.
 Storage—Light-resistant.

Netilmicin Sulfate

ネチルマイシン硫酸塩



$C_{21}H_{41}N_5O_7 \cdot 2\frac{1}{2}H_2SO_4$: 720.78

3-Deoxy-4-C-methyl-3-methylamino- β -L-arabinopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,3,4,6-tetrahydroxy- α -D-glycero-hex-4-enopyranosyl-(1 \rightarrow 4)]-2-deoxy-1-N-ethyl-D-streptamine hemipentasulfate
 [56391-57-2]

Netilmicin Sulfate is the sulfate of a derivative of sisomicin.

It contains not less than 595 μ g (potency) and not more than 720 μ g (potency) per mg, calculated on the dried basis. The potency of Netilmicin Sulfate is expressed as mass (potency) of netilmicin ($C_{21}H_{41}N_5O_7$: 475.58).

Description Netilmicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 30 mg of Netilmicin Sulfate in 3 mL of water, and add 0.2 mL of bromine TS: the solution is immediately decolorized.

(2) Dissolve 15 mg each of Netilmicin Sulfate and Netilmicin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 5 minutes: the principal spots from the sample solution and the standard solution exhibit a red-purple to red-brown color and show the same R_f value.

(3) A solution of Netilmicin Sulfate (1 in 100) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +88 – +96° (0.1 g calculated on the dried basis, water, 10 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of Netilmicin Sulfate in 5 mL of water: the pH of this solution is between 3.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Netilmicin Sulfate in 5 mL of water: the solution is clear and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Netilmicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Netilmicin Sulfate, calculated on the dried basis, in water to make 5 mL, and use this solution as the sample solution. Pipet 0.5 mL, 1 mL, and 1.5 mL of the sample solution, add water to each to make exactly 50 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (3), and the total amount of the intensity of the spots other than the principal spot from the sample solution is not more than 6%.

Loss on drying <2.41> Not more than 15.0% (0.15 g, in vacuum not exceeding 0.67 kPa, 110°C, 3 hours). Sampling should be carried out in a manner to avoid moisture absorption.

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Netilmicin Sulfate Reference Standard equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Netilmicin Sulfate equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentra-

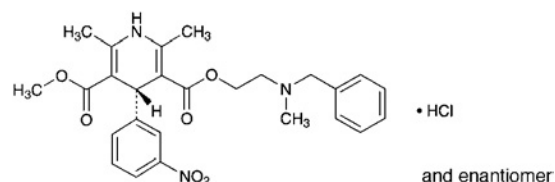
tion sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, not exceeding 5°C, under nitrogen or argon atmosphere.

Nicardipine Hydrochloride

ニカルジピン塩酸塩



$C_{26}H_{29}N_3O_6 \cdot HCl$: 515.99

2-[Benzyl(methyl)amino]ethyl methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate monohydrochloride [54527-84-3]

Nicardipine hydrochloride, when dried, contains not less than 98.5% of $C_{26}H_{29}N_3O_6 \cdot HCl$.

Description Nicardipine Hydrochloride occurs as a pale greenish yellow crystalline powder.

It is freely soluble in methanol and in acetic acid (100), sparingly soluble in ethanol (99.5), and slightly soluble in water, in acetonitrile and in acetic anhydride.

A solution of Nicardipine Hydrochloride in methanol (1 in 20) shows no optical rotation.

It is gradually affected by light.

Identification (1) Determine the absorption spectrum of a solution of Nicardipine Hydrochloride in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicardipine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.02 g of Nicardipine Hydrochloride in 10 mL of water and 3 mL of nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 167 – 171°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Nicardipine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 0.10 g of Nicardipine Hydrochloride in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 50 mL, then take exactly 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solu-

tion as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the area of each peak other than the peak of nicardipine from the sample solution is not larger than the peak area of nicardipine from the standard solution, and the total area of each peak other than the peak of nicardipine from the sample solution is not more than twice the peak area of nicardipine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of a solution of perchloric acid (43 in 50,000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 6 minutes.

Time span of measurement: About 4 times as long as the retention time of nicardipine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that of nicardipine obtained from 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Nicardipine Hydrochloride and nifedipine in 50 mL of the mobile phase. When the procedure is run with 10 μL of this solution under the above operating conditions, nicardipine and nifedipine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 3%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to day-light, using light-resistant vessels. Weigh accurately about 0.9 g of Nicardipine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.60 mg of $\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_6\cdot\text{HCl}$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicardipine Hydrochloride Injection

ニカルジピン塩酸塩注射液

Nicardipine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 93% and not more than 107% of the labeled amount of nicardipine hydrochloride ($\text{C}_{26}\text{H}_{29}\text{N}_3\text{O}_6\cdot\text{HCl}$; 515.99).

Method of preparation Prepare as directed under Injections, with Nicardipine Hydrochloride.

Description Nicardipine Hydrochloride Injection occurs as a clear pale yellow liquid.

It is gradually changed by light.

Identification To a volume of Nicardipine Hydrochloride Injection, equivalent to 1 mg of Nicardipine Hydrochloride according to the labeled amount, add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 235 nm and 239 nm, and between 351 nm and 355 nm.

pH <2.54> 3.0 – 4.5

Purity Related substances—Conduct the procedure without exposure to day-light using light-resistant vessels. To a volume of Nicardipine Hydrochloride Injection, equivalent to 5 mg of Nicardipine Hydrochloride according to the labeled amount, add the mobile phase to make 10 mL, and use this solution as the sample solution. To exactly 2 mL of the sample solution add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of these solutions by the automatic integration method: the areas of the peaks other than nicardipine from the sample solution are not more than the peak area of nicardipine from the standard solution, and the total of the areas of the peaks other than nicardipine from the sample solution is not more than 2 times of the peak area of nicardipine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of nicardipine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicardipine obtained from 10 μL of this solution is equivalent to 8 to 12% of that from the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 5 times with 10 μL of the standard solution under the above operating

conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Bacterial endotoxins <4.01> Less than 8.33 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay Conduct the procedure without exposure to day-light using light-resistant vessels. To an exact volume of Nicardipine Hydrochloride Injection, equivalent to about 2 mg of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$), add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of nicardipine hydrochloride for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of nicardipine to that of the internal standard.

Amount (mg) of nicardipine hydrochloride ($C_{26}H_{29}N_3O_6 \cdot HCl$)
 $= W_S \times (Q_T/Q_S) \times (1/25)$

W_S : Amount (mg) of nicardipine hydrochloride for assay

Internal standard solution—A solution of di-*n*-butyl phthalate in methanol (1 in 625).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogen phosphate in water to make 1000 mL. To 320 mL of this solution add 680 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicardipine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, nicardipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

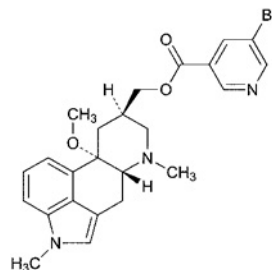
System repeatability: When the test is repeated 5 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of nicardipine is not more than 1.0%.

Containers and storage Containers—Hermetic containers. Colored containers may be used.

Storage—Light-resistant.

Nicergoline

ニセルゴリン



$C_{24}H_{26}BrN_3O_3$: 484.39

[(8*R*,10*S*)-10-Methoxy-1,6-dimethylergolin-8-yl]methyl
 5-bromopyridine-3-carboxylate
 [27848-84-6]

Nicergoline, when dried, contains not less than 98.5% and not more than 101.0% of $C_{24}H_{26}BrN_3O_3$.

Description Nicergoline occurs as white to light yellow, crystals or crystalline powder.

It is soluble in acetonitrile, in ethanol (99.5) and in acetic anhydride, and practically insoluble in water.

It is gradually colored to light brown by light.

Melting point: about 136°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Nicergoline in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nicergoline as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +5.2 – +6.2° (after drying, 0.5 g, ethanol (95), 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nicergoline according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 25 mg of Nicergoline in 25 mL of acetonitrile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetonitrile to make exactly 100 mL. Pipet 10 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak, having the relative retention time of about 0.5 with respect to nicergoline, is not larger than 4 times the peak area of nicergoline from the standard solution, and the area of the peak other than nicergoline and other than the peak mentioned above is not larger than 2.5 times the peak area of nicergoline from

the standard solution. The peak which area is larger than the peak area of nicergoline from the standard solution is not more than two peaks, and the total area of the peaks other than the peak of nicergoline is not larger than 7.5 times the peak area of nicergoline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add acetonitrile to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μ L of this solution is equivalent to 3 to 7% of that with 20 μ L of the solution for system suitability test.

System performance: When the procedure is run with 20 μ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 4.0%.

Loss on drying <2.41> Not more than 0.5% (2 g, in vacuum, 60°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Nicergoline, previously dried, add 10 mL of acetic anhydride, and warm to dissolve. After cooling, add 40 mL of nitrobenzene, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes to blue-green from red through a blue-purple (indicator: 10 drops of neutral red TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.22 mg of C₂₄H₂₆BrN₃O₃

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nicergoline Powder

ニセルゴリン散

Nicergoline Powder contains not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline (C₂₄H₂₆BrN₃O₃; 484.39).

Method of preparation Prepare as directed under Powders, with Nicergoline.

Identification Vigorously shake for 10 minutes a quantity of Nicergoline Powder, equivalent to 10 mg of Nicergoline according to the labeled amount, with 20 mL of diluted ethanol (4 in 5), and centrifuge for 10 minutes. To 2 mL of the supernatant liquid add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity Related substances—Perform the test with 20 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μ L of this solution is equivalent to 3 to 7% of that with 20 μ L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage unit <6.02> The Nicergoline Powder in single-unit container meets the requirement of the Mass variation test.

Particle size <6.03> It meets the requirements of Powders.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Weigh accurately a quantity of Nicergoline Powder, equivalent to about 5 mg of nicergoline (C₂₄H₂₆BrN₃O₃), and perform the test at 50 revolutions per minute according to the Paddle method using 900 mL of 2nd fluid for dissolution test

as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a laminated polyester fiber filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of nicergoline for assay, previously dried in vacuum at 60 °C for 2 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, and add 2nd fluid for dissolution test to make exactly 100 mL. Pipet 10 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances at 225 nm, A_{T1} and A_{S1} , and at 250 nm, A_{T2} and A_{S2} , of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$)

$$= (W_S/W_T) \times \{(A_{T1} - A_{T2}) / (A_{S1} - A_{S2})\} \times (1/C) \times 9$$

W_S : Amount (mg) of nicergoline for assay

W_T : Amount (g) of sample

C : Labeled amount (mg) of nicergoline ($C_{24}H_{26}BrN_3O_3$) in 1 g

Assay Weigh accurately a quantity of Nicergoline Powder, equivalent to about 20 mg of nicergoline ($C_{24}H_{26}BrN_3O_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60 °C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of nicergoline.

$$\begin{aligned} &\text{Amount (mg) of nicergoline (} C_{24}H_{26}BrN_3O_3 \text{)} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of nicergoline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not

more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nicergoline Tablets

ニセルゴリン錠

Nicergoline Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of nicergoline ($C_{24}H_{26}BrN_3O_3$; 484.39).

Method of preparation Prepare as directed under Tablets, with Nicergoline.

Identification Take a quantity of powdered Nicergoline Tablets, equivalent to 10 mg of Nicergoline according to the labeled amount, add 20 mL of ethanol (99.5), shake vigorously for 10 minutes, and filter through a 0.45- μ m pore-size membrane filter. To 2 mL of the filtrate add ethanol (99.5) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 226 nm and 230 nm, and between 286 nm and 290 nm.

Purity Related substances—Perform the test with 20 μ L of the sample solution obtained in the Assay as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of substances other than nicergoline by the area percentage method: the total amount of them is not more than 2.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of nicergoline beginning after the solvent peak.

System suitability—

Test for required detectability: To 1 mL of the standard solution obtained in the Assay add a mixture of acetonitrile and water (17:3) to make 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, add the mixture of acetonitrile and water (17:3) to make exactly 100 mL. Confirm that the peak area of nicergoline obtained with 20 μ L of this solution is equivalent to 3 to 7% of that with 20 μ L of the solution for system suitability test.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicergoline is not more than 1.5%.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

To 1 tablet of Nicergoline Tablets add exactly 25 mL of diluted ethanol (4 in 5), disperse to fine particles with the aid of ultrasonic wave, and shake for 5 minutes. Centrifuge this solution for 10 minutes, pipet exactly 4 mL of the supernatant liquid, add diluted ethanol (4 in 5) to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, and dissolve in exactly 25 mL of diluted ethanol (4 in 5). Pipet 4 mL of this solution, add diluted ethanol (4 in 5) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances at 288 nm, A_{T1} and A_{S1} , and at 340 nm, A_{T2} and A_{S2} , of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of nicergoline (C}_{24}\text{H}_{26}\text{BrN}_3\text{O}_3) \\ = W_S \times \{(A_{T1} - A_{T2}) / (A_{S1} - A_{S2})\} \times (1/2) \end{aligned}$$

W_S : Amount (mg) of nicergoline for assay

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Nicergoline Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of nicergoline ($\text{C}_{24}\text{H}_{26}\text{BrN}_3\text{O}_3$), add exactly 20 mL of a mixture of acetonitrile and water (17:3), vigorously shake for 10 minutes, centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of nicergoline for assay, previously dried in vacuum at 60°C for 2 hours, dissolve in exactly 20 mL of the mixture of acetonitrile and water (17:3), and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of nicergoline.

Amount (mg) of nicergoline ($\text{C}_{24}\text{H}_{26}\text{BrN}_3\text{O}_3$) = $W_S \times (A_T / A_S)$

W_S : Amount (mg) of nicergoline for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 288 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 7.0 with triethylamine. To 350 mL of this solution add 350 mL of methanol and 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nicergoline is about 25 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nicergoline are not less than 8000 and not more than 2.0, respectively.

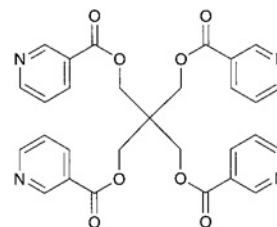
System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of

nicergoline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Niceritrol

ニセリトロール



$\text{C}_{29}\text{H}_{24}\text{N}_4\text{O}_8$: 556.52

Pentaerythritol tetranicotinate

[5868-05-3]

Niceritrol, when dried, contains not less than 99.0% of $\text{C}_{29}\text{H}_{24}\text{N}_4\text{O}_8$.

Description Niceritrol occurs as a white to pale yellowish white powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in chloroform, soluble in *N,N*-dimethylformamide, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Niceritrol in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Niceritrol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 162 – 165°C

Purity (1) Chloride <1.03>—To 2.0 g of Niceritrol add 50 mL of water, and warm at 70°C for 20 minutes, while shaking occasionally. After cooling, filter, and to 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Niceritrol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Niceritrol according to Method 3, and perform the test. Use 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Pyridine—Dissolve 0.5 g of Niceritrol in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pyridine, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N,N*-

dimethylformamide to make exactly 100 mL, then pipet 0.5 mL of this solution, add *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of pyridine in both solutions: the peak area of pyridine from the sample solution is not larger than the peak area of pyridine from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with polyethylene glycol 20M for gas chromatography coated at the ratio of 10% on acid-treated and silanized siliceous earth for gas chromatography (150 to 180 μ m in particle diameter).

Column temperature: A constant temperature of about 160°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of pyridine is about 2 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, the number of theoretical steps of the peak of pyridine is not less than 1500 steps.

System repeatability: When the test is repeated 6 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pyridine is not more than 3.0%.

(5) Free acids—Transfer about 1 g of Niceritrol, weighed accurately, to a separator, dissolve in 20 mL of chloroform, and extract with 20 mL and then 10 mL of water while shaking well. Combine the whole extracts, and titrate <2.50> with 0.01 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination, make any necessary correction, and calculate the amount of free acid by the following equation: it is not more than 0.1%.

$$\begin{aligned} \text{Each mL of 0.01 mol/L sodium hydroxide VS} \\ = 1.231 \text{ mg of } C_6H_5NO_2 \end{aligned}$$

(6) Related substances—Dissolve 0.10 g of Niceritrol in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet exactly 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Niceritrol, previously dried, add exactly 25 mL of 0.5 mol/L sodium hydroxide VS,

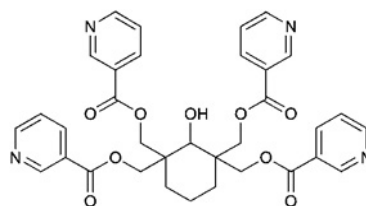
boil gently for 20 minutes under a reflux condenser with a carbon dioxide absorber (soda lime). After cooling, titrate <2.50> immediately the excess sodium hydroxide with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.5 mol/L sodium hydroxide VS} \\ = 69.57 \text{ mg of } C_{29}H_{24}N_4O_8 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Nicomol

ニコモール



$C_{34}H_{32}N_4O_9$: 640.64
(2-Hydroxycyclohexane-1,1,3,3-tetrayl)tetramethyl tetranicotinate [27959-26-8]

Nicomol, when dried, contains not less than 98.0% of $C_{34}H_{32}N_4O_9$.

Description Nicomol occurs as a white, crystalline powder. It is odorless and tasteless.

It is soluble in chloroform, and practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in dilute nitric acid.

Identification (1) Mix 0.01 g of Nicomol with 0.02 g of 1-chloro-2,4-dinitrobenzene, add 2 mL of dilute ethanol, heat in a water bath for 5 minutes, cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color develops.

(2) Dissolve 0.1 g of Nicomol in 5 mL of dilute hydrochloric acid, and add 5 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Nicomol in 1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nicomol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 181 – 185°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Nicomol in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Nicomol add 50 mL of freshly boiled and cooled water, shake for 5 minutes, filter, and to 25

mL of the filtrate add 0.60 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: a red color develops.

(3) Chloride <1.03>—Dissolve 0.6 g of Nicomol in 15 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.01 mol/L hydrochloric acid VS add 15 mL of dilute nitric acid and water to make 50 mL (not more than 0.024%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicomol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nicomol according to Method 3, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.20 g of Nicomol in 20 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 2 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, ethanol (95), acetonitrile and ethyl acetate (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1.5 g of Nicomol, previously dried, add exactly 40 mL of 0.5 mol/L sodium hydroxide VS, and boil gently under a reflux condenser connected to a carbon dioxide absorption tube (soda lime) for 10 minutes. After cooling, titrate <2.50> immediately the excess sodium hydroxide with 0.25 mol/L sulfuric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.5 mol/L sodium hydroxide VS} \\ = 80.08 \text{ mg of } C_{34}H_{32}N_4O_9 \end{aligned}$$

Containers and storage Containers—Tight containers.

Nicomol Tablets

ニコモール錠

Nicomol Tablets contain not less than 95% and not more than 105% of the labeled amount of nicomol ($C_{34}H_{32}N_4O_9$; 640.64).

Method of preparation Prepare as directed under Tablets, with Nicomol.

Identification To a portion of powdered Nicomol Tablets,

equivalent to 0.5 g of Nicomol according to the labeled amount, add 20 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Proceed with the residue as directed in the Identification (1) and (2) under Nicomol.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Nicomol Tablets at 75 revolutions per minute according to the Paddle method using 900 mL of the 1st fluid for dissolution test. Take 20 mL or more of the dissolved solution 60 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.8 μ m. Discard the first 10 mL of the filtrate, pipet 2 mL of the subsequent, add the 1st fluid for dissolution test to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in the 1st fluid for dissolution test to make exactly 100 mL, then pipet 2 mL of this solution, add the 1st fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Nicomol Tablets in 60 minutes is not less than 75%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the labeled} \\ \text{amount of nicomol (} C_{34}H_{32}N_4O_9 \text{)} \\ = W_S \times (A_T/A_S) \times (1/C) \times 225 \end{aligned}$$

W_S : Amount (mg) of nicomol for assay

C : Labeled amount (mg) of nicomol ($C_{34}H_{32}N_4O_9$) in 1 tablet

Assay Weigh accurately not less than 20 Nicomol Tablets and powder. Weigh accurately a portion of the powder, equivalent to about 1 g of nicomol ($C_{34}H_{32}N_4O_9$), add 100 mL of 1 mol/L hydrochloric acid TS, shake well, add water to make exactly 500 mL, and filter. Discard the first 50 mL of the filtrate, pipet 2 mL of the subsequent filtrate, add 50 mL of 1 mol/L hydrochloric acid TS and water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of nicomol for assay, previously dried at 105°C for 4 hours, dissolve in 50 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add 20 mL of 1 mol/L hydrochloric acid TS and water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 262 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

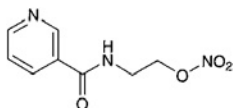
$$\begin{aligned} \text{Amount (mg) of nicomol (} C_{34}H_{32}N_4O_9 \text{)} \\ = W_S \times (A_T/A_S) \times (25/2) \end{aligned}$$

W_S : Amount (mg) of nicomol for assay

Containers and storage Containers—Tight containers.

Nicorandil

ニコランジル

C₈H₉N₃O₄: 211.17*N*-[2-(Nitrooxy)ethyl]pyridine-3-carboxamide [65141-46-0]

Nicorandil contains not less than 98.5% and not more than 101.0% of C₈H₉N₃O₄, calculated on the anhydrous basis.

Description Nicorandil occurs as white crystals.

It is freely soluble in methanol, in ethanol (99.5) and in acetic acid (100), soluble in acetic anhydride, and sparingly soluble in water.

Melting point: about 92°C (with decomposition).

Identification Determine the infrared absorption spectrum of Nicorandil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Sulfate <1.14>—Dissolve 2.0 g of Nicorandil in 20 mL of dilute ethanol, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of dilute ethanol and 1 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.010%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Nicorandil according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Nicorandil in 10 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of *N*-(2-hydroxyethyl)isonicotinamide nitric ester, having the relative retention time of about 0.86 with respect to nicorandil, is not more than 0.5% of the peak area of nicorandil, the area of all other peaks is less than 0.1%, and the sum area of the peaks other than nicorandil and *N*-(2-hydroxyethyl)isonicotinamide nitric ester is not more than 0.25% of the total peak area.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran, triethylamine and trifluoroacetic acid (982:10:5:3).

Flow rate: Adjust the flow rate so that the retention time of nicorandil is about 18 minutes.

Time span of measurement: About 3 times as long as the retention time of nicorandil beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 500 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of nicorandil obtained with 10 μL of this solution is equivalent to 2 to 8% of that with 10 μL of the solution for system suitability test.

System performance: Dissolve 10 mg of *N*-(2-hydroxyethyl)isonicotinamide nitric ester in the mobile phase to make 100 mL. To 1 mL of this solution add 10 mL of the sample solution. When the procedure is run with this solution under the above operating conditions, *N*-(2-hydroxyethyl)isonicotinamide nitric ester and nicorandil are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of nicorandil is not more than 1.5%.

Water <2.48> Not more than 0.1% (2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nicorandil, dissolve in 30 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

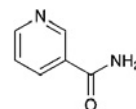
Each mL of 0.1 mol/L perchloric acid VS
= 21.12 mg of C₈H₉N₃O₄

Containers and storage Containers—Tight containers.

Storage—At a temperature between 2°C and 8°C.

Nicotinamide

ニコチン酸アミド

C₆H₆N₂O: 122.12

Pyridine-3-carboxamide [98-92-0]

Nicotinamide, when dried, contains not less than 98.5% and not more than 102.0% of C₆H₆N₂O.

Description Nicotinamide occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

Nicotinamide is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

Identification (1) Mix 5 mg of Nicotinamide with 0.01 g of 1-chloro-2,4-dinitrobenzene, heat gently for 5 to 6 seconds, and fuse the mixture. Cool, and add 4 mL of potassi-

um hydroxide-ethanol TS: a red color is produced.

(2) To 0.02 g of Nicotinamide add 5 mL of sodium hydroxide TS, and boil carefully: the gas evolved turns moistened red litmus paper blue.

(3) Dissolve 0.02 g of Nicotinamide in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinamide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH <2.54> Dissolve 1.0 g of Nicotinamide in 20 mL of water: the pH of this solution is between 6.0 and 7.5.

Melting point <2.60> 128 – 131°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Nicotinamide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Take 0.5 g of Nicotinamide, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Take 1.0 g of Nicotinamide, and perform the test. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.019%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Nicotinamide according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(5) Readily carbonizable substances <1.15>—Take 0.20 g of Nicotinamide, and perform the test. The solution has no more color than Matching Fluid A.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Nicotinamide and Nicotinamide Reference Standard, both previously dried, dissolve separately in 3 mL of water, and add the mobile phase to make them exactly 100 mL. Pipet 8 mL each of these solutions, and add the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of nicotinamide to that of the internal standard.

$$\text{Amount (g) of nicotinamide (C}_6\text{H}_6\text{N}_2\text{O)} = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of dried Nicotinamide Reference Standard

Internal standard solution—A solution of nicotinic acid (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle di-

ameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1 g of sodium 1-heptane sulfonate in water to make 1000 mL. To 700 mL of this solution add 300 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of nicotinamide is about 7 minutes.

System suitability—

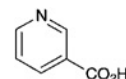
System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, nicotinic acid and nicotinamide are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nicotinamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Nicotinic Acid

ニコチン酸



$\text{C}_6\text{H}_5\text{NO}_2$: 123.11

Pyridine-3-carboxylic acid [59-67-6]

Nicotinic Acid, when dried, contains not less than 99.5% of $\text{C}_6\text{H}_5\text{NO}_2$.

Description Nicotinic Acid occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is sparingly soluble in water, slightly soluble in ethanol (95), and very slightly soluble in diethyl ether.

It dissolves in sodium hydroxide TS and in sodium carbonate TS.

Identification (1) Triturate 5 mg of Nicotinic Acid with 0.01 g of 1-chloro-2,4-dinitrobenzene, and fuse the mixture by gentle heating for 5 to 6 seconds. Cool, and add 4 mL of potassium hydroxide-ethanol TS: a dark red color is produced.

(2) Dissolve 0.02 g of Nicotinic Acid in water to make 1000 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nicotinic Acid Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

pH <2.54> Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the pH of this solution is between 3.0 and 4.0.

Melting point <2.60> 234 – 238°C

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Nicotinic Acid in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of Nico-

tinic Acid. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 1.0 g of Nicotinic Acid in 3 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS and 3 mL of dilute hydrochloric acid, and dilute with water to make 50 mL (not more than 0.019%).

(4) Nitro compounds—Dissolve 1.0 g of Nicotinic Acid in 8 mL of sodium hydroxide TS, and add water to make 20 mL: the solution has no more color than Matching Fluid A.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Nicotinic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 1 hour).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nicotinic Acid, previously dried, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 12.31 mg of C₆H₅NO₂

Containers and storage Containers—Well-closed containers.

Nicotinic Acid Injection

ニコチン酸注射液

Nicotinic Acid Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 110% of the labeled amount of nicotinic acid (C₆H₅NO₂; 123.11).

Method of preparation Prepare as directed under Injections, with Nicotinic Acid. It may contain Sodium Carbonate or Sodium Hydroxide as a solubilizer.

Description Nicotinic Acid Injection is a clear, colorless liquid.

pH: 5.0 – 7.0

Identification (1) To a volume of Nicotinic Acid Injection, equivalent to 0.1 g of Nicotinic Acid according to the labeled amount, add 0.3 mL of dilute hydrochloric acid, and evaporate on a water bath to 2 mL. After cooling, collect the crystals formed, wash with small portions of ice-cold water until the last washing shows no turbidity on the addition of silver nitrate TS, and dry at 105°C for 1 hour: the crystals melt <2.60> between 234°C and 238°C. With the crystals, proceed as directed in the Identification (1) under Nicotinic Acid.

(2) Dissolve 0.02 g of the dried crystals obtained in (1) in water to make 1000 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 261 nm and 263 nm, and a minimum between 235 nm and 239 nm. Separately, de-

termine the absorbances of this solution, A_1 and A_2 , at each wavelength of maximum and minimum absorption, respectively: the ratio A_2/A_1 is between 0.35 and 0.39.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Nicotinic Acid Injection, equivalent to about 0.1 g of nicotinic acid (C₆H₅NO₂), and add the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Nicotinic Acid Reference Standard, previously dried at 105°C for 1 hour, and dissolve in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of nicotinic acid to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of nicotinic acid (C}_6\text{H}_5\text{NO}_2\text{)} \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Nicotinic Acid Reference Standard

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.1 g of sodium 1-octane sulfonate in a mixture of 0.05 mol/L sodium dihydrogenphosphate TS, pH 3.0 and methanol (4:1) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 9 minutes.

System suitability—

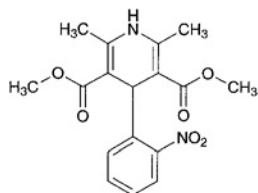
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, nicotinic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of nicotinic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Nifedipine

ニフェジピン

C₁₇H₁₈N₂O₆; 346.33

Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [21829-25-4]

Nifedipine contains not less than 98.0% and not more than 102.0% of C₁₇H₁₈N₂O₆, calculated on the dried basis.

Description Nifedipine occurs as a yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetone and in dichloromethane, sparingly soluble in methanol, in ethanol (95) and in acetic acid (100), slightly soluble in diethyl ether, and practically insoluble in water.

It is affected by light.

Identification (1) Dissolve 0.05 g of Nifedipine in 5 mL of ethanol (95), and add 5 mL of hydrochloric acid and 2 g of zinc powder. Allow to stand for 5 minutes, and filter. Perform the test with the filtrate as directed under Qualitative Tests <1.09> for primary aromatic amines: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Nifedipine in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Nifedipine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 172 – 175°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Nifedipine in 5 mL of acetone: the solution is clear and yellow.

(2) Chloride <1.03>—To 2.5 g of Nifedipine add 12 mL of dilute acetic acid and 13 mL of water, and heat to boil. After cooling, filter, and discard the first 10 mL of the filtrate. To 5 mL of the subsequent filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—To 4 mL of the filtrate obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution.

Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.054%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Nifedipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nifedipine according to Method 3, and perform the test (not more than 2 ppm).

(6) Basic substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 5.0 g of Nifedipine in 80 mL of a mixture of acetone and acetic acid (100) (5:3), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction. Not more than 1.9 mL of 0.02 mol/L perchloric acid VS is consumed.

(7) Dimethyl-2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridinedicarboxylate—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.15 g of Nifedipine in dichloromethane to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of dimethyl 2,6-dimethyl-4-(2-nitrosophenyl)-3,5-pyridine-dicarboxylate for thin-layer chromatography in exactly 10 mL of dichloromethane. Measure exactly 1 mL of this solution, add dichloromethane to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution, corresponding to that from the standard solution, is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay The procedure should be performed under protection from direct sunlight in light-resistant vessels. Weigh accurately about 0.12 g of Nifedipine, and dissolve in methanol to make exactly 200 mL. Measure exactly 5 mL of this solution, and add methanol to make exactly 100 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 350 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

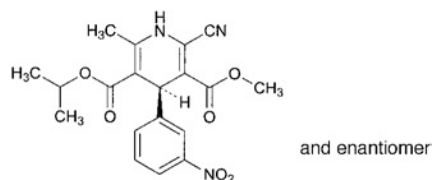
$$\begin{aligned} \text{Amount (mg) of C}_{17}\text{H}_{18}\text{N}_2\text{O}_6 \\ = (A/142.3) \times 40,000 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nilvadipine

ニルバジピン



$C_{19}H_{19}N_3O_6$; 385.37

3-Methyl 5-(1-methylethyl) (4*RS*)-2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate [75530-68-6]

Nilvadipine contains not less than 98.0% and not more than 102.0% of $C_{19}H_{19}N_3O_6$.

Description Nilvadipine occurs as a yellow crystalline powder.

It is freely soluble in acetonitrile, soluble in methanol, sparingly soluble in ethanol (99.5), and practically insoluble in water.

A solution of Nilvadipine in acetonitrile (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Nilvadipine in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nilvadipine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nilvadipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Nilvadipine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 167 – 171°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nilvadipine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 20 mg of Nilvadipine in 20 mL of acetonitrile, and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount of each related substance is not more than 0.3%, and the total of them is not more than 0.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (32:27:18).

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nilvadipine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. Confirm that the peak area of nilvadipine obtained from 5 μ L of this solution is equivalent to 7 to 13% of that obtained from 5 μ L of the solution for system suitability test.

System performance: When the procedure is run with 5 μ L of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine is not less than 3300 and not more than 1.3, respectively.

System repeatability: Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 10 mL. When the test is repeated 6 times with 5 μ L of this solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Loss on drying <2.41> Not more than 0.1% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg each of Nilvadipine and Nilvadipine Reference Standard, dissolve in methanol to make exactly 25 mL. Pipet 10 mL each of these solutions, add exactly 20 mL of the internal standard solution, 20 mL of water and methanol to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 5 μ L each of the sample solution and standard solution as directed under the Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of nilvadipine to that of the internal standard.

$$\text{Amount (mg) of } C_{19}H_{19}N_3O_6 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Nilvadipine Reference Standard

Internal standard solution—A solution of acenaphthene in methanol (1 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phos-

phoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 5 μL of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nilvadipine Tablets

ニルバジピン錠

Nilvadipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nilvadipine ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_6$; 385.37).

Method of preparation Prepare as directed under Tablets, with Nilvadipine.

Identification To a quantity of powdered Nilvadipine Tablets, equivalent to 1 mg of Nilvadipine according to the labeled amount, add 100 mL of ethanol (99.5), shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Determine the absorption spectrum of the sample solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 239 nm and 243 nm and a maximum having a broad-ranging absorption between 371 nm and 381 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Nilvadipine Tablets add V mL of a mixture of acetonitrile and water (7:3) so that each mL of the solution contains about 0.2 mg of nilvadipine ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_6$) according to the labeled amount, add exactly V mL of the internal standard solution, and disperse the particles with the aid of ultrasonic waves. Centrifuge for 10 minutes, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine Reference Standard, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

$$\begin{aligned} & \text{Amount (mg) of nilvadipine (C}_{19}\text{H}_{19}\text{N}_3\text{O}_6) \\ & = W_S \times (Q_T/Q_S) \times (V/100) \end{aligned}$$

W_S : Amount (mg) of Nilvadipine Reference Standard

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Dissolution <6.10> Perform the test according to the follow-

ing method: it meets the requirement.

Perform the test with 1 tablet of Nilvadipine Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding 0.5 μm . Discard the first 10 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add exactly 1 mL of methanol, and use this solution as the sample solution. Separately, weigh accurately an amount of Nilvadipine Reference Standard, equivalent to 10 times the labeled amount of Nilvadipine Tablets, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 10 mL of water, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of nilvadipine: the dissolution rate in 30 minutes is not less than 85%.

Dissolution rate (%) with respect to the labeled amount of nilvadipine ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_6$)

$$= W_S \times (A_T/A_S) \times (1/C) \times 9$$

W_S : Amount (mg) of Nilvadipine Reference Standard

C : Labeled amount (mg) of nilvadipine ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 242 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of phosphate buffer solution, pH 7.4, methanol and acetonitrile (7:7:6)

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nilvadipine are not less than 2000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nilvadipine is not more than 1.5%.

Assay Weigh accurately not less than 20 Nilvadipine Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 5 mg of nilvadipine ($\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_6$), add 10 mL of a mixture of acetonitrile and water (7:3) and exactly 25 mL of the internal standard solution, shake for 15 minutes, and add the mixture of acetonitrile and water (7:3) to make 50 mL. Centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 20 mg of Nilvadipine Reference Standard, dissolve in the mixture of acetonitrile and water (7:3) to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 25 mL of the internal standard solution and the mixture of

acetonitrile and water (7:3) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of nilvadipine to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of nilvadipine (C}_{19}\text{H}_{19}\text{N}_3\text{O}_6) \\ = W_S \times (Q_T/Q_S) \times (1/4) \end{aligned}$$

W_S : Amount (mg) of Nilvadipine Reference Standard

Internal standard solution—A solution of acenaphthene in acetonitrile (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.5 g of diammonium hydrogen phosphate in 1000 mL of water, add 10 mL of tetrabutylammonium hydroxide TS, adjust the pH to 7.0 with diluted phosphoric acid (1 in 10), and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of nilvadipine is about 12 minutes.

System suitability—

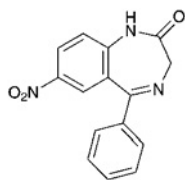
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, nilvadipine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of nilvadipine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Nitrazepam

ニトラゼパム



$\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_3$; 281.27

7-Nitro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
[146-22-5]

Nitrazepam, when dried, contains not less than 99.0% of $\text{C}_{15}\text{H}_{11}\text{N}_3\text{O}_3$.

Description Nitrazepam occurs as white to yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetic acid (100), soluble in acetone

and in chloroform, slightly soluble in methanol, in ethanol (95) and in ethanol (99.5), very slightly soluble in diethyl ether, and practically insoluble in water.

Melting point: about 227°C (with decomposition).

Identification (1) To 3 mL of a solution of Nitrazepam in methanol (1 in 500) add 0.1 mL of sodium hydroxide TS: a yellow color is produced.

(2) To 0.02 g of Nitrazepam add 15 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and filter: the filtrate responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Neutralize 0.5 mL of the filtrate obtained in (2) with sodium hydroxide TS, add 2 mL of ninhydrin TS, and heat on a water bath: a purple color is produced.

(4) Determine the absorption spectrum of a solution of Nitrazepam in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Nitrazepam in 20 mL of acetone: the solution is clear and pale yellow to light yellow in color.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Nitrazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nitrazepam according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Nitrazepam in a 10 mL of mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and chloroform (1:1) to make exactly 20 mL, pipet 2 mL of this solution, add a mixture of methanol and chloroform (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of nitromethane and ethyl acetate (17:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Nitrazepam, previously dried, and dissolve in 40 mL of acetic acid (100). Titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

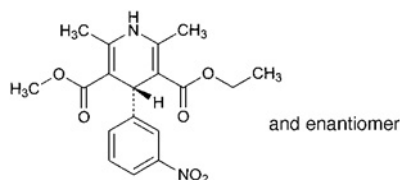
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 28.13 \text{ mg of C}_{15}\text{H}_{11}\text{N}_3\text{O}_3 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nitrendipine

ニトレンジピン



$C_{18}H_{20}N_2O_6$; 360.36

3-Ethyl 5-methyl (4*RS*)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
[39562-70-4]

Nitrendipine, when dried, contains not less than 98.5% and not more than 101.0% of $C_{18}H_{20}N_2O_6$.

Description Nitrendipine occurs as a yellow crystalline powder.

It is soluble in acetonitrile, sparingly soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually colored to brownish yellow by light.

A solution of Nitrendipine in acetonitrile (1 in 50) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Nitrendipine in methanol (1 in 80,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Nitrendipine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 157 – 161°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Nitrendipine according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure rapidly using light-resistant vessels. Dissolve 40 mg of Nitrendipine in 5 mL of acetonitrile, add the mobile phase to make 25 mL, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test immediately with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area by the automatic integration method, and calculate the amount of related substances by the following equation: the amount of a related substance, having the relative retention time of about 0.8 with respect to nitrendipine, is not more than 1.0%, a related substance, having the relative retention time of about 1.3, is not more than 0.25%, and other related substances are not more than 0.2%, respectively. The total amount of the substances other

than nitrendipine is not more than 2.0%.

$$\text{Amount (\% of related substance)} = A_T/A_S$$

A_T : Each peak area other than nitrendipine obtained from the sample solution

A_S : Peak area of nitrendipine obtained from the standard solution

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5).

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of nitrendipine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of nitrendipine obtained with 10 μ L of this solution is equivalent to 14 to 26% of that with 10 μ L of the standard solution.

System performance: Dissolve 10 mg of Nitrendipine and 3 mg of propyl parahydroxybenzoate in 5 mL of acetonitrile, and add the mobile phase to make 100 mL. When the procedure is run with 5 μ L of this solution under the above operating conditions, propyl parahydroxybenzoate and nitrendipine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Nitrendipine, previously dried, dissolve in 60 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 100), add 50 mL of water, and titrate <2.50> with 0.1 mol/L serium (IV) tetraammonium sulfate VS until the red-orange color of the solution vanishes (indicator: 3 drops of 1,10-phenanthroline TS). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L serium (IV) tetraammonium sulfate VS
= 18.02 mg of $C_{18}H_{20}N_2O_6$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nitrendipine Tablets

ニトレンジピン錠

Nitrendipine Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$; 360.36).

Method of preparation Prepare as directed under Tablets, with Nitrendipine.

Identification Shake a quantity of powdered Nitrendipine Tablets, equivalent to 5 mg of Nitrendipine according to the labeled amount, with 70 mL of methanol, then add methanol to make 100 mL, and centrifuge. To 5 mL of the supernatant liquid add methanol to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, and between 350 nm and 354 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure using light-resistant vessels. To 1 tablet of Nitrendipine Tablets add 15 mL of diluted acetonitrile (4 in 5), stir until the tablet is completely disintegrated, and further stir for 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 20 mL, and centrifuge. Pipet V mL of the supernatant liquid, equivalent to about 1 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 5 mL of the internal standard solution, then add diluted acetonitrile (4 in 5) to make 25 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} & \text{Amount (mg) of nitrendipine (C}_{18}\text{H}_{20}\text{N}_2\text{O}_6\text{)} \\ & = W_S \times (Q_T/Q_S) \times (1/V) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of nitrendipine for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Conduct this procedure using light-resistant vessels. Perform the test with 1 tablet of Nitrendipine Tablets at 100 revolutions per minute according to the Paddle method, using 900 mL of the dissolution medium (a solution of polysorbate 80 (3 in 5000) for a 5-mg tablet, and a solution of polysorbate 80 (3 in 2000) for a 10-mg tablet). Withdraw 20 mL or more of the dissolution medium 45 minutes after starting the test, and filter through a membrane filter with a pore size not exceeding $0.45 \mu\text{m}$. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add the dissolution medium to make exactly V' mL so that each mL contains about $5.6 \mu\text{g}$ of nitrendipine ($C_{18}H_{20}N_2O_6$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of nitrendipine for assay, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 100 mL, then pipet 5 mL of this solution, and add the dissolution medium to make exactly 50 mL. Pipet 5 mL of this solution, add the dissolution medium to make exactly 25 mL, and use this solution as the standard solution.

Perform the test with exactly $20 \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of nitrendipine. The dissolution rate in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of nitrendipine ($C_{18}H_{20}N_2O_6$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S : Amount (mg) of nitrendipine for assay

C : Labeled amount (mg) of nitrendipine ($C_{18}H_{20}N_2O_6$) in 1 tablet

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 356 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5 \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 25°C .

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5)

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 9 minutes.

System suitability—

System performance: When the procedure is run with $20 \mu\text{L}$ of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of nitrendipine are not less than 5000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $20 \mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 2.0%.

Assay Conduct this procedure using light-resistant vessels. To 20 tablets of Nitrendipine Tablets add 150 mL of diluted acetonitrile (4 in 5), stir until the tablets completely disintegrate, and stir for further 10 minutes. Add diluted acetonitrile (4 in 5) to make exactly 200 mL, and centrifuge. Pipet a volume of the supernatant liquid, equivalent to about 2 mg of nitrendipine ($C_{18}H_{20}N_2O_6$), add exactly 10 mL of the internal standard solution and diluted acetonitrile (4 in 5) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of nitrendipine for assay, previously dried at 105°C for 2 hours, and dissolve in diluted acetonitrile (4:5) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 10 mL of the internal standard solution and diluted acetonitrile (4:5) to make 50 mL, and use this solution as the standard solution. Perform the test with $10 \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of nitrendipine to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of nitrendipine (C}_{18}\text{H}_{20}\text{N}_2\text{O}_6\text{)} \\ & = W_S \times (Q_T/Q_S) \times (1/50) \end{aligned}$$

W_S : Amount (mg) of nitrendipine for assay

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted acetonitrile (4 in 5) (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25° C.

Mobile phase: A mixture of water, tetrahydrofuran and acetonitrile (14:6:5)

Flow rate: Adjust the flow rate so that the retention time of nitrendipine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and nitrendipine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of nitrendipine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nitrogen

窒素

N₂: 28.01

Nitrogen contains not less than 99.5 vol% of N₂.

Description Nitrogen is a colorless gas and is odorless.

Nitrogen (1 mL) dissolves in 65 mL of water and in 9 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

Nitrogen (1000 mL) at 0°C and at a pressure of 101.3 kPa weighs about 1.251 g.

It is inert and does not support combustion.

Identification The flame of a burning wood splinter is extinguished immediately in an atmosphere of Nitrogen.

Purity Carbon dioxide—Maintain the containers of Nitrogen at a temperature between 18°C and 22°C for more than 6 hours before the test, and correct the volume to be at 20°C and 101.3 kPa.

Pass 1000 mL of Nitrogen into 50 mL of barium hydroxide TS in a Nessler tube during 15 minutes through a delivery tube with an orifice approximately 1 mm in diameter, keeping the end of the tube at a distance of 2 mm from the bottom of the Nessler tube: any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

Assay Collect the sample as directed under Purity. Introduce 1.0 mL of Nitrogen into a gas-measuring tube or syringe for gas chromatography from a metal cylinder with a pressure-reducing valve, through a directly connected poly-

vinyl chloride tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak area A_T of oxygen. Separately, introduce 1.0 mL of oxygen into the gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard gas mixture. Proceed with 1.0 mL of this mixture in the same manner under Nitrogen, and measure the peak area A_S of oxygen.

$$\text{Amount (vol\%)} \text{ of N}_2 = 100 - (A_T/A_S)$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with zeolite for gas chromatography (250 to 350 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium

Flow rate: Adjust the flow rate so that the retention time of oxygen is about 3 minutes.

Selection of column: Introduce 1.0 mL of oxygen into the gas mixer, add Nitrogen to make 100 mL, and mix thoroughly. Proceed with 1.0 mL of this mixture under the above operating conditions. Use a column giving well-resolved peaks of oxygen and nitrogen in this order.

System repeatability: Repeat the test 5 times according to the above conditions with the standard gas mixture. Relative standard deviation of peak area of oxygen is not more than 2.0%.

Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

Nitroglycerin Tablets

ニトログリセリン錠

Nitroglycerin Tablets contain not less than 80% and not more than 120% of the labeled amount of nitroglycerin (C₃H₅N₃O₉; 227.09).

Method of preparation Prepare as directed under Tablets, with nitroglycerin.

Identification (1) Weigh a quantity of powdered Nitroglycerin Tablets, equivalent to 6 mg of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, shake thoroughly with 12 mL of diethyl ether, filter, and use the filtrate as the sample solution. Evaporate 5 mL of the sample solution, dissolve the residue in 1 to 2 drops of sulfuric acid, and add 1 drop of diphenylamine TS: a deep blue color develops.

(2) Evaporate 5 mL of the sample solution obtained in (1), add 5 drops of sodium hydroxide TS, heat over a low flame, and concentrate to about 0.1 mL. Cool, heat the residue with 0.02 g of potassium hydrogen sulfate: the odor of acrolein is perceptible.

Purity Free nitrate ion—Transfer an accurately measured quantity of powdered Nitroglycerin Tablets, equivalent to 20 mg of nitroglycerin (C₃H₅N₃O₉) according to the labeled amount, to a separator, add 40 mL of isopropylether and 40 mL of water, shake for 10 minutes, and allow the layers to

separate. Collect the aqueous layer, add 40 mL of isopropylether, shake for 10 minutes, collect the aqueous layer, filter, and use the filtrate as the sample solution. Separately, transfer 10 mL of Standard Nitric Acid Solution to a separator, add 30 mL of water and 40 mL of the isopropyl ether layer of the first extraction of the sample solution, shake for 10 minutes, continue the procedure in the same manner as the sample solution, and use the solution so obtained as the standard solution. Transfer 20 mL each of the sample solution and the standard solution to Nessler tubes, respectively, shake well with 30 mL of water and 0.06 g of Griess-Romijn's nitric acid reagent, allow to stand for 30 minutes, and observe the tubes horizontally: the sample solution has no more color than the standard solution.

Uniformity fo dosage units <6.02> Perform the test according to the following method: it meets the requirements of the Content uniformity test.

Transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, and add exactly V mL of acetic acid (100) to provide a solution containing about 30 μg of nitroglycerin ($\text{C}_3\text{H}_5\text{N}_3\text{O}_9$) per mL. Shake vigorously for 1 hour, and after disintegrating the tablet, centrifuge, and use the supernatant liquid as the sample solution. When the tablet does not disintegrate during this procedure, transfer 1 tablet of Nitroglycerin Tablets to a glass-stoppered centrifuge tube, wet the tablet with 0.05 mL of acetic acid (100), and grind down it with a glass rod. While rinsing the glass rod, add acetic acid (100) to make exactly V mL of a solution containing about 30 μg of nitroglycerin ($\text{C}_3\text{H}_5\text{N}_3\text{O}_9$) per mL. Shake for 1 hour, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 5 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, add 2 mL each of salicylic acid TS shake, allow to stand for 15 minutes, and add 10 mL each of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

$$\begin{aligned} \text{Amount (mg) of nitroglycerin (C}_3\text{H}_5\text{N}_3\text{O}_9) \\ = W_S \times (A_T/A_S) \times (V/2000) \times 0.7487 \end{aligned}$$

WS: Amount (mg) of potassium nitrate

Calculate the average content from the contents of 10 tablets: it meets the requirements of the test when each content deviates from the average content by not more than 25%. When there is 1 tablet showing a deviation exceeding 25% and not exceeding 30%, determine the content of an additional 20 tablets in the same manner. Calculate the 30 deviations from the new average of all 30 tablets: it meets the requirements of the test when 1 tablet may deviate from the average content by between 25% and 30%, but no tablet deviates by more than 30%.

Disintegration <6.09> It meets the requirement, provided

that the time limit of the test is 2 minutes, and the use of the disks is omitted.

Assay Weigh accurately and disintegrate, by soft pressing, not less than 20 Nitroglycerin Tablets. Weigh accurately a portion of the powder, equivalent to about 3.5 mg of nitroglycerin ($\text{C}_3\text{H}_5\text{N}_3\text{O}_9$), add exactly 50 mL of acetic acid (100), shake for 1 hour, filter, and use this filtrate as the sample solution. Separately, weigh accurately about 90 mg of potassium nitrate, previously dried at 105°C for 4 hours, dissolve in 5 mL of water, and add acetic acid (100) to make exactly 100 mL. Pipet 10 mL of the solution, add acetic acid (100) to make exactly 100 mL, and use this solution as the standard solution. Measure exactly 2 mL each of the sample solution and the standard solution, to each solution add 2 mL of salicylic acid TS, shake, allow to stand for 15 minutes, and add 10 mL of water. Render the solution alkaline with about 12 mL of a solution of sodium hydroxide (2 in 5) while cooling in ice, and add water to make exactly 50 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 2 mL of acetic acid (100) in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 410 nm, respectively.

$$\begin{aligned} \text{Amount (mg) of nitroglycerin (C}_3\text{H}_5\text{N}_3\text{O}_9) \\ = W_S \times (A_T/A_S) \times (1/20) \times 0.7487 \end{aligned}$$

W_S : Amount (mg) of potassium nitrate

Containers and storage Containers—Tight containers. Storage—Light-resistant, and not exceeding 20°C.

Nitrous Oxide

亜酸化窒素

N_2O : 44.01

Nitrous Oxide contains not less than 97.0 vol% of N_2O .

Description Nitrous Oxide is a colorless gas at room temperature and at atmospheric pressure, and is odorless.

1 mL of Nitrous Oxide dissolves in 1.5 mL of water and in 0.4 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa. It is soluble in diethyl ether and in fatty oils.

1000 mL of Nitrous Oxide at 0°C and at a pressure of 101.3 kPa weighs about 1.96 g.

Identification (1) A glowing splinter of wood held in Nitrous Oxide: it bursts into flame immediately.

(2) Transfer 1 mL each of Nitrous Oxide and nitrous oxide directly from metal cylinders with a pressure-reducing valve to gas measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the conditions of the Assay: the retention time of the main peak from Nitrous Oxide coincides with that of nitrous oxide.

Purity Maintain the containers of Nitrous Oxide between 18°C and 22°C for more than 6 hours before the test, and

correct the volume at 20°C and at a pressure of 101.3 kPa.

(1) Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 100 mL of Nitrous Oxide through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) Carbon dioxide—Pass 1000 mL of Nitrous Oxide through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that produced in the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3) Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 2000 mL of Nitrous Oxide through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) Potassium permanganate-reducing substance—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.10 mL of 0.02 mol/L potassium permanganate VS to each of the tubes, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the manner as directed in (1): the color of solution A is the same as that of solution B.

(5) Chloride <1.03>—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, shake, and use these as solution A and solution B, respectively. Pass 1000 mL of Nitrous Oxide through solution A in the same manner as directed in (1): the turbidity of solution A is the same as that of solution B.

(6) Carbon monoxide—Introduce 5.0 mL of Nitrous Oxide into a gas-cylinder or a syringe for gas chromatography from a metal cylinder holding gas under pressure and fitted with a pressure-reducing valve, through a directly connected polyvinyl tube. Perform the test with this according to the Gas Chromatography <2.02> under the following conditions: no peak is observed at the same retention time as that of carbon monoxide.

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 300 to 500 μm zeolite for gas chromatography (0.5 nm in pore size).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of carbon monoxide is about 20 minutes.

Selection of column: To 0.1 mL each of carbon monoxide

and air in a gas mixer add carrier gas to make 100 mL, and mix well. Proceed with 5.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of oxygen, nitrogen and carbon monoxide in this order.

Detection sensitivity: Adjust the sensitivity so that the peak height of carbon monoxide obtained from 5.0 mL of the mixed gas used in the selection of column is about 10 cm.

Assay Withdraw Nitrous Oxide as directed in the Purity.

Introduce 1.0 mL of Nitrous Oxide into a gas-measuring tube or syringe for gas chromatography from a metal cylinder under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test with this solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of air. Separately, introduce 3.0 mL of nitrogen into a gas mixer, add carrier gas to make exactly 100 mL, mix thoroughly, and use this as the standard mixed gas. Proceed with 1.0 mL of this mixture as directed in the case of Nitrous Oxide, and determine the peak area A_S of nitrogen in the same manner.

$$\text{Amount (vol\%)} \text{ of } \text{N}_2\text{O} = 100 - 3 \times (A_T/A_S)$$

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with silica gel for gas chromatography (300 to 500 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 2 minutes.

Selection of column: To 3.0 mL of nitrogen in a gas mixer add Nitrous Oxide to make 100 mL, and mix well. Proceed with 1.0 mL of the mixed gas under the above operating conditions. Use a column giving well-resolved peaks of nitrogen and nitrous oxide in this order.

System repeatability: Repeat the test five times with the standard mixed gas under the above operating conditions: the relative standard deviation of the peak area of nitrogen is not more than 2.0%.

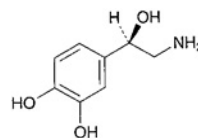
Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

Noradrenaline

Norepinephrine

ノルアドレナリン



and enantiomer

$\text{C}_8\text{H}_{11}\text{NO}_3$; 169.18

4-[(1*RS*)-2-Amino-1-hydroxyethyl]benzene-1,2-diol
[51-41-2]

Noradrenaline, when dried, contains not less than

98.0% of *dl*-norepinephrine ($C_8H_{11}NO_3$).

Description Noradrenaline occurs as a white to light brown or slightly reddish brown, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

It gradually changes to brown by air and by light.

Identification (1) Determine the absorption spectrum of a solution of Noradrenaline in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noradrenaline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Noradrenaline in 10 mL of 0.1 mol/L hydrochloric acid TS, and add water to make 100 mL: the solution is clear and colorless.

(2) Arterenone—Dissolve 50 mg of Noradrenaline in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL. Determine the absorbance of the solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.1.

(3) Adrenaline—Dissolve 10.0 mg of Noradrenaline in 2.0 mL of diluted acetic acid (100) (1 in 2). Pipet 1 mL of this solution, add water to make 10 mL, then mix with 0.3 mL of a solution of sodium nitrite (1 in 100), and allow to stand for 1 minute: the solution has no more color than the following control solution.

Control solution: Dissolve 2.0 mg of Adrenaline Bitartrate Reference Standard and 90 mg of Noradrenaline Bitartrate Reference Standard in water to make exactly 10 mL. Measure exactly 1 mL of this solution, add 1.0 mL of diluted acetic acid (100) (1 in 2) and water to make 10 mL, and proceed in the same manner.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, silica gel, 18 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Noradrenaline, previously dried, dissolve in 50 mL of acetic acid for nonaqueous titration by warming, if necessary, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from blue-purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.92 mg of $C_8H_{11}NO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, under nitrogen atmosphere, and in a cold place.

Noradrenaline Injection

Noradrenaline Hydrochloride Injection

Norepinephrine Hydrochloride Injection

Norepinephrine Injection

ノルアドレナリン注射液

Noradrenaline Injection is an aqueous solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of *dl*-noradrenaline ($C_8H_{11}NO_3$; 169.18).

Method of preparation Dissolve Noradrenaline in 0.01 mol/L hydrochloric acid TS, and prepare as directed under Injections.

Description Norepinephrine Injection is a clear, colorless liquid.

It gradually becomes a pale red color by light and by air.
pH: 2.3 – 5.0

Identification Transfer a volume of Noradrenaline Injection, equivalent to 1 mg of Noradrenaline according to the labeled amount, to each of two test tubes A and B, and add 1 mL of water to each tube. Add 10 mL of potassium hydrogen phthalate buffer solution, pH 3.5, to A, and 10 mL of phosphate buffer solution, pH 6.5, to B. To each of these solutions add 1.0 mL of iodine TS, allow to stand for 5 minutes, and add 2.0 mL of sodium thiosulfate TS: no color or a pale red color develops in test tube A, and a deep red-purple color develops in test tube B.

Purity (1) Arterenone—Measure a volume of Noradrenaline Injection, equivalent to 10 mg of Noradrenaline according to the labeled amount, add water to make exactly 20 mL, and determine the absorbance of this solution at 310 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance is not more than 0.10.

(2) Adrenaline—Measure a volume of Noradrenaline Injection, equivalent to 5 mg of Noradrenaline according to the labeled amount, add 1 mL of diluted acetic acid (100) (1 in 2) and water to make exactly 10 mL, and proceed as directed in the Purity (3) under Noradrenaline.

Extractable volume <6.05> It meets the requirement.

Assay Pipet a volume of Noradrenaline Injection, equivalent to about 5 mg of *dl*-noradrenaline ($C_8H_{11}NO_3$), add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Noradrenaline Bitartrate Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 24 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution, add 0.2 mL each of starch TS, then add iodine TS dropwise with swirling until a persistent blue color is produced. Add 2 mL of iodine TS, and shake. Adjust the pH of the solution to 6.5 with 0.05 mol/L disodium hydrogenphosphate TS, add 10 mL of phosphate buffer solution, pH 6.5, and shake. Immediately after allowing to stand for 3 minutes, add sodium thiosulfate TS drop-

wise until a red-purple color develops, then add water to make exactly 50 mL. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 515 nm within 5 minutes as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } dl\text{-noradrenaline (C}_8\text{H}_{11}\text{NO}_3) \\ = W_S \times (A_T/A_S) \times 0.5016 \end{aligned}$$

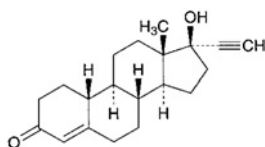
W_S : Amount (mg) of Noradrenaline Bitartrate Reference Standard

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Norethisterone

ノルエチステロン



$C_{20}H_{26}O_2$: 298.42

17-Hydroxy-19-nor-17 α -pregn-4-en-20-yn-3-one [68-22-4]

Norethisterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{20}H_{26}O_2$.

Description Norethisterone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is soluble in chloroform, sparingly soluble in ethanol (95) and in tetrahydrofuran, slightly soluble in diethyl ether, and very slightly soluble in water.

It is affected by light.

Identification (1) To 2 mg of Norethisterone add 2 mL of sulfuric acid: the solution shows a red-brown color and a yellow-green fluorescence. Add 10 mL of water to this solution cautiously: a yellow color develops and a yellow-brown precipitate is formed.

(2) To 25 mg of Norethisterone add 3.5 mL of a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate trihydrate in 25 mL of methanol. Heat under a reflux condenser on a water bath for 5 hours, cool, and add 15 mL of water. Collect the precipitate formed, wash with 1 to 2 mL of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 5 hours: the crystals melt <2.60> between 112°C and 118°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-23 - -27^\circ$ (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

Melting point <2.60> 203 – 209°C

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.2 g of Norethisterone, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric

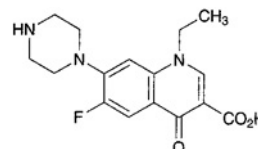
titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 29.84 \text{ mg of } C_{20}H_{26}O_2 \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Norfloxacin

ノルフロキサシン



$C_{16}H_{18}FN_3O_3$: 319.33

1-Ethyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid [70458-96-7]

Norfloxacin, when dried, contains not less than 99.0% of $C_{16}H_{18}FN_3O_3$.

Description Norfloxacin occurs as a white to pale yellow crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in ethanol (99.5) and in acetone, very slightly soluble in methanol, and practically insoluble in water.

It dissolves in dilute hydrochloric acid TS and in sodium hydroxide TS.

It is hygroscopic.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Norfloxacin in a solution of sodium hydroxide (1 in 250) to make 100 mL. To 5 mL of this solution add a solution of sodium hydroxide (1 in 250) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.44>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve a suitable amount of Norfloxacin in a suitable amount of acetone, evaporate the acetone under reduced pressure, and dry the residue. Determine the infrared absorption spectrum of the residue so obtained as directed in the potassium bromide method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Sulfate <1.14>—Dissolve 1.0 g of Norfloxacin in 7 mL of 0.5 mol/L sodium hydroxide TS and 23 mL of water, and add 1 drop of phenolphthalein TS. Add gradually diluted hydrochloric acid (1 in 3) to this solution until the red color disappears, then add 0.5 mL of dilute hydrochloric acid, and cool in ice for 30 minutes. Filter through a glass filter (G4), and wash the residue with 10 mL of water. Combine the filtrate and the washing, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.50 mL of 0.005 mol/L sulfuric acid

VS add 7 mL of 0.5 mol/L sodium hydroxide TS and 1 drop of phenolphthalein TS, add diluted hydrochloric acid (1 in 3) until the red color disappears, then add 1.5 mL of dilute hydrochloric acid, 1 or 2 drops of bromophenol blue TS and water to make 50 mL (not more than 0.024%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Norfloxacin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Norfloxacin according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.10 g of Norfloxacin in 50 mL of a mixture of methanol and acetone (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of methanol and acetone (1:1) to make exactly 100 mL. Pipet 2 mL of this solution, add a mixture of methanol and acetone (1:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography (5–7 μ m in particle diameter). Develop with a mixture of methanol, chloroform, toluene, diethylamine and water (20:20:10:7:4) to a distance of about 9 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm and 366 nm): the number of the spot other than the principal spot from the sample solution is not more than 2 and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Norfloxacin, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

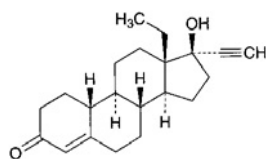
Each mL of 0.1 mol/L perchloric acid VS
= 31.93 mg of C₁₆H₁₈FN₃O₃

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Norgestrel

ノルゲストレル



C₂₁H₂₈O₂: 312.45

13-Ethyl-17-hydroxy-18,19-dinor-17 α -pregn-4-en-20-yn-3-one [6533-00-2]

Norgestrel, when dried, contains not less than 98.0%

of C₂₁H₂₈O₂.

Description Norgestrel occurs as white crystals or crystalline powder.

It is soluble in tetrahydrofuran and in chloroform, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 1 mg of Norgestrel in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. With this solution, examine under ultraviolet light (main wavelength: 365 nm): the solution shows a red-orange fluorescence.

(2) Determine the infrared absorption spectrum of Norgestrel, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 206–212°C

Purity (1) Heavy metals <1.07>—Take 1.0 g of Norgestrel, heat gently to carbonize, cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. After cooling, add 1 mL of sulfuric acid, proceed with this solution according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 30 mg of Norgestrel in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.2 g of Norgestrel, previously dried, dissolve in 40 mL of tetrahydrofuran, add 10 mL of a solution of silver nitrate (1 in 20), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.25 mg of C₂₁H₂₈O₂

Containers and storage Containers—Well-closed containers.

Norgestrel and Ethinylestradiol Tablets

ノルゲストレル・エチニルエストラジオール錠

Norgestrel and Ethinylestradiol Tablets contain not less than 90% and not more than 110% of the labeled amount of norgestrel ($C_{21}H_{28}O_2$: 312.45) and ethinylestradiol ($C_{20}H_{24}O_2$: 296.40).

Method of preparation Prepare as directed under Tablets, with Norgestrel and Ethinylestradiol.

Identification (1) Weigh a quantity of Norgestrel and Ethinylestradiol Tablets, equivalent to 10 mg of Norgestrel according to the labeled amount, previously powdered, add 10 mL of chloroform, shake for 10 minutes, and filter. To 2 mL of the filtrate add 6 mL of sodium hydroxide TS, shake vigorously, and centrifuge. Take 1 mL of the chloroform layer, evaporate on a water bath to dryness, dissolve the residue in 2 mL of ethanol (95), and add 1 mL of sulfuric acid: a red-purple color develops. Examine under ultraviolet light (main wavelength: 365 nm): this solution shows a red-orange fluorescence (norgestrel).

(2) Take 1 mL of the filtrate obtained in (1), evaporate on a water bath to dryness, add 1 mL of boric acid-methanol buffer solution to the residue, shake, and cool in ice. Add 1 mL of ice-cold diazo TS, shake, add 1 mL of sodium hydroxide TS, and shake: a red-orange color develops (ethinylestradiol).

(3) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 10 mg of Norgestrel Reference Standard and 1 mg of Ethinylestradiol Reference Standard, respectively, in 10 mL of chloroform, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1,2-dichloroethane, methanol and water (368:32:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of *p*-toluenesulfonate in ethanol (95) (1 in 5) on the plate, and heat at 105°C for 5 minutes. Examine under ultraviolet light (main wavelength: 365nm): two spots from the sample solution show the similar color tone and *R_f* value to each spot from the standard solutions (1) and (2).

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Add 2 mL of diluted methanol (7 in 10) to 1 tablet of Norgestrel and Ethinylestradiol Tablets, add exactly 2 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μ m, and use this filtrate as the sample solution. Separately, weigh accurately quantities of Norgestrel Reference Standard and of Ethinylestradiol Reference Standard, equivalent to 100 times each of the labeled amounts, dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, and use

this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb} , of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

$$\begin{aligned} \text{Amount (mg) of norgestrel (C}_{21}\text{H}_{28}\text{O}_2) \\ = W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/100) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2) \\ = W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/100) \end{aligned}$$

W_{Sa} : Amount (mg) of Norgestrel Reference Standard

W_{Sb} : Amount (mg) of Ethinylestradiol Reference Standard

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Norgestrel and Ethinylestradiol Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 50 mL or more of the dissolved solution 45 minutes after starting the test, and membrane filter through a membrane filter with a pore size not exceeding 0.8 μ m. Discard the first 10 mL of the filtrate, transfer exactly 30 mL of the subsequent into a chromatography column [prepared by packing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μ m in particle diameter) in a tube about 1 cm in inside diameter]. After washing the column with 15 mL of water, elute with 3 mL of methanol, and evaporate the effluent on a water bath to dryness at about 40°C with the aid of a current air. Dissolve the residue in exactly 2 mL of diluted methanol (7 in 10), and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Norgestrel Reference Standard and about 2.5 mg of Ethinylestradiol Reference Standard dissolve in diluted methanol (7 in 10) to make exactly 100 mL, then pipet 3 mL of this solution, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Tb} , of norgestrel and ethinylestradiol from the sample solution, and the peak areas, A_{Sa} and A_{Sb} , of norgestrel and ethinylestradiol from the standard solution.

The dissolution rate of Norgestrel and Ethinylestradiol Tablets in 45 minutes is not less than 70%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ \text{of norgestrel (C}_{21}\text{H}_{28}\text{O}_2) \\ = W_{Sa} \times (A_{Ta}/A_{Sa}) \times (1/C_a) \times (9/5) \end{aligned}$$

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ \text{of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2) \end{aligned}$$

$$= W_{Sb} \times (A_{Tb}/A_{Sb}) \times (1/C_b) \times (9/5)$$

W_{Sa} : Amount (mg) of Norgestrel Reference Standard

W_{Sb} : Amount (mg) of Ethinylestradiol Reference Standard

C_a : Labeled amount (mg) of norgestrel ($C_{21}H_{28}O_2$) in 1 tablet

C_b : Labeled amount (mg) of ethinylestradiol ($C_{20}H_{24}O_2$) in 1 tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

Proceed as directed in the system suitability in the Assay.

Assay Weigh accurately not less than 20 Norgestrel and Ethinylestradiol Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 1 mg of norgestrel ($C_{21}H_{28}O_2$), add 4 mL of diluted methanol (7 in 10), add exactly 4 mL of the internal standard solution, shake for 20 minutes, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.2 μm , and use this filtrate as the sample solution. Separately, weigh accurately about 50 mg of Norgestrel Reference Standard and about 5 mg of Ethinylestradiol Reference Standard, and dissolve in diluted methanol (7 in 10) to make exactly 200 mL. Pipet 4 mL of this solution, add exactly 4 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the sample solution and also the ratios, Q_{Sa} and Q_{Sb} , of the peak areas of norgestrel and ethinylestradiol to the peak area of the internal standard of the standard solution.

$$\begin{aligned} &\text{Amount (mg) of norgestrel (C}_{21}\text{H}_{28}\text{O}_2\text{)} \\ &= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/50) \end{aligned}$$

$$\begin{aligned} &\text{Amount (mg) of ethinylestradiol (C}_{20}\text{H}_{24}\text{O}_2\text{)} \\ &= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/50) \end{aligned}$$

W_{Sa} : Amount (mg) of Norgestrel Reference Standard

W_{Sb} : Amount (mg) of Ethinylestradiol Reference Standard

Internal standard solution—A solution of diphenyl in diluted methanol (7 in 10) (1 in 50,000).

Operating conditions—

Detector: Norgestrel—An ultraviolet absorption photometer (wavelength: 241 nm).

Ethinylestradiol—A fluorophotometer (excitation wavelength: 281 nm, fluorescence wavelength: 305 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile and water (11:9).

Flow rate: Adjust the flow rate so that the retention time of norgestrel is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20

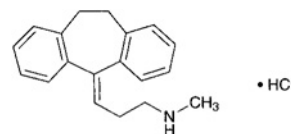
μL of the standard solution under the above operating conditions, ethinylestradiol, norgestrel and the internal standard are eluted in this order, and the resolution between the peaks of norgestrel and the internal standard is not less than 8.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethinylestradiol and norgestrel to that of the internal standard are not more than 1.0%, respectively.

Containers and storage Containers—Tight containers.

Nortriptyline Hydrochloride

ノルトリプチリン塩酸塩



$C_{19}H_{21}N.HCl$: 299.84

3-(10,11-Dihydro-5H-dibenzo[*a,d*]cyclohepten-5-ylidene)-*N*-methylpropylamine monohydrochloride [894-71-3]

Nortriptyline Hydrochloride, when dried, contains not less than 98.5% of $C_{19}H_{21}N.HCl$.

Description Nortriptyline Hydrochloride occurs as a white to yellowish white, crystalline powder. It is odorless, or has a faint, characteristic odor.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in water, and practically insoluble in diethyl ether.

The pH of a solution of Nortriptyline Hydrochloride (1 in 100) is about 5.5.

Melting point: 215 – 220°C

Identification (1) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 mL of bromine TS: the color of the test solution disappears.

(2) To 5 mL of a solution of Nortriptyline Hydrochloride (1 in 100) add 1 to 2 drops of a solution of quinhydrone in methanol (1 in 40): a red color gradually develops.

(3) Determine the absorption spectrum of a solution of Nortriptyline Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Nortriptyline Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Nortriptyline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Nortriptyline Hydrochloride in 10 mL of water: the solution is clear and colorless to very light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Nortriptyline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Nortriptyline Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Nortriptyline Hydrochloride in 20 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 4 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, methanol and diethylamine (8:1:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Nortriptyline Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 29.98 \text{ mg of } C_{19}H_{21}N.HCl \end{aligned}$$

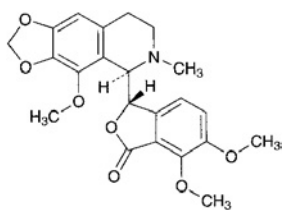
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine

Narcotine

ノスカピン



$C_{22}H_{23}NO_7$: 413.42

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one [J28-62-1]

Noscapine, when dried, contains not less than 98.5% of $C_{22}H_{23}NO_7$.

Description Noscapine occurs as white crystals or crystal-

line powder. It is odorless and tasteless.

It is very soluble in acetic acid (100), slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Noscapine in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Noscapine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +42 – +48° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid TS, 25 mL, 100 nm).

Melting point <2.60> 174 – 177°C

Purity (1) Chloride <1.03>—Dissolve 0.7 g of Noscapine in 20 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test with this solution. Prepare the control solution as follows: To 0.4 mL of 0.01 mol/L hydrochloric acid add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.02%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Noscapine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Morphine—Dissolve 10 mg of Noscapine in 1 mL of water and 5 mL of 1-nitroso-2-naphthol TS with shaking, add 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the solution with 10 mL of chloroform, centrifuge, and collect the aqueous layer: the solution so obtained has no more color than a pale red.

(4) Related substances—Dissolve 0.7 g of Noscapine in 50 mL of acetone, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add acetone to make exactly 50 mL. Pipet 5 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (60:60:9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute bismuth subnitrate-potassium iodide TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.8 g of Noscapine, previously dried, dissolve in 30 mL of acetic acid (100) and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and

make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 41.34 mg of $C_{22}H_{23}NO_7$

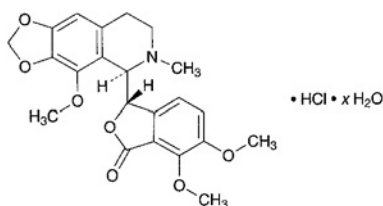
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Noscapine Hydrochloride Hydrate

Narcotine Hydrochloride

ノスカピン塩酸塩水和物



$C_{22}H_{23}NO_7 \cdot HCl \cdot xH_2O$

(3*S*)-6,7-Dimethoxy-3-[(5*R*)-4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-*g*]isoquinolin-5-yl]isobenzofuran-1(3*H*)-one monohydrochloride hydrate [912-60-7, anhydride]

Noscapine Hydrochloride Hydrate, when dried, contains not less than 98.0% of noscapine hydrochloride $C_{22}H_{23}NO_7 \cdot HCl$: 449.88.

Description Noscapine Hydrochloride Hydrate occurs as colorless or white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in acetic acid (100), and in acetic anhydride, soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of formaldehyde-sulfuric acid TS: a purple color, changing to yellow-brown, is produced.

(2) To 1 mg of Noscapine Hydrochloride Hydrate add 1 drop of a solution of ammonium vanadate (V) in sulfuric acid (1 in 200): an orange color is produced.

(3) Dissolve 0.02 g of Noscapine Hydrochloride Hydrate in 1 mL of water, and add 3 drops of sodium acetate TS: a white, flocculent precipitate is produced.

(4) Dissolve 1 mg of Noscapine Hydrochloride Hydrate in 1 mL of diluted sulfuric acid (1 in 35), shake with 5 drops of a solution of disodium chlomotropate dihydrate (1 in 50), and add 2 mL of sulfuric acid dropwise: a purple color is produced.

(5) Dissolve 0.1 g of Noscapine Hydrochloride Hydrate in 10 mL of water, make the solution alkaline with ammonia TS, and shake with 10 mL of chloroform. Separate the chloroform layer, wash with 5 mL of water, and filter. Distil most of the filtrate on a water bath, add 1 mL of ethanol (99.5), and evaporate to dryness. Dry the residue at 105°C for 4 hours: the residue so obtained melts <2.60> between 174°C and 177°C.

(6) Make a solution of Noscapine Hydrochloride Hydrate (1 in 50) alkaline with ammonia TS, and filter the

precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Purity Morphine—Dissolve 10 mg of Noscapine Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

Loss on drying <2.41> Not more than 9.0% (0.5 g, 120°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately about 0.5 g of Noscapine Hydrochloride Hydrate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.99 mg of $C_{22}H_{23}NO_7 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Nystatin

ナイスタチン

Nystatin is a mixture of polyene macrolide substances having antifungal activity produced by the growth of *Streptomyces noursei*.

It contains not less than 4600 units (potency) per mg, calculated on the dried basis. The potency of Nystatin is expressed as the unit of nystatin ($C_{47}H_{75}NO_{17}$: 926.09), and one unit corresponds to 0.27 μg of nystatin ($C_{47}H_{75}NO_{17}$).

Description Nystatin occurs as a white to light yellow-brown powder.

It is soluble in formamide, sparingly soluble in methanol, slightly soluble in ethanol (95), and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 1 mg of Nystatin in 5 mL of water and 1 mL of sodium hydroxide TS, heat for 2 minutes, and cool. To this solution add 3 mL of a solution of 4-aminoacetophenone in methanol (1 in 200) and 1 mL of hydrochloric acid: a red-purple color develops.

(2) To 10 mg of Nystatin add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), heat at not exceeding 50°C to dissolve, then add diluted methanol (4 in 5) to make 500 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Nys-

tatin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Nystatin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 5.0% (0.3 g, in vacuum, 60°C, 3 hours).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Saccharomyces cerevisiae* ATCC 9763

(ii) Culture medium—Use the medium 2) Medium for test organism [12] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin Reference Standard equivalent to about 60,000 units, previously dried at 40°C for 2 hours in vacuum (not more than 0.67 kPa), dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

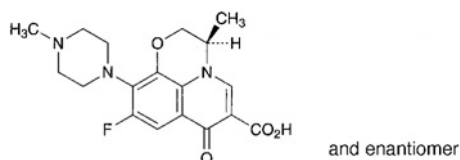
(iv) Sample solutions—Use a light-resistant container. Weigh accurately an amount of Nystatin equivalent to about 60,000 units, dissolve in formamide to make a solution of 3000 units per mL, and use this solution as the sample stock solution. Take exactly a suitable amount of the sample stock solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 300 units and 150 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Ofloxacin

オフロキサシン



$C_{18}H_{20}FN_3O_4$: 361.37

(3*R*S)-9-Fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-[1,4]benzooxazine-6-carboxylic acid [82419-36-1]

Ofloxacin, when dried, contains not less than 99.0% and not more than 101.0% of ofloxacin ($C_{18}H_{20}FN_3O_4$).

Description Ofloxacin occurs as pale yellowish white to light yellowish white, crystals or crystalline powder.

It is freely soluble in acetic acid (100), slightly soluble in water, and very slightly soluble in acetonitrile and in ethanol (99.5).

A solution of Ofloxacin in sodium hydroxide TS (1 in 20) does not show optical rotation.

It is changed in color by light.

Melting point: about 265°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ofloxacin in 0.1 mol/L hydrochloric acid TS (1 in 150,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ofloxacin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ofloxacin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Conduct this procedure without exposure to light. Dissolve 10 mg of Ofloxacin in 50 mL of a mixture of water and acetonitrile (6:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add a mixture of water and acetonitrile (6:1) to make exactly 20 mL. Pipet 1 mL of this solution, add a mixture of water and acetonitrile (6:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than ofloxacin obtained from the sample solution is not more than 0.4 times the peak area of ofloxacin from the standard solution, and the total area of the peaks other than ofloxacin from the sample solution is not more than the peak area from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 294 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 7.0 g of sodium perchlorate monohydrate and 4.0 g of ammonium acetate in 1300 mL of water, adjust the pH to 2.2 with phosphoric acid, and add 240 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of ofloxacin is about 20 minutes.

Time span of measurement: About 1.8 times as long as the retention time of ofloxacin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure 1 mL of the standard solution, and add a mixture of water and acetonitrile

(6:1) to make exactly 20 mL. Confirm that the peak area of ofloxacin obtained from 10 μ L of this solution is equivalent to 4 to 6% of that from 10 μ L of the standard solution.

System performance: To 0.5 mL of the sample solution add 1 mL of a solution of ofloxacin demethyl substance in a mixture of water and acetonitrile (6:1) (1 in 20,000) and a mixture of water and acetonitrile (6:1) to make 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, ofloxacin demethyl substance and ofloxacin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ofloxacin is not more than 2.0%.

Loss on drying <2.41> Not less than 0.2% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Ofloxacin, previously dried, dissolve in 100 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.14 mg of C₁₈H₂₀FN₃O₄

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Olive Oil

Oleum Olivae

オリブ油

Olive Oil is the fixed oil obtained by expression from the ripe fruit of *Olea europaea* Linné (*Oleaceae*).

Description Olive Oil is a light yellow oil. It has a faint odor, which is not rancid, and has a bland taste.

It is miscible with diethyl ether, with petroleum diethyl ether and with carbon disulfide.

It is slightly soluble in ethanol (95).

The whole or a part of it congeals between 0°C and 6°C.

Congeaing point of the fatty acids: 17 – 26°C

Specific gravity <1.13> d_{25}^{25} : 0.908 – 0.914

Acid value <1.13> Not more than 1.0.

Saponification value <1.13> 186 – 194

Unsaponifiable matters <1.13> Not more than 1.5%.

Iodine value <1.13> 79 – 88

Purity (1) Drying oil—Mix 2 mL of Olive Oil with 10 mL of diluted nitric acid (1 in 4), add 1 g of powdered sodium nitrite little by little with thorough shaking, and allow to stand in a cold place for 4 to 10 hours: the mixture congeals to a white solid.

(2) Peanut oil—Weigh exactly 1.0 g of Olive Oil, dissolve in 60 mL of sulfuric acid-hexane-methanol TS, boil for 2.5

hours on a water bath under a reflux condenser, cool, transfer to a separator, and add 100 mL of water. Wash the flask with 50 mL of petroleum ether, add the washing to the separator, shake, allow to stand, and separate the petroleum ether layer. Extract the water layer with another 50 mL of petroleum ether, and combine the petroleum ether layer with the former petroleum ether solution. Wash the petroleum ether solution repeatedly with 20-mL portions of water until the washings show no more acidity to methyl orange TS. Then add 5 g of anhydrous sodium sulfate, shake, filter, wash anhydrous sodium sulfate with two 10-mL portions of petroleum ether, filter the washings using the former separator, combine the filtrates, distil the petroleum ether on a water bath, passing nitrogen. Dissolve the residue in acetone to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 0.067 g of methyl behenate in acetone to make exactly 50 mL. Pipet 2 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Measure the peak heights, H_T and H_S , of methyl behenate of respective solutions: H_T is not higher than H_S .

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with silanized siliceous earth for gas chromatography (150 to 180 μ m in particle diameter), coated with polyethylene glycol 20 mol/L in a ratio of 5%.

Column temperature: A constant temperature of about 220°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of methyl behenate is about 18 minutes.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of methyl behenate obtained from 2 μ L of the standard solution is 5 to 10 mm.

Containers and storage Containers—Tight containers.

Powdered Opium

Opium Pulveratum

アヘン末

Powdered Opium is a homogeneous powder of opium obtained from *Papaver somniferum* Linné (*Papaveraceae*). Starch or Lactose Hydrate may be added.

Powdered Opium contains not less than 9.5% and not more than 10.5% of morphine (C₁₇H₁₉NO₃: 285.34).

Description Powdered Opium occurs as a yellow-brown to dark brown powder.

Identification (1) To 0.1 g of Powdered Opium add 5 mL of diluted ethanol (7 in 10), dissolve by treating with ultrasonic waves for 10 minutes, and add diluted ethanol (7 in 10) to make 10 mL. Filter this solution, and use the filtrate as the sample solution. Separately, dissolve 25 mg of Morphine Hydrochloride Hydrate, 12 mg of Codeine Phosphate Hy-

drate, 2 mg of Papaverine Hydrochloride, and 12 mg of Noscapine Hydrochloride Hydrate separately in 25 mL of diluted ethanol (7 in 10), and use these solutions as the standard solution (1), the standard solution (2), the standard solution (3) and the standard solution (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia water (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: each spot from the sample solution shows the same color tone and *R_f* value of each spot obtained from the standard solution (1), the standard solution (2), the standard solution (3), and the standard solution (4) (morphine, codeine, papaverine and noscapine), respectively.

(2) To 0.1 g of Powdered Opium add 5 mL of water, and shake the mixture for 5 minutes. Filter, to the filtrate add 1 mL of a solution of hydroxylammonium chloride (3 in 10) and 1 drop of iron (III) chloride TS, and shake: a red-brown color is produced. To this solution add immediately 5 mL of diethyl ether, and shake: the diethyl ether layer has no red-purple color (meconic acid).

Loss on drying <2.41> Not more than 8.0% (1 g, 105°C, 5 hours).

Assay Place about 5 g of Powdered Opium, accurately weighed, in a mortar, and triturate it with exactly 10 mL of water. Add 2 g of calcium hydroxide and exactly 40 mL of water, and stir the mixture for 20 minutes. Filter, and shake 30 mL of the filtrate with 0.1 g of magnesium sulfate heptahydrate for 1 minute. To the mixture add 0.3 g of calcium hydroxide, shake for 1 minute, and allow to stand for 1 hour. Filter, place 20 mL of the filtrate, exactly measured, in a glass-stoppered flask, and add 10 mL of diethyl ether and 0.3 g of ammonium chloride. Shake vigorously with caution. When crystals begin to separate out, shake for 30 minutes with a mechanical shaker, and set aside overnight at a temperature of 5°C to 10°C. Decant the diethyl ether layer and filter first, and then the water layer through filter paper 7 cm in diameter. Wash the adhering crystals in the flask with three 5-mL portions of water saturated with diethyl ether, and wash the crystals on the filter paper with each of these washings. Wash the top of the glass-stoppered flask and the upper part of the filter paper with final 5 mL of water saturated with diethyl ether. Transfer the crystals and the filter paper to a beaker. Dissolve the crystals remaining in the glass-stoppered flask with the aid of 15 mL of 0.05 mol/L sulfuric acid VS, accurately measured, and pour the solution into the beaker. Wash the glass-stoppered flask with four 5-mL portions of water, and add the washings to the solution in the beaker. Titrate <2.50> the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS (indicator: 4 drops of methyl red-methylene blue TS).

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of C₁₇H₁₉NO₃

Containers and storage Containers—Tight containers.

Diluted Opium Powder

アヘン散

Diluted Opium Powder contains not less than 0.90% and not more than 1.10% of morphine (C₁₇H₁₉NO₃; 285.34).

Method of preparation

Powdered Opium	100 g
Starch or a suitable diluent	a sufficient quantity
To make	
	1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Diluted Opium Powder occurs as a light brown powder.

Identification (1) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (1) under Powdered Opium.

(2) Proceed with 1 g of Diluted Opium Powder as directed in the Identification (2) under Powdered Opium.

Assay Place about 50 g of Diluted Opium Powder, accurately weighed, in a glass-stoppered flask, and stir with 250 mL of dilute ethanol in a water bath at 40°C for 1 hour. Filter the mixture through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, and add 50 mL of dilute ethanol. Stir the mixture in a water bath at 40°C for 10 minutes, and filter through the same glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Evaporate the combined filtrate in a mortar to dryness on a water bath. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, and, after cooling, triturate it with exactly 10 mL of water. Proceed with this solution as directed in Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of C₁₇H₁₉NO₃

Containers and storage Containers—Tight containers.

Opium Tincture

アヘンチンキ

Opium Tincture contains not less than 0.93 w/v% and not more than 1.07 w/v% of morphine (C₁₇H₁₉NO₃; 285.34).

Method of preparation

Powdered Opium	100 g
35 vol% Ethanol	a sufficient quantity
To make	
	1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water in place of 35 vol% Ethanol.

Description Opium Tincture is a dark red-brown liquid.

It is affected by light.

Identification (1) To 1 mL of Opium Tincture add diluted ethanol (7 in 10) to make 10 mL, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (1) under Powdered Opium.

(2) Evaporate 1 mL of Opium Tincture to dryness on a water bath, and proceed with the residue as directed in the Identification (2) under Powdered Opium.

Alcohol number <1.01> Not less than 3.5 (Method 1).

Assay Evaporate 50 mL of Opium Tincture, accurately measured, on a water bath to dryness. Add 10 mL of ethanol (99.5) to the residue, evaporate to dryness again, cool, and triturate with exactly 10 mL of water. Proceed with this solution as directed in the Assay under Powdered Opium.

$$\begin{aligned} \text{Each mL of 0.05 mol/L sulfuric acid VS} \\ = 28.53 \text{ mg of } C_{17}H_{19}NO_3 \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Opium Alkaloids Hydrochlorides

アヘンアルカロイド塩酸塩

Opium Alkaloids Hydrochlorides consist of the hydrochlorides of some of the main alkaloids obtained from opium.

It contains not less than 47.0% and not more than 52.0% of morphine ($C_{17}H_{19}NO_3$: 285.34), and not less than 35.0% and not more than 41.0% of other opium alkaloids.

Description Opium Alkaloids Hydrochlorides occur as a white to light brown powder.

It is soluble in water, and slightly soluble in ethanol (99.5).

It is colored by light.

Identification (1) Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 10 mL of diluted ethanol (1 in 2), and use this solution as the sample solution. Separately, dissolve 60 mg of Morphine Hydrochloride Hydrate, 40 mg of Noscapine Hydrochloride Hydrate, 10 mg of Codein Phosphate Hydrate and 10 mg of Papaverine Hydrochloride in 10 mL each of diluted ethanol (1 in 2), and use these solutions as the standard solutions (1), (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene, ethanol (99.5) and ammonia solution (28) (20:20:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): each spot from the sample solution is the same in color tone and R_f value with the corresponding spot from the standard solutions (1), (2), (3) and (4) (morphine, noscapine, codeine and papaverine).

(2) A solution of Opium Alkaloids Hydrochlorides (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Opium Alkaloids Hydrochlorides in 50 mL of water: the pH of the solution is between 3.0

and 4.0.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Opium Alkaloids Hydrochlorides in 10 mL of water: the solution is clear, and its absorbance <2.24> at 420 nm is not more than 0.20.

(2) Meconic acid—Dissolve 0.1 g of Opium Alkaloids Hydrochlorides in 2 mL of water, and pour into a polyethylene column 1 cm in inside diameter, packed with about 0.36 g of aminopropylsilanized silica gel for pretreatment (55–105 μ m in particle diameter) and previously washed through with 5 mL of water. Then, wash the column with 5 mL of water, 5 mL of methanol and 10 mL of 0.1 mol/L hydrochloric acid in this order, then elute with 2 mL of 1 mol/L hydrochloric acid, and use the eluate as the test solution. To the test solution add 2 mL of dilute sodium hydroxide TS and 1 drop of iron (III) chloride TS: no red color develops.

Loss on drying <2.41> Not more than 6.0% (0.5 g, 120°C, 8 hours).

Residue on ignition <2.44> Not more than 0.5% (0.5 g).

Assay Weigh accurately about 0.1 g of Opium Alkaloids Hydrochlorides, and dissolve in water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of morphine hydrochloride for assay, dissolve in water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of morphine, codeine, papaverine, thebaine, narceine and noscapine, A_{T1} , A_{T2} , A_{T3} , A_{T4} , A_{T5} and A_{T6} , from the sample solution, and the peak area of morphine, A_S , from the standard solution.

$$\begin{aligned} \text{Amount (mg) of morphine (} C_{17}H_{19}NO_3 \text{)} \\ = W_S \times (A_{T1}/A_S) \times 0.8867 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of other opium alkaloids} \\ = W_S \\ \times \{(A_{T2} + 0.29A_{T3} + 0.20A_{T4} + 0.19A_{T5} + A_{T6})/A_S\} \\ \times 0.8867 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

The relative retention time of codeine, papaverine, thebaine, narceine and noscapine with respect to morphine obtained under the following operating conditions are as follows.

Component	Relative retention time
codeine	1.1
papaverine	1.9
thebaine	2.5
narceine	2.8
noscapine	3.6

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: Dissolve 60 mg of Morphine Hydrochloride Hydrate, 10 mg of Codeine Phosphate Hydrate, 10 mg of Papaverine Hydrochloride and 40 mg of Noscapine Hydrochloride Hydrate in water to make 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, morphine, codeine, papaverine and noscapine are eluted in this order with the complete separation between these peaks and with the resolution between the peaks of morphine and codeine being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of morphine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Opium Alkaloids Hydrochlorides Injection

アヘンアルカロイド塩酸塩注射液

Opium Alkaloids Hydrochlorides Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine (C₁₇H₁₉NO₃: 285.34).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Water for Injection	a sufficient quantity
<hr/>	
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids Hydrochlorides Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification To 1 mL of Opium Alkaloids Hydrochlorides Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution, and proceed as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

Extractable volume <6.05> It meets the requirements.

Assay Pipet 2 mL of Opium Alkaloids Hydrochlorides Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay (1) under Opium Alkaloids Hydrochlorides.

Amount (mg) of morphine (C₁₇H₁₉NO₃)

$$= W_S \times (Q_T / Q_S) \times 0.8867$$

W_S: Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrine hydrochloride (1 in 500).

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Opium Alkaloids and Atropine Injection

アヘンアルカロイド・アトロピン注射液

Opium Alkaloids and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine (C₁₇H₁₉NO₃: 285.34), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate Hydrate [(C₁₇H₂₃NO₃)₂·H₂SO₄·H₂O: 694.84].

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection	a sufficient quantity
<hr/>	
	To make 1000 mL

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids and Atropine Injection is a colorless or light brown, clear liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Atropine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Opium Alkaloids and Atropine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Atropine Sulfate Reference Standard in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.2 R_f value among the several spots from the sample solution and an

orange colored spot from the standard solution show the same color tone, and have the same R_f value (atropine).

Extractable volume <6.05> It meets the requirements.

Assay (1) Morphine—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, then add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3) \\ &= W_S \times (Q_T/Q_S) \times 0.8867 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of ethylefrine hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Atropine sulfate hydrate—Pipet 2 mL of Opium Alkaloids and Atropine Injection, add exactly 2 mL of the internal standard solution, and add 10 mL of diluted dilute hydrochloric acid (1 in 10). Shake this solution with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this so-

lution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate Reference Standard (determine previously loss on drying <2.41> in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use this solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of atropine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of atropine sulfate hydrate} \\ &[(\text{C}_{17}\text{H}_{23}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4 \cdot \text{H}_2\text{O}] \\ &= W_S \times (Q_T/Q_S) \times (1/50) \times 1.027 \end{aligned}$$

W_S : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μ m siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, the internal standard and atropine are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of atropine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Opium Alkaloids and Scopolamine Injection

アヘンアルカロイド・スコポラミン注射液

Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 1.80 w/v% and not more than 2.20 w/v% of morphine (C₁₇H₁₉NO₃: 285.34) and not less than 0.054 w/v% and not more than 0.066 w/v% of scopolamine hydrobromide hydrate (C₁₇H₂₁NO₄·HBr·3H₂O: 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	40 g
Scopolamine Hydrobromide Hydrate	0.6 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Opium Alkaloids and Scopolamine Injection is a clear, colorless to light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of water and 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5) to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide Reference Standard in 100 mL of water. To 2 mL of this solution add 2 mL of ammonia TS, proceed with this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 Rf value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same Rf value (scopolamine).

Extractable volume <6.05> It meets the requirements.

Assay (1) Morphine—Pipet 1 mL of Opium Alkaloids and Scopolamine Injection, add 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3) \\ &= W_S \times (Q_T/Q_S) \times 0.8867 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrin

hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 2 mL of Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scopolamine Hydrobromide Reference Standard (determine previously its loss on drying <2.41> in the same manner as directed under Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use thus obtained solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of scopolamine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of scopolamine hydrobromide hydrate} \\ &(\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HBr} \cdot 3\text{H}_2\text{O}) \\ &= W_S \times (Q_T/Q_S) \times (1/50) \times 1.1406 \end{aligned}$$

W_S : Amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μm siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Weak Opium Alkaloids and Scopolamine Injection

弱アヘンアルカロイド・スコポラミン注射液

Weak Opium Alkaloids and Scopolamine Injection is an aqueous solution for injection.

It contains not less than 0.90 w/v% and not more than 1.10 w/v% of morphine ($\text{C}_{17}\text{H}_{19}\text{NO}_3$; 285.34) and not less than 0.027 w/v% and not more than 0.033 w/v% of scopolamine hydrobromide hydrate ($\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HBr} \cdot 3\text{H}_2\text{O}$; 438.31).

Method of preparation

Opium Alkaloids Hydrochlorides	20 g
Scopolamine Hydrobromide Hydrate	0.3 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Weak Opium Alkaloids and Scopolamine Injection is a clear, colorless or light brown liquid.

It is affected by light.

pH: 2.5 – 3.5

Identification (1) To 1 mL of Opium Alkaloids and Scopolamine Injection add 1 mL of ethanol (99.5), mix, and use this solution as the sample solution. Proceed with the sample solution as directed in the Identification (1) under Opium Alkaloids Hydrochlorides.

(2) To 2 mL of Weak Opium Alkaloids and Scopolamine Injection add 2 mL of ammonia TS, extract with 10 mL of diethyl ether, and filter the diethyl ether layer. Evaporate the filtrate on a water bath to dryness, add 1 mL of ethanol (99.5)

to the residue, and heat to dissolve. Allow to stand this solution in an ice water for 30 minutes with occasional shaking. After crystals are formed, use the supernatant liquid as the sample solution. Separately, dissolve 0.03 g of Scopolamine Hydrobromide Reference Standard in 100 mL of water, proceed with 2 mL of this solution in the same manner as for the sample solution, and use a solution so obtained as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol and ammonia water (28) (200:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a spot of about 0.7 R_f value among the several spots from the sample solution and an orange colored spot from the standard solution show the same color tone, and have the same R_f value (scopolamine).

Extractable volume <6.05> It meets the requirements.

Assay (1) Morphine—Pipet 2 mL of Weak Opium Alkaloids and Scopolamine Injection, add exactly 10 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of morphine hydrochloride for assay, dissolve in exactly 10 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Perform the test with 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of morphine to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of morphine (C}_{17}\text{H}_{19}\text{NO}_3) \\ = W_S \times (Q_T \times Q_S) \times 0.8867 \end{aligned}$$

W_S : Amount (mg) of morphine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of etilefrin hydrochloride (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 500 mL of diluted phosphoric acid (1 in 1000), and adjust the pH to 3.0 with sodium hydroxide TS. To 240 mL of this solution add 70 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of morphine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, morphine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating

conditions, the relative standard deviation of the ratios of the peak area of morphine to that of the internal standard is not more than 2.0%.

(2) Scopolamine hydrobromide hydrate—Pipet 4 mL of Weak Opium Alkaloids and Scopolamine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10), and shake with two 10-mL portions of dichloromethane. Remove the dichloromethane layer, to the water layer add 2 mL of ammonia TS, add immediately 20 mL of dichloromethane, shake vigorously, filter the dichloromethane extract through a filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloroethane and 0.5 mL of bis-trimethyl silyl acetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 60 mg of Scopolamine Hydrobromide Reference Standard (separately determine its loss on drying <2.41> in the same manner as directed under Scopolamine Hydrobromide Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of scopolamine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of scopolamine hydrobromide hydrate} \\ &(\text{C}_{17}\text{H}_{21}\text{NO}_4 \cdot \text{HBr} \cdot 3\text{H}_2\text{O}) \\ &= W_S \times (Q_T/Q_S) \times (1/50) \times 1.1406 \end{aligned}$$

W_S : Amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1.5 m in length, packed with 180 to 250 μ m siliceous earth for gas chromatography coated in 1 to 3% with 50% phenyl-methyl silicone polymer for gas chromatography.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating conditions, the internal standard and scopolamine are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of scopolamine to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Orange Oil

Oleum Aurantii

オレンジ油

Orange Oil is the essential oil obtained by expression from the peel of the edible fruit of *Citrus* species (*Rutaceae*).

Description Orange Oil is a yellow to yellow-brown liquid. It has a characteristic, aromatic odor, and a slightly bitter taste.

It is miscible with an equal volume of ethanol (95) with turbidity.

Refractive index <2.45> n_D^{20} : 1.472 – 1.474

Optical rotation <2.49> α_D^{20} : +85 – +99° (100 mm).

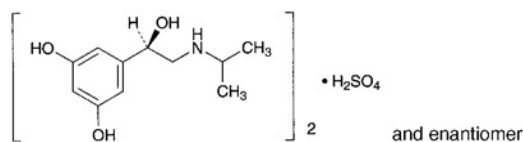
Specific gravity <1.13> d_{20}^{20} : 0.842 – 0.848

Purity Heavy metals <1.07>—Proceed with 1.0 mL of Orange Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Orciprenaline Sulfate

オルシプレナリン硫酸塩



($\text{C}_{11}\text{H}_{17}\text{NO}_3$)₂· H_2SO_4 : 520.59

5-[(1*RS*)-1-Hydroxy-2-[(1-methylethyl)amino]ethyl] benzene-1,3-diol hemisulfate [5874-97-5]

Orciprenaline Sulfate contains not less than 98.5% of ($\text{C}_{11}\text{H}_{17}\text{NO}_3$)₂· H_2SO_4 , calculated on the dried basis.

Description Orciprenaline Sulfate occurs as white crystals or crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Orciprenaline Sulfate (1 in 20) shows no optical rotation.

Melting point: about 220°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Or-

ciprenaline Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Orciprenaline Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Orciprenaline Sulfate in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3 mL of Matching Fluid T add 1 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Orciprenaline Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Orciprenalone—Dissolve 0.200 g of Orciprenaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at 328 nm is not more than 0.075.

Loss on drying <2.41> Not more than 1.5% (1 g, in vacuum, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

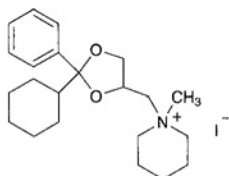
Assay Weigh accurately about 0.7 g of Orciprenaline Sulfate, dissolve in 100 mL of acetic acid (100) by warming on a water bath, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.06 mg of $(C_{11}H_{17}NO_3)_2 \cdot H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxapium Iodide

オキサピウムヨウ化物



$C_{22}H_{34}INO_2$: 471.42

1-(2-Cyclohexyl-2-phenyl-1,3-dioxolan-4-ylmethyl)-1-methylpiperidinium iodide [6577-41-9]

Oxapium Iodide, when dried, contains not less than 98.5% of $C_{22}H_{34}INO_2$.

Description Oxapium Iodide occurs as a white, crystalline powder.

It is soluble in acetonitrile, in methanol and in ethanol (95), slightly soluble in water, in acetic anhydride and in acetic acid (100), and practically insoluble in diethyl ether.

A solution of Oxapium Iodide in methanol (1 in 100) does not show optical rotation.

Identification (1) Determine the infrared absorption spectrum of Oxapium Iodide, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.1 g of Oxapium Iodide in 10 mL of methanol, and add 2 mL of dilute nitric acid and 2 mL of silver nitrate TS: a greenish yellow precipitate is formed.

Melting point <2.60> 198 – 203°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Oxapium Iodide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Oxapium Iodide in 100 mL of a mixture of water and acetonitrile (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of water and acetonitrile (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of each solution by the automatic integration method: the total area of the peaks other than the peak of oxapium from the sample solution is not larger than the area of the peak from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of 20°C to 30°C.

Mobile phase: To 57 mL of acetic acid (100) and 139 mL of triethylamine add water to make 1000 mL. To 50 mL of this solution add 500 mL of acetonitrile, 10 mL of dilute acetic acid and 440 mL of water.

Flow rate: Adjust the flow rate so that the retention time of oxapium is about 4 minutes.

Selection of column: Dissolve 0.05 g of Oxapium Iodide and 3 mg of benzophenone in 100 mL of the mobile phase. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of oxapium and benzophenone in this order with the resolution between these peaks being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of oxapium obtained from 50 μ L of the standard solution composes 5 to 15% of the full scale.

Time span of measurement: About 6 times as long as the retention time of oxapium beginning after the peak of iodide ion.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Oxapium Iodide, previously dried, dissolve in 50 mL of a mixture of acetic an-

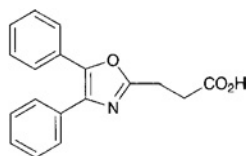
hydride and acetic acid (100) (9:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, platinum electrode). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 47.14 mg of C₂₂H₃₄INO₂

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxaprozin

オキサプロジン



C₁₈H₁₅NO₃: 293.32
3-(4,5-Diphenyloxazol-2-yl)propanoic acid [21256-18-8]

Oxaprozin, when dried, contains not less than 98.5% of C₁₈H₁₅NO₃.

Description Oxaprozin occurs as a white to yellowish white crystalline powder.

It is sparingly soluble in methanol and in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually affected by light.

Identification Determine the infrared absorption spectrum of Oxaprozin, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (285 nm): 455 – 495 (after drying, 10 mg, methanol, 1000 mL).

Melting point <2.60> 161 – 165°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Oxaprozin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Oxaprozin according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Oxaprozin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 5 mL, 3 mL and 1 mL of this solution, add methanol to each to make exactly 10 mL, and use these solutions as the standard solutions (2), (3) and (4), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl

acetate and acetic acid (100) (99:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the total intensity of the spots other than the principal spot from the sample solution is not more than 1.0% calculated on the basis of intensities of the spots from the standard solutions (1), (2), (3) and (4).

Loss on drying <2.41> Not more than 0.3% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

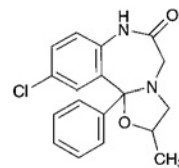
Assay Weigh accurately about 0.5 g of Oxaprozin, previously dried, dissolve in 50 mL of ethanol (95), and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.33 mg of C₁₈H₁₅NO₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxazolam

オキサゾラム



C₁₈H₁₇ClN₂O₂: 328.79
10-Chloro-2-methyl-11b-phenyl-2,3,7,11b-tetrahydro[1,3]oxazolo[3,2-d][1,4]benzodiazepin-6(5H)-one [24143-17-7]

Oxazolam, when dried, contains not less than 99.0% of C₁₈H₁₇ClN₂O₂.

Description Oxazolam occurs as white crystals or crystalline powder.

It is odorless and tasteless.

It is freely soluble in acetic acid (100), soluble in 1,4-dioxane and in dichloromethane, slightly soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It gradually changes in color by light.

Melting point: about 187°C (with decomposition).

Identification (1) Dissolve 0.01 g of Oxazolam in 10 mL of ethanol (95) by heating, and add 1 drop of hydrochloric acid: a light yellow color develops, and the solution shows a yellow-green fluorescence under ultraviolet light (main wavelength: 365 nm). Add 1 mL of sodium hydroxide TS to this solution: the color and fluorescence of this solution disappear immediately.

(2) Dissolve 0.01 g of Oxazolam in 5 mL of dilute hydrochloric acid by heating in a water bath for 10 minutes. After cooling, 1 mL of this solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Place 2 g of Oxazolam in a 200-mL flask, add 50 mL of ethanol (95) and 25 mL of 6 mol/L hydrochloric acid TS,

and boil under a reflux condenser for 5 hours. After cooling, neutralize with a solution of sodium hydroxide (1 in 4), and extract with 30 mL of dichloromethane. Dehydrate with 3 g of anhydrous sodium sulfate, filter, and evaporate the dichloromethane of the filtrate. Dissolve the residue in 20 mL of methanol by heating on a water bath, and cool immediately in an ice bath. Collect the crystals, and dry in vacuum at 60°C for 1 hour: the crystals melt <2.60> between 96°C and 100°C.

(4) Determine the absorption spectrum of a solution of Oxazolam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Proceed with Oxazolam as directed under Flame Coloration Test <1.04> (2), and perform the test: a green color appears.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (246 nm): 410 – 430 (after drying, 1 mg, ethanol (95), 100 mL).

Purity (1) Chloride <1.03>—To 1.0 g of Oxazolam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 25 mL of this filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.20 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Oxazolam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Place 1.0 g of Oxazolam in a Kjeldahl flask, add 5 mL of sulfuric acid and 5 mL of nitric acid, and heat gently. Repeat the addition of 2 to 3 mL of nitric acid at times, and continue to heat until a colorless to light yellow solution is obtained. After cooling, add 15 mL of saturated ammonium oxalate monohydrate solution, heat the solution until dense white fumes are evolved, and evaporate to a volume of 2 to 3 mL. After cooling, dilute with water to 10 mL, and perform the test with this solution as the test solution (not more than 2 ppm).

(4) Related substances—Dissolve 0.05 g of Oxazolam in 10 mL of dichloromethane, and use this solution as the sample solution. Pipet 1 mL of this solution, add dichloromethane to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Immediately air-dry, develop the plate with a mixture of toluene and acetone (8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.65 g of Oxazolam, previously dried, dissolve in 100 mL of a mixture of acetic acid

(100) and 1,4-dioxane (1:1). Titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

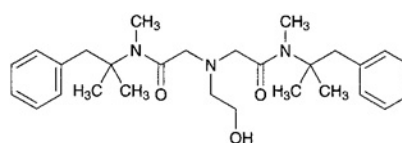
Each mL of 0.1 mol/L perchloric acid VS
= 32.88 mg of $\text{C}_{18}\text{H}_{17}\text{ClN}_2\text{O}_2$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Oxethazaine

Oxetacaine

オキセサゼイン



$\text{C}_{28}\text{H}_{41}\text{N}_3\text{O}_3$: 467.64

2,2'-(2-Hydroxyethylimino)bis[N-(1,1-dimethyl-2-phenylethyl)-N-methylacetamide] [126-27-2]

Oxethazaine, when dried, contains not less than 98.5% of $\text{C}_{28}\text{H}_{41}\text{N}_3\text{O}_3$.

Description Oxethazaine occurs as a white to pale yellowish white, crystalline powder.

Identification (1) Determine the absorption spectrum of a solution of Oxethazaine in ethanol (95) (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxethazaine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 101 – 104°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Oxethazaine in 20 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Oxethazaine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.40 g of Oxethazaine in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of

the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, tetrahydrofuran, methanol and ammonia solution (28) (24:10:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) 2-Aminoethanol—To 1.0 g of Oxethazaine add methanol to make exactly 10 mL, then add 0.1 mL of a solution of 1-fluoro-2,4-dinitrobenzene in methanol (1 in 25), shake well, and heat at 60°C for 20 minutes: the solution has no more color than the following control solution.

Control solution: To 0.10 g of 2-aminoethanol add methanol to make exactly 200 mL, pipet 1 mL of this solution, and add methanol to make exactly 10 mL. Proceed as directed above.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

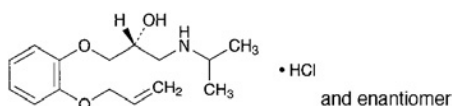
Assay Weigh accurately about 0.9 g of Oxethazaine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 46.76 mg of C₂₈H₄₁N₃O₃

Containers and storage Containers—Tight containers.

Oxprenolol Hydrochloride

オクスプレノロール塩酸塩



C₁₅H₂₃NO₃·HCl: 301.81
(2*RS*)-1-[2-(Allyloxy)phenoxy]-3-(1-methylethyl)aminopropan-2-ol monohydrochloride
[6452-73-9]

Oxprenolol Hydrochloride, when dried, contains not less than 98.5% of C₁₅H₂₃NO₃·HCl.

Description Oxprenolol Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) To 2 mL of a solution of Oxprenolol Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 2 mL of sodium hydroxide TS: a blue-purple color develops. To this solution add 1 mL of diethyl ether, shake well, and allow to stand: a red-purple color develops in the diethyl ether layer, and a blue-purple color develops in the water layer.

(2) To 3 mL of a solution of Oxprenolol Hydrochloride (1 in 150) add 3 drops of Reinecke salt TS: a light red

precipitate is formed.

(3) Determine the infrared absorption spectrum of Oxprenolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Oxprenolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the pH of this solution is between 4.5 and 6.0.

Melting point <2.60> 107 – 110°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Oxprenolol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Oxprenolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Oxprenolol Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.25 g of Oxprenolol Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 4 mL of the sample solution, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate in a developing chamber saturated with ammonia vapor with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxprenolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

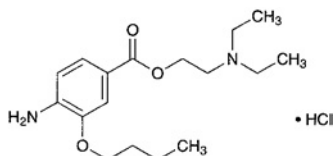
Each mL of 0.1 mol/L perchloric acid VS
= 30.18 mg of C₁₅H₂₃NO₃·HCl

Containers and storage Containers—Tight containers.

Oxybuprocaine Hydrochloride

Benoxinate Hydrochloride

オキシブプロカイン塩酸塩



$C_{17}H_{28}N_2O_3 \cdot HCl$: 344.88

2-(Diethylamino)ethyl 4-amino-3-butyloxybenzoate monohydrochloride [5987-82-6]

Oxybuprocaine Hydrochloride, when dried, contains not less than 99.0% of $C_{17}H_{28}N_2O_3 \cdot HCl$.

Description Oxybuprocaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a saline taste. It exhibits anesthetic properties when placed on the tongue.

It is very soluble in water, freely soluble in ethanol (95) and in chloroform, and practically insoluble in diethyl ether.

The pH of a solution of Oxybuprocaine Hydrochloride (1 in 10) is between 5.0 and 6.0.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Oxybuprocaine Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water. This solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Dissolve 0.1 g of Oxybuprocaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: an oily substance is produced. Rub the inner surface of the container with a glass rod: white crystals are formed. Collect the crystals so obtained, recrystallize from water, and dry in a desiccator (in vacuum, phosphorus (V) oxide) for 5 hours: the crystals melt <2.60> between 103°C and 106°C.

(3) Determine the absorption spectrum of a solution of Oxybuprocaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Oxybuprocaine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 158 – 162°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Oxybuprocaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Oxybuprocaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.25 g of Oxybuprocaine Hydrochloride in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 20 mL. Pipet 1 mL of this solution, add chloroform to make exactly 50 mL,

and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and formic acid (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.6 g of Oxybuprocaine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

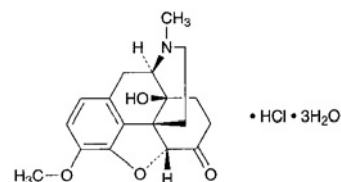
Each mL of 0.1 mol/L perchloric acid VS
= 34.49 mg of $C_{17}H_{28}N_2O_3 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Oxycodone Hydrochloride Hydrate

オキシコドン塩酸塩水和物



$C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$: 405.87

(5R)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one monohydrochloride trihydrate [124-90-3, anhydride]

Oxycodone Hydrochloride Hydrate contains not less than 98.0% of $C_{18}H_{21}NO_4 \cdot HCl$ (mol. wt.: 351.83), calculated on the anhydrous basis.

Description Oxycodone Hydrochloride Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), sparingly soluble in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Oxycodone Hydrochloride Hydrate in 10 mL of water is between 3.8 and 5.8.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Oxycodone Hydrochloride Hydrate (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spec-

trum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Oxycodone Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Oxycodone Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-140 - -149^\circ$ (0.5 g, calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Oxycodone Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 1 mL of water, add 5 mL of 1-nitroso-2-naphthole TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. To this solution add 1 mL of a solution of sodium nitrite (1 in 5000), and warm at 40°C for 5 minutes. After cooling, add 10 mL of chloroform, shake, centrifuge, and separate the water layer: the color of the solution is not more intense than a pale red.

(3) Codeine—Dissolve 10 mg of Oxycodone Hydrochloride Hydrate in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and warm: no blue color is produced. Add 1 drop of nitric acid: no red color develops.

(4) Thebaine—Dissolve 0.10 g of Oxycodone Hydrochloride Hydrate in 2 mL of diluted hydrochloric acid (1 in 10), and heat the solution in a water bath for 25 minutes. After cooling, add 0.5 mL of 4-aminoantipyrine hydrochloride TS and 0.5 mL of a solution of potassium hexacyanoferrate (III) (1 in 100), and shake. Then shake the solution with 2 mL of ammonia TS and 3 mL of chloroform: no red color develops in the chloroform layer.

Water <2.48> 12 – 15% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Oxycodone Hydrochloride Hydrate, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 35.18 \text{ mg of } C_{18}H_{21}NO_4 \cdot HCl \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Compound Oxycodone Injection

Compound Hycodenone Injection

複方オキシコドン注射液

Compound Oxycodone Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more

than 0.86 w/v% of oxycodone hydrochloride hydrate ($C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O$: 405.87), and not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate ($C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O$: 275.73).

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Compound Oxycodone Injection is a clear, colorless to pale yellow liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification (1) To 1 mL of Compound Oxycodone Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

Extractable volume <6.05> It meets the requirement

Assay Pipet 2 mL of Compound Oxycodone Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

$$\begin{aligned} \text{Amount (mg) of oxycodone hydrochloride hydrate} \\ (C_{18}H_{21}NO_4 \cdot HCl \cdot 3H_2O) \\ = W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times 1.1536 \times (1/25) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of hydrocotarnine hydrochloride hydrate} \\ (C_{12}H_{15}NO_3 \cdot HCl \cdot H_2O) \\ = W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times 1.0699 \times (1/25) \end{aligned}$$

W_{Sa} : Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

W_{Sb} : Amount (mg) of hydrocotarnine hydrochloride for assay

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogen phosphate TS add 0.05 mol/L sodium dihydrogen phosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone is about 8 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarnine in this order, with complete separation of these peaks.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Compound Oxycodone and Atropine Injection

Hycoato Injection

複方オキシコドン・アトロピン注射液

Compound Oxycodone and Atropine Injection is an aqueous solution for injection.

It contains not less than 0.74 w/v% and not more than 0.86 w/v% of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄·HCl·3H₂O: 405.87), not less than 0.18 w/v% and not more than 0.22 w/v% of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃·HCl·H₂O: 275.73), and not less than 0.027 w/v% and not more than 0.033 w/v% of atropine sulfate hydrate [(C₁₇H₂₃NO₃)₂·H₂SO₄·H₂O: 694.83].

Method of preparation

Oxycodone Hydrochloride Hydrate	8 g
Hydrocotarnine Hydrochloride Hydrate	2 g
Atropine Sulfate Hydrate	0.3 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Compound Oxycodone and Atropine Injection is a colorless or pale yellow, clear liquid.

It is affected by light.

pH: 2.5 – 4.0

Identification (1) To 1 mL of Compound Oxycodone and Atropine Injection add 1 mL of 2,4-dinitrophenylhydrazine-ethanol TS: a yellow precipitate is formed (oxycodone).

(2) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath, and dissolve the residue in

2 mL of sulfuric acid: a yellow color is produced. Heat the solution: it changes to red, and then to deep orange-red (hydrocotarnine).

(3) Evaporate 1 mL of Compound Oxycodone and Atropine Injection on a water bath. Dissolve the residue in 3 mL of sulfuric acid, add 2 drops of a solution of tannic acid in ethanol (95) (1 in 20), and allow to stand: a deep green color is produced (hydrocotarnine).

(4) To 1 mL of Compound Oxycodone and Atropine Injection add 0.5 mL of 2,4-dinitrophenylhydrazine-ethanol TS, and allow to stand for 1 hour. Centrifuge, and add acetone to the supernatant liquid until no more precipitate is produced. Allow to stand for 20 minutes, and centrifuge. To the supernatant liquid add potassium hydroxide TS until the liquid is light purple. Shake the liquid with 5 mL of dichloromethane, and separate the dichloromethane layer. Take 0.5 mL of the dichloromethane layer, and evaporate to dryness on a water bath. Add 5 drops of fuming nitric acid to the residue, and evaporate to dryness on a water bath. Cool, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced (atropine).

Extractable volume <6.05> It meets the requirement.

Assay (1) Oxycodone hydrochloride hydrate and hydrocotarnine hydrochloride hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of oxycodone hydrochloride for assay and about 0.1 g of hydrocotarnine hydrochloride for assay previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Calculate the ratios, Q_{Ta} and Q_{Sb} , of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of oxycodone and hydrocotarnine to that of the internal standard from the standard solution.

Amount (mg) of oxycodone hydrochloride hydrate (C₁₈H₂₁NO₄·HCl·3H₂O)

$$= W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times 1.1536 \times (1/25)$$

Amount (mg) of hydrocotarnine hydrochloride hydrate (C₁₂H₁₅NO₃·HCl·H₂O)

$$= W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times 1.0699 \times (1/25)$$

W_{Sa} : Amount (mg) of oxycodone hydrochloride for assay, calculated on the anhydrous basis

W_{Sb} : Amount (mg) of hydrocotarnine hydrochloride for assay

Internal standard solution—Dissolve 0.02 g of phenacetin in 10 mL of ethanol (95), and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized polyvinyl alcohol gel polymer for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 500 mL of 0.05 mol/L disodium hydrogenphosphate TS add 0.05 mol/L sodium dihydrogenphosphate TS, and adjust the pH to 8.0. To 300 mL of this solution add 200 mL of acetonitrile, and mix.

Flow rate: Adjust the flow rate so that the retention time of oxycodone hydrochloride is about 8 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions, and use a column giving elution of the internal standard, oxycodone and hydrocotarine in this order with complete separation of these peaks.

(2) Atropine sulfate hydrate—Pipet 2 mL of Compound Oxycodone and Atropine Injection, and add exactly 2 mL of the internal standard solution. To this solution add 10 mL of diluted dilute hydrochloric acid (1 in 10) and 2 mL of ammonia TS, immediately add 20 mL of dichloromethane, shake vigorously, filter the dichloromethane layer through filter paper on which 5 g of anhydrous sodium sulfate is placed, and evaporate the filtrate to dryness under reduced pressure. To the residue add 0.5 mL of 1,2-dichloromethane and 0.5 mL of bis-trimethylsilylacetamide, stopper tightly, warm in a water bath at 60°C for 15 minutes, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Atropine Sulfate Reference Standard (separately determine its loss on drying <2.41> in the same manner as directed under Atropine Sulfate Hydrate), and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, and add exactly 2 mL of the internal standard solution. Proceed with this solution in the same manner as directed for the sample solution, and use so obtained solution as the standard solution. Perform the test with 2 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of atropine to that of the internal standards.

$$\begin{aligned} & \text{Amount (mg) of atropine sulfate hydrate} \\ & [(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4 \cdot H_2O] \\ & = W_S \times (Q_T/Q_S) \times (1/50) \times 1.027 \end{aligned}$$

W_S : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of homatropine hydrobromide (1 in 4000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 1.5 m in length, packed with 180- to 250- μ m siliceous earth for gas chromatography coated with 1 to 3% of 50% phenyl-methylsilicone polymer.

Column temperature: A constant temperature of about 210°C.

Carrier gas: Nitrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of atropine is about 5 minutes.

Selection of column: Proceed with 2 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and atropine in this order with the resolution between these peaks being not less than 3.

Containers and storage Containers—Hermetic containers,

and colored containers may be used.

Storage—Light-resistant.

Oxydol

オキシドール

Oxydol contains not less than 2.5 w/v% and not more than 3.5 w/v% of hydrogen peroxide (H₂O₂: 34.01). It contains suitable stabilizers.

Description Oxydol occurs as a clear, colorless liquid. It is odorless or has an odor resembling that of ozone.

It gradually decomposes upon standing or upon vigorous agitation.

It rapidly decomposes when in contact with oxidizing substances as well as reducing substances.

It, when alkalized, decomposes with effervescence.

It is affected by light.

pH: 3.0 – 5.0

Specific gravity d_{20}^{20} : about 1.01

Identification 1 mL of Oxydol responds to the Qualitative Tests <1.09> for peroxide.

Purity (1) Acidity—To 25.0 mL of Oxydol add 2 drops of phenolphthalein TS and 2.5 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Heavy metals <1.07>—To 5.0 mL of Oxydol add 20 mL of water and 2 mL of ammonia TS, evaporate on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid by heating, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2 mL of dilute acetic acid, 2.5 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(3) Arsenic <1.11>—To 1.0 mL of Oxydol add 1 mL of ammonia TS, evaporate on a water bath to dryness, take the residue, prepare the test solution according to Method 1, and perform the test (not more than 2 ppm).

(4) Organic stabilizer—Extract 100 mL of Oxydol with 50-mL, 25-mL and 25-mL portions of a mixture of chloroform and diethyl ether (3:2) successively, combine the extracts in a tared vessel, and evaporate the combined extract on a water bath. Dry the residue over silica gel to constant mass: the mass of the residue is not more than 50 mg.

(5) Nonvolatile residue—Evaporate 20.0 mL of Oxydol on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 20 mg.

Assay Pipet 1.0 mL of Oxydol, transfer it to a flask containing 10 mL of water and 10 mL of dilute sulfuric acid, and titrate <2.50> with 0.02 mol/L potassium permanganate VS.

$$\begin{aligned} & \text{Each mL of 0.02 mol/L potassium permanganate VS} \\ & = 1.701 \text{ mg of H}_2\text{O}_2 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 30°C.

Oxygen

酸素

O₂: 32.00

Oxygen contains not less than 99.5 v/v% of O₂.

Description Oxygen is a colorless gas, and is odorless.

1 mL of Oxygen dissolves in 32 mL of water, and in 7 mL of ethanol (95) at 20°C and at a pressure of 101.3 kPa.

1000 mL of Oxygen at 0°C and at a pressure of 101.3 kPa weighs about 1.429 g.

Identification (1) Put a glowing splinter of wood into Oxygen: it bursts into flame immediately.

(2) Transfer 1 mL each of Oxygen and oxygen directly from metal cylinders with a pressure-reducing valve to gas-measuring tubes or syringes for gas chromatography, using a polyvinyl chloride induction tube. Perform the test with these gases as directed under Gas Chromatography <2.02> according to the conditions of Purity (5): the retention time of principal peak from Oxygen coincides with that of oxygen.

Purity Keep the containers of Oxygen between 18°C and 22°C for not less than 6 hours before carrying out the following tests, and calculate the volume to be used with reference to the gas at 20°C and at 101.3 kPa.

(1) Acidity or alkalinity—To 400 mL of freshly boiled and cooled water add 0.3 mL of methyl red TS and 0.3 mL of bromothymol blue TS, and boil for 5 minutes. Transfer 50 mL of this solution to each of three Nessler tubes marked A, B and C. Add 0.10 mL of 0.01 mol/L hydrochloric acid VS to tube A, 0.20 mL of 0.01 mol/L hydrochloric acid VS to tube B, stopper each of the tubes, and cool. Pass 1000 mL of Oxygen through the solution in tube A for 15 minutes, employing delivery tube with an orifice approximately 1 mm in diameter and extending to within 2 mm of the bottom of the Nessler tube: the color of the solution in tube A is not deeper orange-red than that of the solution in tube B and not deeper yellow-green than that of the solution in tube C.

(2) Carbon dioxide—Pass 1000 mL of Oxygen through 50 mL of barium hydroxide TS in a Nessler tube, in the same manner as directed in (1): any turbidity produced does not exceed that of the following control solution.

Control solution: To 50 mL of barium hydroxide TS in a Nessler tube add 1 mL of a solution of 0.1 g of sodium hydrogen carbonate in 100 mL of freshly boiled and cooled water.

(3) Oxidizing substances—Transfer 15 mL of potassium iodide-starch TS to each of two Nessler tubes marked A and B, add 1 drop of acetic acid (100) to each of the tubes, mix, and use these as solution A and solution B, respectively. Pass 2000 mL of Oxygen through solution A for 30 minutes in the same manner as directed in (1): the color of solution A is the same as that of the stoppered, untreated solution B.

(4) Chloride <1.03>—Pour 50 mL of water into each of two Nessler tubes marked A and B, add 0.5 mL of silver nitrate TS to each of the tubes, mix, and use these as solution A and solution B, respectively. Pass 1000 mL of Oxygen through solution A in the same manner as directed in (1): the turbidity

of solution A is the same as that of solution B.

(5) Nitrogen—Introduce 1.0 mL of Oxygen into a gas-measuring tube or syringe for gas chromatography from a metal hermetic container under pressure through a pressure-reducing valve and a directly connected polyvinyl tube. Perform the test as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak area A_T of nitrogen. Introduce 0.50 mL of nitrogen into the gas mixer, draw carrier gas into the mixer to make exactly 100 mL, and allow to mix thoroughly. Perform the test in the same manner with 1.0 mL of this mixture as directed above, and determine the peak area A_S of nitrogen: A_T is not larger than A_S .

Operating conditions—

Detector: A thermal-conductivity detector.

Column: A column about 3 mm in inside diameter and about 3 m in length, packed with 250- to 355- μ m zeolite for gas chromatography (0.5 mm).

Column temperature: A constant temperature of about 50°C.

Carrier gas: Hydrogen or helium.

Flow rate: Adjust the flow rate so that the retention time of nitrogen is about 5 minutes.

Selection of column: Introduce 0.5 mL of nitrogen into a gas mixer, add Oxygen to make 100 mL, and mix well. Proceed with 1.0 mL of the mixture under the above operating conditions. Use a column giving well-resolved peaks of Oxygen and nitrogen in this order.

Assay (i) Apparatus—The apparatus is shown diagrammatically in the accompanying figure. A is a 100-mL gas buret having a two-way stopcock a, b - c, d - e and e - f are graduated in 0.1 mL, and c - d is graduated in 2 mL. A is properly connected with a leveling tube B by a thick rubber tube. Fill ammonium chloride-ammonia TS up to the middle of A and B. Place in the absorption ball g of the gas pipette C a coil of copper wire, not more than 2 mm in diameter, which extends to the uppermost portion of the bulb, add 125 mL of ammonium chloride-ammonia TS, and stopper with a rubber stopper i. Connect C with A using the thick rubber tube.

(ii) Procedure—Open a, set B downward and draw the liquid in g to the stopcock opening a. Then close a. Open a to the intake tube h, and fill A and h with ammonium chloride-ammonia TS by lifting B. Close a, connect h with a container of Oxygen, open a, set B downward and measure accurately 100 mL of Oxygen. Open a toward C, and transfer the Oxygen to g by lifting B. Close a, and rock C gently for 5 minutes. Open a, draw the residual gas back into A by setting B downward, and measure the volume of the residual gas. Repeat the procedure until the volume of residual gas is constant, and designate this as V (mL). With fresh ammonium chloride-ammonia TS in C, repeat the procedure at least four times, and measure the volume of residual gas. Calculate V and the volume of Oxygen used as the sample with reference to the gas at 20°C and at 101.3 kPa.

$$\begin{aligned} & \text{Volume (mL) of oxygen (O}_2\text{)} \\ &= \text{calculated volume of the sample (mL)} \\ & \quad - \text{calculated volume of } V \text{ (mL)} \end{aligned}$$

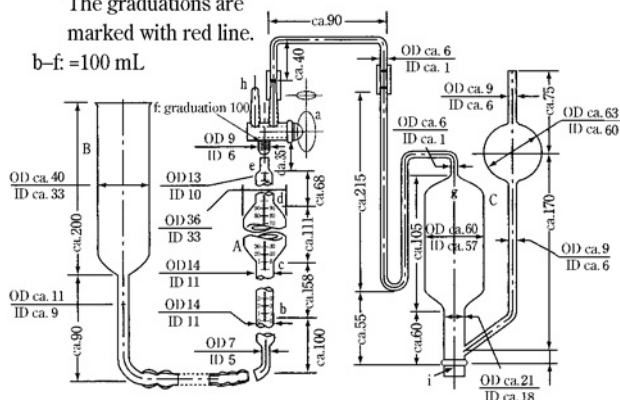
Containers and storage Containers—Metal cylinders.

Storage—Not exceeding 40°C.

b-c: calibrated in 0.1 mL
 c-d: calibrated in 2 mL
 d-e: calibrated in 0.1 mL
 e-f: calibrated in 0.1 mL

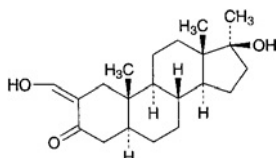
The graduations are marked with red line.

b-f: =100 mL



Oxymetholone

オキシメトロン



$C_{21}H_{32}O_3$: 332.48

17β-Hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one [434-07-1]

Oxymetholone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{32}O_3$.

Description Oxymetholone occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in 1,4-dioxane, sparingly soluble in methanol, in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It is gradually colored and decomposed by light.

Identification (1) Dissolve 2 mg of Oxymetholone in 1 mL of ethanol (95), and add 1 drop of iron (III) chloride TS: a purple color develops.

(2) Dissolve 0.01 g of Oxymetholone in methanol to make 50 mL. To 5 mL of the solution add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Oxymetholone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +34 – +38° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 175 – 182°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Oxymetholone in 25 mL of 1,4-dioxane: the solution is clear, and shows a colorless to pale yellow color.

(2) Related substances—Dissolve 50 mg of Oxymetholone in 5 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the spot. Develop immediately the plate with a mixture of toluene and ethanol (99.5) (49:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 100°C for 3 to 5 minutes: any spot other than the principal spot and starting point obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

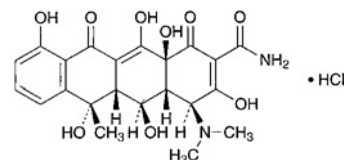
Assay Weigh accurately about 40 mg of Oxymetholone, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 5 mL of this solution, and add methanol to make exactly 50 mL. To exactly measured 5 mL of this solution add 5 mL of sodium hydroxide-methanol TS and methanol to make exactly 50 mL. Determine the absorbance *A* of this solution at the wavelength of maximum absorption at about 315 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared by adding methanol to 5 mL of sodium hydroxide-methanol TS to make 50 mL, as the blank.

$$\begin{aligned} \text{Amount (mg) of } C_{21}H_{32}O_3 \\ = (A/541) \times 50,000 \end{aligned}$$

Containers and storage Containers—Tight containers.
 Storage—Light-resistant.

Oxytetracycline Hydrochloride

オキシテトラサイクリン塩酸塩



$C_{22}H_{24}N_2O_9 \cdot HCl$: 496.89

(4*S*,4*aR*,5*S*,5*aR*,6*S*,12*aS*)-4-Dimethylamino-3,5,6,10,12,12*a*-hexahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide monohydrochloride [2058-46-0]

Oxytetracycline Hydrochloride is the hydrochloride

of a tetracycline substance having antibacterial activity produced by the growth of *Streptomyces rimosus*.

It contains not less than 880 μg (potency) and not more than 945 μg (potency) per mg, calculated on the dried basis. The potency of Oxytetracycline Hydrochloride is expressed as mass (potency) of oxytetracycline ($\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_9$; 460.43).

Description Oxytetracycline Hydrochloride occurs as yellow, crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Oxytetracycline Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of Oxytetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 20 mg of Oxytetracycline Hydrochloride in 3 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-188 - -200^\circ$ (0.25 g calculated on the dried basis, 0.1 mol/L hydrochloric acid, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Oxytetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 50 ppm).

(2) Related substances—Dissolve 20 mg of Oxytetracycline Hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of 4-epioxytetracycline in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as 4-epioxytetracycline stock solution. Separately, dissolve 20 mg of tetracycline hydrochloride in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and use this solution as tetracycline hydrochloride stock solution. Separately, dissolve 8 mg of β -apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as β -apooxytetracycline stock solution. Pipet 1 mL of 4-epioxytetracycline stock solution, 4 mL of tetracycline hydrochloride stock solution and 40 mL of β -apooxytetracycline stock solution, add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 4-epioxytetracycline and tetracycline obtained from the sample solution are not more than each of the peak area obtained from the standard solution, and the total area of the peaks, α -apooxytetracycline having the relative retention time of about 2.1 with respect to oxytetracycline, β -apooxytetracycline and the peaks, which appear between α -apooxytetracycline and β -apooxytetracycline, is not more than the peak area of β -apooxytetracycline from the standard solution. The peak area of 2-acetyl-2-decarboxamide oxytetracycline, which appears after the prin-

cipal peak, obtained from the sample solution is not more than 4 times the peak area of 4-epioxytetracycline from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (8 μm in particle diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase A: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 100 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 30 g of *t*-butanol and water to make 1000 mL.

Mobile phase B: Mix 60 mL of 0.33 mol/L potassium dihydrogen phosphate TS, 50 mL of a solution of tetrabutylammonium hydrogensulfate (1 in 100), 10 mL of a solution of disodium dihydrogen ethylenediamine tetraacetate dihydrate (1 in 2500) and 200 mL of water, and adjust the pH to 7.5 with 2 mol/L sodium hydroxide TS. To this solution add 100 g of *t*-butanol and water to make 1000 mL.

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 20	70 → 10	30 → 90
20 - 35	10 → 20	90 → 80

Flow rate: 1.0 mL/min

Time span of measurement: About 3.5 times as long as the retention time of oxytetracycline beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Pipet 4 mL of this solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 20 mL. Confirm that the peak area of 4-epioxytetracycline obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Dissolve 8 mg of α -apooxytetracycline in 5 mL of 0.01 mol/L sodium hydroxide TS, add 0.01 mol/L hydrochloric acid TS to make 100 mL, and use this solution as α -apooxytetracycline stock solution. Mix 3 mL of the sample solution, 2 mL of 4-epioxytetracycline stock solution, 6 mL of tetracycline hydrochloride stock solution, 6 mL of β -apooxytetracycline stock solution and 6 mL of α -apooxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-epioxytetracycline, oxytetracycline, tetracycline, α -apooxytetracycline and β -apooxytetracycline are eluted in this order with the resolutions between the peaks, 4-epioxytetracycline and oxytetracycline, oxytetracycline and tetracycline, and α -apooxytetracycline and β -apooxytetracycline being not less than 4, not less than 5 and not less than 4, respectively,

and the symmetry coefficient of the peak of oxytetracycline is not more than 1.3.

System repeatability: Pipet 1 mL of 4-epioxytetracycline stock solution, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. When the test is repeated 6 times with 20 μ L of this solution under the above operating conditions, the relative standard deviation of the peak area of 4-epioxytetracycline is not more than 2.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Weigh accurately an amount of Oxytetracycline Hydrochloride and Oxytetracycline Hydrochloride Reference Standard, equivalent to about 50 mg (potency), and dissolve each in diluted hydrochloric acid (1 in 100) to make exactly 50 mL. Pipet 5 mL each of these solutions, add diluted methanol (3 in 20) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of oxytetracycline.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of oxytetracycline (C}_{22}\text{H}_{24}\text{N}_2\text{O}_9) \\ = W_S \times (A_T/A_S) \times 1000$$

W_S : Amount [mg (potency)] of Oxytetracycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 263 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with strongly acidic ion exchange resin for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 3.402 g of potassium dihydrogen phosphate and 9.306 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 700 mL of water, add 300 mL of methanol, and adjust the pH to 4.5 with dilute hydrochloric acid.

Flow rate: Adjust the flow rate so that the retention time of oxytetracycline is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the theoretical plates and the symmetrical coefficient of the peak of oxytetracycline are not less than 1000 and not more than 2.0, respectively.

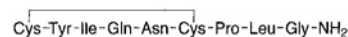
System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Oxytocin

オキシトシン



$\text{C}_{43}\text{H}_{66}\text{N}_{12}\text{O}_{12}\text{S}_2$: 1007.19
[50-56-6]

Oxytocin is a synthetic peptide having the property of causing the contraction of uterine smooth muscle.

It contains not less than 540 oxytocin Units and not more than 600 oxytocin Units per mg, calculated on the dehydrated and de-acetic acid basis.

Description Oxytocin occurs as a white powder.

It is very soluble in water, and freely soluble in ethanol (99.5).

It dissolves in hydrochloric acid TS.

The pH of a solution prepared by dissolving 0.10 g of Oxytocin in 10 mL of freshly boiled and cooled water is between 4.0 and 6.0.

It is hygroscopic.

Identification Determine the absorption spectrum of a solution of Oxytocin (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Constituent amino acids Put about 1 mg of Oxytocin in a test tube for hydrolysis, add 6 mol/L hydrochloric acid TS to dissolve, replace the air in the tube with Nitrogen, seal the tube under reduced pressure, and heat at 110 to 115°C for 16 hours. After cooling, open the tube, evaporate the hydrolyzate to dryness under reduced pressure, add 2 mL of 0.02 mol/L hydrochloric acid TS to dissolve the residue, and use this solution as the sample solution. Separately, weigh accurately about 27 mg of L-aspartic acid, about 24 mg of L-threonine, about 21 mg of L-serine, about 29 mg of L-glutamic acid, about 23 mg of L-proline, about 15 mg of glycine, about 18 mg of L-alanine, about 23 mg of L-valine, about 48 mg of L-cystine, about 30 mg of methionine, about 26 mg of L-isoleucine, about 26 mg of L-leucine, about 36 mg of L-tyrosine, about 33 mg of phenylalanine, about 37 mg of L-lysine hydrochloride, about 42 mg of L-histidine hydrochloride monohydrate and about 42 mg of L-arginine hydrochloride, dissolve them in 10 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the respective molar ratios with respect to leucine: 0.95 – 1.05 for aspartic acid, 0.95 – 1.05 for glutamic acid, 0.95 – 1.05 for proline, 0.95 – 1.05 for glycine, 0.80 – 1.10 for isoleucine, 0.80 – 1.05 for tyrosine and 0.80 – 1.05 for cystine, and not more than 0.01 each for others.

Operating conditions—

Detector: A visible spectrophotometer (wavelength: 440 nm and 570 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 8 cm in length, packed with strongly acidic ion-exchange resin for liquid chromatography (sodium type) composed with a sulfonated polystyrene copolymer (3 μm in particle diameter).

Column temperature: A constant temperature of about 57°C.

Chemical reaction bath temperature: A constant temperature of about 130°C.

Color developing time: About 1 minute.

Mobile phase: Prepare mobile phases A, B and C according to the following table.

Mobile phase	A	B	C
Citric acid monohydrate	19.80 g	22.00 g	6.10 g
Trisodium citrate dihydrate	6.19 g	7.74 g	26.67 g
Sodium chloride	5.66 g	7.07 g	54.35 g
Ethanol (99.5)	260.0 mL	20.0 mL	—
Benzyl alcohol	—	—	5.0 mL
Thiodiglycol	5.0 mL	5.0 mL	—
Lauromacrogol solution (1 in 4)	4.0 mL	4.0 mL	4.0 mL
Caprylic acid	0.1 mL	0.1 mL	0.1 mL
Water	a sufficient amount	a sufficient amount	a sufficient amount
Total amount	2000 mL	1000 mL	1000 mL
pH	3.3	3.2	4.9

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 – 9	100	0	0
9 – 25	0	100	0
25 – 61	0	100 → 0	0 → 100
61 – 80	0	0	100

Reaction reagent: Mix 407 g of lithium acetate dihydrate, 245 mL of acetic acid (100) and 801 mL of 1-methoxy-2-propanol, add water to make 2000 mL, stir for more than 10 minutes while passing Nitrogen, and use this solution as Solution A. Separately, to 1957 mL of 1-methoxy-2-propanol add 77 g of ninhydrin and 0.134 g of sodium borohydride, stir for more than 30 minutes while passing Nitrogen, and use this solution as Solution B. Mix Solution A and Solution B before use.

Flow rate of mobile phase: About 0.26 mL per minute.

Flow rate of reaction reagent: About 0.3 mL per minute.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are eluted in this order with the resolutions between

the peaks of threonine and serine, glycine and alanine, and isoleucine and leucine being not less than 1.5, 1.4 and 1.2, respectively.

System repeatability: When the test is repeated 3 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of aspartic acid, proline, valine and arginine are not more than 2.0%, respectively.

Purity (1) Acetic acid—Weigh accurately about 15 mg of Oxytocin, dissolve in the internal standard solution to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of acetic acid (100), add the internal standard solution to make exactly 100 mL. Pipet 2 mL of this solution, add the internal standard solution to make exactly 200 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of acetic acid to that of the internal standard: the amount of acetic acid is not less than 6.0% and not more than 10.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of acetic acid (C}_2\text{H}_4\text{O}_2\text{)} \\ = (W_S/W_T) \times (Q_T/Q_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of acetic acid (100)

W_T : Amount (mg) of the sample

Internal standard solution—A solution of propionic acid in the mobile phase (1 in 10,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 0.7 mL of phosphoric acid add 900 mL of water, adjust the pH to 3.0 with 8 mol/L sodium hydroxide TS, and add water to make 1000 mL. To 950 mL of this solution add 50 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 3 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 14.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of acetic acid to that of the internal standard is not more than 2.0%.

(2) Related substances—Dissolve 25 mg of Oxytocin in 100 mL of the mobile phase A, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of them by the area percentage method: the amount

of each peak other than Oxytocin is not more than 1.5%, and the total of them is not more than 5.0%.

Operating conditions—

Detector, column, column temperature, mobile phase, flowing of mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of oxytocin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of oxytocin obtained from 50 μ L of this solution is equivalent to 5 to 15% of that from 50 μ L of the solution for system suitability test.

System performance: Dissolve an adequate amount of oxytocin and vasopressin in the mobile phase A, so that each mL contains about 0.1 mg each of them. When the procedure is run with 50 μ L of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 50 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

Water <2.48> Not more than 5.0% (50 mg, coulometric titration).

Assay Weigh accurately an amount of Oxytocin, equivalent to about 13,000 Units, dissolve in the mobile phase A to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 1 bottle of the Oxytocin Reference Standard in the mobile phase A to make a known concentration solution containing each mL contains about 130 Units, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of oxytocin.

Units per mg of Oxytocin, calculated on the dehydrated and de-acetic acid basis

$$= (W_S / W_T) \times (A_T / A_S) \times 100$$

W_S : Units per mL of the standard solution

W_T : Amount (mg) of sample, calculated on the dehydrated and de-acetic acid basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	70→40	30→60
30 – 30.1	40→70	60→30
30.1 – 45	70	30

Flow rate: About 1.0 mL per minute.

System suitability—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 20 mL of the mobile phase A. When the procedure is run with 25 μ L of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—At 2 to 8°C.

Oxytocin Injection

オキシトシン注射液

Oxytocin Injection is an aqueous solution for injection.

It contains not less than 90.0% and not more than 110.0% of the labeled oxytocin Units.

Method of preparation Prepare as directed under Injections, with Oxytocin.

Description Oxytocin Injection is a colorless, clear liquid.

pH <2.54> 2.5 – 4.5

Bacterial endotoxins <4.01> Less than 10 EU/oxytocin Unit.

Extractable volume <6.05> It meets the requirement

Foreign insoluble matter <6.06> Perform the test according to the Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Measure exactly a portion of Oxytocin Injection according to the labeled Units, dilute with the diluent so that each mL contains about 1 Unit, and use this solution as the sample solution. Separately, dissolve 1 bottle of Oxytocin Reference Standard in the mobile phase A to make exactly 20 mL. Pipet a suitable volume of this solution, dilute with the diluent to make a known concentration solution so that each

mL contains about 1 Unit, and use this solution as the standard solution. Perform the test with exactly 100 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of oxytocin.

$$\begin{aligned} &\text{Units per mL of Oxytocin Injection} \\ &= W_S \times (A_T/A_S) \times (b/a) \end{aligned}$$

W_S : Units per mL of the standard solution

a : Volume (mL) of sample

b : Total volume of the sample solution prepared by diluting with the diluent

Diluent: Dissolve 5 g of chlorobutanol, 1.1 g of sodium acetate trihydrate, 5 g of acetic acid (100) and 6 mL of ethanol (99.5) in water to make 1000 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 15.6 g of sodium dihydrogen phosphate dihydrate in 1000 mL of water.

Mobile phase B: A mixture of water and acetonitrile (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 30	70→40	30→60
30 – 30.1	40→70	60→30
30.1 – 45	70	30

Flow rate: About 1.0 mL per minute.

System suitability—

System performance: Dissolve 2 mg each of oxytocin and vasopressin in 100 mL of the mobile phase A. When the procedure is run with 100 μ L of this solution under the above operating conditions, vasopressin and oxytocin are eluted in this order with the resolution between these peaks being not less than 14, and the symmetry factor of the peak of oxytocin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 100 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of oxytocin is not more than 2.0%.

Containers and storage Containers—Hermetic containers.

Storage—In a cold place, and avoid freezing.

Pancreatin

パンクレアチン

Pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog, and has amylolytic, proteolytic and lipolytic activities.

It contains not less than 2800 starch saccharifying activity units, not less than 28,000 proteolytic activity units, and not less than 960 lipolytic activity units per g.

It is usually diluted with suitable excipients.

Description Pancreatin occurs as a white to light yellow powder. It has a characteristic odor.

Purity (1) Rancidity—Pancreatin has no unpleasant or rancid odor and is tasteless.

(2) Fat—Add 20 mL of diethyl ether to 1.0 g of Pancreatin, extract with occasional shaking for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, combine the washing with the filtrate, evaporate the diethyl ether, and dry the residue at 105°C for 2 hours: the mass of the residue does not exceed 20 mg.

Loss on drying <2.41> Not more than 4.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 5% (1 g).

Assay (1) Starch digestive activity <4.03>

(i) Substrate solution—Use potato starch TS for amylolytic activity test, prepared by adding 10 mL of phosphate buffer solution for pancreatin instead of 10 mL of 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 100 mL. Pipet 10 mL of this solution, and add ice-cold water to make exactly 100 mL.

(iii) Procedure—Proceed as directed in (i) Assay for starch digestive activity under Digestion Test.

(2) Protein digestive activity <4.03>

(i) Substrate solution—Use the substrate solution 2 described in (2) Assay for protein digestive activity under Digestion Test after adjusting the pH to 8.5.

(ii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and add ice-cold water to make exactly 200 mL.

(iii) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test, using trichloroacetic acid TS B as the precipitation reagent.

(3) Fat digestive activity <4.03>

(i) Emulsifier—Prepare with 18 g of polyvinyl alcohol I and 2 g of polyvinyl alcohol II as directed in (3) Assay for fat digestive activity under Digestion Test.

(ii) Substrate solution—Use the substrate solution described in (3) Assay for fat digestive activity under the Digestion Test.

(iii) Sample solution—Weigh accurately about 0.1 g of Pancreatin, add a suitable amount of ice-cold water, stir, and

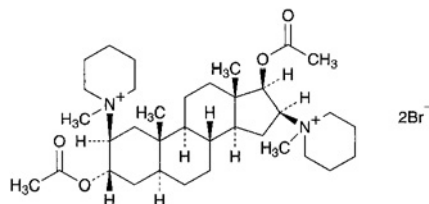
add ice-cold water to make exactly 100 mL.

(iv) Procedure—Proceed as directed in (3) Assay for fat digestive activity under Digestion Test, using phosphate buffer solution, pH 8.0, as the buffer solution.

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Pancuronium Bromide

パンクロニウム臭化物



$C_{35}H_{60}Br_2N_2O_4$: 732.67

1,1'-(3 α ,17 β -Diacetoxy-5 α -androstan-2 β ,16 β -diyl)bis(1-methylpiperidinium) dibromide [15500-66-0]

Pancuronium Bromide contains not less than 98.0% and not more than 102.0% of $C_{35}H_{60}Br_2N_2O_4$, calculated on the dehydrated basis.

Description Pancuronium Bromide occurs as a white crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95) and in acetic anhydride.

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Pancuronium Bromide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Pancuronium Bromide (1 in 100) responds to the Qualitative Tests <1.09> (1) for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: +38 – +42° (0.75 g calculated on the dehydrated basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution of Pancuronium Bromide (1 in 100) is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pancuronium Bromide in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 50 mg of Pancuronium Bromide in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (1). Separately, weigh exactly 5 mg of dacturionium bromide for thin-layer chromatography, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, acetonitrile and a solution of sodium iodide (1 in 5) (17:2:1) to a distance of about

12 cm, and air-dry the plate. Spray evenly a solution of sodium nitrite in methanol (1 in 100) on the plate, allow to stand for 2 minutes, and spray evenly potassium bismuth iodide TS on the plate: a spot from the sample solution, corresponding to that from the standard solution (2), has no more color than that from the standard solution (2), and the spots other than the principal spot and the above mentioned spot from the sample solution have no more color than the spot from the standard solution (1).

Water <2.48> Not more than 8.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

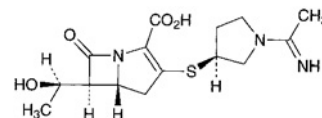
Assay Weigh accurately about 0.2 g of Pancuronium Bromide, dissolve in 50 mL of acetic anhydride by warming, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 36.63 mg of $C_{35}H_{60}Br_2N_2O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Panipenem

パニペネム



$C_{15}H_{21}N_3O_4S$: 339.41

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-[(3*S*)-1-(1-iminoethyl)pyrrolidin-3-ylsulfanyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid [87726-17-8]

Panipenem contains not less than 900 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the anhydrous and desolvent basis. The potency of Panipenem is expressed as mass (potency) of panipenem ($C_{15}H_{21}N_3O_4S$).

Description Panipenem occurs as a white to light yellow, crystalline powder or mass.

It is very soluble in water, freely soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is hygroscopic.

It deliquesces in the presence of moisture.

Identification (1) Dissolve 0.02 g of Panipenem in 2 mL of water, add 1 mL of hydroxylammonium chloride-ethanol TS, allow to stand for 3 minutes, add 1 mL of acidic ammonium iron (III) sulfate TS, and shake: a red-brown color develops.

(2) Determine the absorption spectrum of a solution of Panipenem in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a

maximum between 296 nm and 300 nm.

(3) Determine the infrared absorption spectrum of Panipenem as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 1760 cm^{-1} , 1676 cm^{-1} , 1632 cm^{-1} , 1588 cm^{-1} , 1384 cm^{-1} and 1249 cm^{-1} .

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (298 nm): 280 – 310 (50 mg calculated on the anhydrous and desolvent basis, 0.02 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, 2500 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: +55 – +65° (0.1 g, calculated on the anhydrous and desolvent basis, 0.1 mol/L 3-(N-morpholino)propanesulfonic acid buffer solution, pH 7.0, 10 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of Panipenem in 10 mL of water: the pH of the solution is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Panipenem according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Residual solvents <2.46>—Weigh accurately about 0.2 g of Panipenem, transfer to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution and 2 mL of water to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, pipet 15 mL of ethanol (99.5) and 3 mL of acetone, add water to make exactly 200 mL. Pipet 1 mL and 2 mL of this solution, and add water to them to make exactly 20 mL. Transfer exactly 2 mL each of these solutions to a 20-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution, seal tightly a rubber stopper with aluminum cap, and use these solutions as the standard solution (1) and the standard solution (2). Shake gently in a water bath at a constant room temperature, and allow to stand for 30 minutes. Perform the test with 1 mL of the sample gas in each container as directed under Gas Chromatography <2.02> according to the following condition. Calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of ethanol and acetone to that of the internal standard from the sample solution, the ratios, Q_{Sa1} and Q_{Sb1} , of the peak area of ethanol and acetone to that of the internal standard from the standard solution (1), and the ratios, Q_{Sa2} and Q_{Sb2} , of the peak area of ethanol and acetone to that of the internal standard from the standard solution (2). Calculate the amount of the ethanol and acetone by the following formula: ethanol is not more than 5.0% and acetone is not more than 1.0%.

$$\begin{aligned} &\text{Amount (\%)} \text{ of ethanol in Panipenem} \\ &= 15 \times 0.79 \times \{(Q_{Ta} + Q_{Sa2} - 2Q_{Sa1})/2(Q_{Sa2} - Q_{Sa1})\} \\ &\quad \times (1/1000) \times (100/W) \end{aligned}$$

W : Amount (g) of Panipenem

$$\begin{aligned} &\text{Amount (\%)} \text{ of acetone in Panipenem} \\ &= 3 \times 0.79 \times \{(Q_{Tb} + Q_{Sb2} - 2Q_{Sb1})/2(Q_{Sb2} - Q_{Sb1})\} \\ &\quad \times (1/1000) \times (100/W) \end{aligned}$$

W : amount (g) of Panipenem

0.79: Specific gravity (d_{20}^{20}) of ethanol (99.5) and acetone

Internal standard solution—A solution of 1-propanol (1 in

400).

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: A glass column 1 mm in inside diameter and 40 m in length, coated with porous polymer bead for gas chromatography.

Column temperature: A constant temperature of about 140°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of 1-propanol is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 mL of the gas of the standard solution (2) under the above operating conditions, ethanol, acetone and the internal standard are eluted in this order with the resolution between ethanol and acetone being not less than 4.

System repeatability: When the test is repeated 6 times with 1 mL of the gas of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of ethanol to that of the internal standard is not more than 5.0%.

(4) Related substances—Being specified separately.

Water <2.48> Weigh accurately about 0.5 g of Panipenem, transfer to a 15-mL narrow-mouthed cylindrical glass bottle, add exactly 2 mL of the internal standard solution to dissolve, seal tightly a rubber stopper with aluminum cap, and use this solution as the sample solution. Separately, weigh accurately 2 g of water, and add the internal standard solution to make exactly 100 mL. Pipet 5 mL and 10 mL of this solution, add the internal standard solution to make exactly 20 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with 1 μL of the sample solution and standard solutions (1) and (2) as directed under Gas Chromatography <2.02> according to the following condition, and calculate the ratios, Q_T , Q_{S1} and Q_{S2} of the peak area of water to that of the internal standard. Calculate the amount of water by the following formula: water is not more than 5.0%.

$$\begin{aligned} &\text{Amount of water (\%)} \\ &= (W_S/W_T) \times \{(Q_T + Q_{S2} - 2Q_{S1})/2(Q_{S2} - Q_{S1})\} \\ &\quad \times (1/100) \times 100 \end{aligned}$$

W_S : Amount (g) of weighed water

W_T : Amount (g) of Panipenem

Internal standard solution—A solution of acetonitrile in methanol (1 in 100).

Operating conditions—

Detector: A thermal conductivity detector

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with porous ethylvinylbenzene-divinylbenzene copolymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of about 125°C.

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of acetonitrile is about 8 minutes.

System suitability—

System performance: When the procedure is run with 1 μL of the standard solution (2) under the above operating conditions, water, methanol, and the internal standard are eluted

in this order with the resolution between water and internal standard being not less than 10.

System repeatability: When the test is repeated 6 times with 1 μL of the standard solution (2) under the above operating conditions, the relative standard deviation of the ratios of the peak area of water to that of the internal standard is not more than 5.0%.

Residue on ignition Being specified separately.

Bacterial endotoxins <4.01> Less than 0.15 EU/mg (potency).

Assay Weigh accurately an amount of Panipenem and Panipenem Reference Standard, equivalent to about 0.1 g (potency), dissolve separately in 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test within 30 minutes after preparation of the solutions with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of panipenem to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of panipenem (C}_{15}\text{H}_{21}\text{N}_3\text{O}_4\text{S)} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Panipenem Reference Standard

Internal standard solution—A solution of sodium *p*-styrenesulfonate in 0.02 mol / L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silicone polymer coated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.02 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 8.0 and acetonitrile (50:1).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, panipenem and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

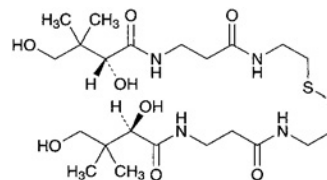
System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of panipenem to that of the internal standard is not more than 2.0%.

Containers and storage Containers—Tight containers.

Storage—Not exceeding -10°C .

Pantethine

パンテチン



$\text{C}_{22}\text{H}_{42}\text{N}_4\text{O}_8\text{S}_2$: 554.72

Bis(2- {3-[(2*R*)-2,4-dihydroxy-3,3-dimethylbutanoylamino]propanoylamino} ethyl) disulfide [16816-67-4]

Pantethine is an aqueous solution containing 80% of pantethine.

Pantethine contains not less than 98.0% of pantethine ($\text{C}_{22}\text{H}_{42}\text{N}_4\text{O}_8\text{S}_2$), calculated on the anhydrous basis.

Description Pantethine is a clear, colorless to pale yellow viscous liquid.

It is miscible with water, with methanol and with ethanol (95).

It is decomposed by light.

Identification (1) To 0.7 g of Pantethine add 5 mL of sodium hydroxide TS, shake, and add 1 to 2 drops of copper (II) sulfate TS: a blue-purple color develops.

(2) To 0.7 g of Pantethine add 3 mL of water, shake, add 0.1 g of zinc powder and 2 mL of acetic acid (100), and boil for 2 to 3 minutes. After cooling, add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

(3) To 1.0 g of Pantethine add 500 mL of water, and shake. To 5 mL of this solution add 3 mL of 1 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. After cooling, add 7 mL of a solution of hydroxylammonium chloride in sodium hydroxide TS (3 in 140), and allow to stand for 5 minutes. Add 3 drops of 2,4-dinitrophenol TS, and add 1 mol/L hydrochloric acid TS dropwise until the solution has no color, and then add 1 mL of iron (III) chloride TS: a red-purple color develops.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+15.0 - +18.0^\circ$ (1 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Pantethine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Pantethine according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.6 g of Pantethine in 10 mL of water, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-

layer chromatography. Develop the plate with 2-butanone saturated with water to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand for about 10 minutes in iodide vapor: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Mercapto compounds—To 1.5 g of Pantethine add 20 mL of water, shake, add 1 drop of ammonia TS and 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS: a red color is not developed.

Water <2.48> 18 – 22% (0.2 g, volumetric titration, direct titration).

Residue on Ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.3 g of Pantethine, add water to make exactly 20 mL. Transfer exactly 5 mL of this solution in an iodine bottle, and add exactly 25 mL of 0.05 mol/L bromine VS and 100 mL of water. Add 5 mL of diluted sulfuric acid (1 in 5) rapidly, stopper tightly immediately, and warm at 40 to 50°C for 15 minutes with occasional shaking. After cooling, carefully add 5 mL of a solution of potassium iodide (2 in 5), then immediately stopper tightly, shake, add 100 mL of water and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

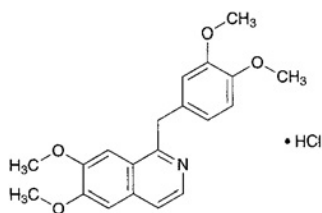
$$\begin{aligned} \text{Each mL of 0.05 mol/L bromine VS} \\ = 5.547 \text{ mg of } C_{22}H_{42}N_4O_8S_2 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a temperature not exceeding 10°C.

Papaverine Hydrochloride

パパペリン塩酸塩



$C_{20}H_{21}NO_4 \cdot HCl$: 375.85

6,7-Dimethoxy-1-(3,4-dimethoxybenzyl)isoquinoline monohydrochloride [61-25-6]

Papaverine Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{21}NO_4 \cdot HCl$.

Description Papaverine Hydrochloride occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in acetic acid (100), slightly soluble in ethanol (95), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Papaverine Hydrochloride (1 in 50) is between 3.0 and 4.0.

Identification (1) To 1 mg of Papaverine Hydrochloride add 1 drop of formaldehyde-sulfuric acid TS: a colorless to light yellow-green color is produced, and it gradually changes

to deep red, then to brown.

(2) Dissolve 0.02 g of Papaverine Hydrochloride in 1 mL of water, and add 3 drops of sodium acetate TS: a white precipitate is produced.

(3) Dissolve 1 mg of Papaverine Hydrochloride in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and examine under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence.

(4) Dissolve 0.1 g of Papaverine Hydrochloride in 10 mL of water, make alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath, and dry the residue at 105°C for 3 hours: the residue so obtained melts <2.60> between 145°C and 148°C.

(5) Alkalify a solution of Papaverine Hydrochloride (1 in 50) with ammonia TS, and filter the precipitate. Acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests <1.09> (2) for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Papaverine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Morphine—Dissolve 10 mg of Papaverine Hydrochloride in 1 mL of water, add 5 mL of 1-nitroso-2-naphthol TS and 2 mL of a solution of potassium nitrate (1 in 10), and warm at 40°C for 2 minutes. Add 1 mL of a solution of sodium nitrate (1 in 5000), and warm at 40°C for 5 minutes. After cooling, shake the mixture with 10 mL of chloroform, centrifuge, and separate the aqueous layer: the solution so obtained has no more color than a pale red color.

(3) Readily carbonizable substances <1.15>—Perform the test with 0.12 g of Papaverine Hydrochloride: the solution has no more color than Matching Fluid S or P.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Papaverine Hydrochloride, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 37.59 \text{ mg of } C_{20}H_{21}NO_4 \cdot HCl \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Papaverine Hydrochloride Injection

パパペリン塩酸塩注射液

Papaverine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$: 375.85).

Method of preparation Prepare as directed under Injections, with Papaverine Hydrochloride.

Description Papaverine Hydrochloride Injection is a clear, colorless liquid.

pH: 3.0 – 5.0

Identification (1) To 1 mL of Papaverine Hydrochloride Injection add 3 drops of sodium acetate TS: a white precipitate is produced.

(2) Dilute a volume of Papaverine Hydrochloride Injection, equivalent to 0.1 g of Papaverine hydrochloride according to the labeled amount, with water to 10 mL, render the solution alkaline with ammonia TS, and shake with 10 mL of diethyl ether. Draw off the diethyl ether layer, wash with 5 mL of water, and filter. Evaporate the filtrate on a water bath to dryness, and dry the residue at 105°C for 3 hours: the residue so obtained melts <2.60> between 145°C and 148°C.

(3) Proceed with 1 mg each of the residue obtained in (2) as directed in the Identification (1) and (3) under Papaverine Hydrochloride.

(4) Alkalify 2 mL of Papaverine Hydrochloride Injection with ammonia TS, filter the precipitate off, and acidify the filtrate with dilute nitric acid: the solution responds to Qualitative Tests <1.09> (2) for chloride.

Extractable volume <6.05> It meets the requirement.

Assay Dilute an exactly measured volume of Papaverine Hydrochloride Injection, equivalent to about 0.2 g of papaverine hydrochloride ($C_{20}H_{21}NO_4 \cdot HCl$), with water to 10 mL, render the solution alkaline with ammonia TS, and extract with 20-mL, 15-mL, 10-mL and 10-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and re-extract the washings with two 5-mL portions of chloroform. Combine all the chloroform extracts, and distil the chloroform on a water bath. Dissolve the residue in 30 mL of acetic acid (100), and titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 18.79 mg of $C_{20}H_{21}NO_4 \cdot HCl$

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Paraffin

パラフィン

Paraffin is a mixture of solid hydrocarbons obtained from petroleum.

Description Paraffin occurs as a colorless or white, more or less transparent, crystalline mass. It is odorless and tasteless.

Paraffin is sparingly soluble in diethyl ether and practically insoluble in water, in ethanol (95) and in ethanol (99.5).

Specific gravity d_{20}^{20} : about 0.92 (proceed as directed in the Specific gravity (2) under Fats and Fatty Oils <1.13>).

Identification (1) Heat Paraffin strongly in a porcelain dish, and ignite: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 g of Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Melting point <2.60> 50 – 75°C (Method 2).

Purity (1) Acidity or alkalinity—Boil 10.0 g of Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS in a water bath for 5 minutes, and shake vigorously: a red color is not produced. Add 0.20 mL of 0.02 mol/L sodium hydroxide VS to this solution, and shake: a red color is produced.

(2) Heavy metals <1.07>—Ignite 2.0 g of Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(4) Sulfur compounds—To 4.0 g of Paraffin add 2 mL of ethanol (99.5), further add 2 drops of a clear saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and heat for 10 minutes at 70°C with occasional shaking: no dark brown color develops in the aqueous layer.

(5) Readily carbonizable substances—Melt 5.0 g of Paraffin placed in a Nessler tube at a temperature near the melting point. Add 5 mL of sulfuric acid for readily carbonizable substances, and warm at 70°C for 5 minutes in a water bath. Remove the tube from the water bath, immediately shake vigorously and vertically for 3 seconds, and warm for 1 minute in a water bath at 70°C. Repeat this procedure five times: the color of the sulfuric acid layer is not darker than that of the following control solution.

Control solution: Add 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution, 0.5 mL of Cupric Sulfate Colorimetric Stock Solution and 5 mL of liquid paraffin to 3.0 mL of Ferric Chloride Colorimetric Stock Solution, and shake vigorously.

Containers and storage Containers—Well-closed containers.

Liquid Paraffin

流動パラフィン

Liquid Paraffin is a mixture of liquid hydrocarbons obtained from petrolatum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description Liquid Paraffin is a colorless, transparent, oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C.

Identification (1) Heat Liquid Paraffin strongly in a por-

celain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> d_{20}^{20} : 0.860 – 0.890

Viscosity <2.53> Not less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Liquid Paraffin to a small beaker, and heat on a water bath: a foreign odor is not perceptible.

(2) **Acidity or alkalinity**—Shake vigorously 10 mL of Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide: a red color develops.

(3) **Heavy metals** <1.07>—Ignite 2.0 g of Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) **Arsenic** <1.11>—Prepare the test solution with 1.0 g of Liquid Paraffin, according to Method 3 except that after addition of 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), add 1.5 mL of hydrogen peroxide (30), fire to burn, and perform the test (not more than 2 ppm).

(5) **Solid paraffin**—Transfer 50 mL of Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) **Sulfur compounds**—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) **Polycyclic aromatic hydrocarbons**—Take 25 mL of Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution obtained as the sample solution. Transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to another 50-mL separator, shake vigorously for 2 minutes with 5.0 mL

of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 2 minutes. Transfer the lower layer to a 10-mL glass-stoppered centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution thus obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) **Readily carbonizable substances**—Transfer 5 mL of Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the Liquid Paraffin layer remains unchanged in color, and the sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Ferric Chloride Colorimetric Stock Solution with 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution and 0.50 mL of Cupric Sulfate Colorimetric Stock Solution.

Containers and storage Containers—Tight containers.

Light Liquid Paraffin

軽質流動パラフィン

Light Liquid Paraffin is a mixture of hydrocarbons obtained from petroleum.

Tocopherols of a suitable form may be added at a concentration not exceeding 0.001% as a stabilizer.

Description Light Liquid Paraffin is a clear, colorless oily liquid, nearly free from fluorescence. It is odorless and tasteless.

It is freely soluble in diethyl ether, and practically insoluble in water and in ethanol (95).

Boiling point: above 300°C

Identification (1) Heat Light Liquid Paraffin strongly in a porcelain dish, and fire: it burns with a bright flame and the odor of paraffin vapor is perceptible.

(2) Heat 0.5 of Light Liquid Paraffin with 0.5 g of sulfur with shaking carefully: the odor of hydrogen sulfide is perceptible.

Specific gravity <2.56> d_{20}^{20} : 0.830 – 0.870

Viscosity <2.53> Less than 37 mm²/s (Method 1, 37.8°C).

Purity (1) Odor—Transfer a suitable amount of Light Liquid Paraffin to a small beaker, and heat on a water bath: no foreign odor is perceptible.

(2) **Acidity or alkalinity**—Shake vigorously 10 mL of Light Liquid Paraffin with 10 mL of hot water and 1 drop of phenolphthalein TS: no red color develops. Shake this solution with 0.20 mL of 0.02 mol/L sodium hydroxide: a red color develops.

(3) **Heavy metals** <1.07>—Ignite 2.0 g of Light Liquid Paraffin in a crucible, first moderately until charred, then between 450°C and 550°C to ash. Cool, add 2 mL of

hydrochloric acid, and evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Light Liquid Paraffin according to Method 3, and perform the test (not more than 2 ppm).

(5) Solid paraffin—Transfer 50 mL of Light Liquid Paraffin, previously dried at 105°C for 2 hours, to a Nessler tube, and cool in ice water for 4 hours: the turbidity produced, if any, is not deeper than that of the following control solution.

Control solution: To 1.5 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(6) Sulfur compounds—Prepare a saturated solution of lead (II) oxide in a solution of sodium hydroxide (1 in 5), and mix 2 drops of this clear solution with 4.0 mL of Light Liquid Paraffin and 2 mL of ethanol (99.5). Heat at 70°C for 10 minutes with frequent shaking, and cool: no dark brown color develops.

(7) Polycyclic aromatic hydrocarbons—Take 25 mL of Light Liquid Paraffin by a 25-mL measuring cylinder, transfer to a 100-mL separator, and wash out the cylinder with 25 mL of hexane for ultraviolet-visible spectrophotometry. Combine the washings with the liquid in the separator, and shake vigorously. Shake this solution vigorously for 2 minutes with 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, and allow to stand for 15 minutes. Transfer the lower layer to a 50-mL separator, add 2 mL of hexane for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, and centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as the sample solution. Separately, transfer 25 mL of hexane for ultraviolet-visible spectrophotometry to a 50-mL separator, add 5.0 mL of dimethylsulfoxide for ultraviolet-visible spectrophotometry, shake vigorously for 2 minutes, and allow to stand for 2 minutes. Transfer the lower layer to a glass-stoppered 10-mL centrifuge tube, centrifuge between 2500 revolutions per minute and 3000 revolutions per minute for about 10 minutes, and use the clear solution so obtained as a control solution. Immediately determine the absorbance of the sample solution using the control solution as the blank as directed under Ultraviolet-visible Spectrophotometry <2.24>: not more than 0.10 at the wavelength region between 260 nm and 350 nm.

(8) Readily carbonizable substances—Transfer 5 mL of Light Liquid Paraffin to a Nessler tube, and add 5 mL of sulfuric acid for readily carbonizable substances. After heating in a water bath for 2 minutes, remove the tube from the water bath, and immediately shake vigorously and vertically for 5 seconds. Repeat this procedure four times: the liquid paraffin layer remains unchanged in color, and sulfuric acid layer has no more color than the following control solution.

Control solution: Mix 3.0 mL of Ferric Chloride Colorimetric Stock Solution with 1.5 mL of Cobaltous Chloride Colorimetric Stock Solution and 0.50 mL of Cupric Sulfate

Colorimetric Stock Solution.

Containers and storage Containers—Tight containers.

Paraformaldehyde

パラホルムアルデヒド

$(\text{CH}_2\text{O})_n$

Poly(oxyethylene) [30525-89-4]

Paraformaldehyde contains not less than 95.0% of CH_2O : 30.03.

Description Paraformaldehyde occurs as a white powder. It has a slight odor of formaldehyde, but a very strong irritating odor is perceptible when it is heated.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in hot water, in hot dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It sublimes at about 100°C.

Identification (1) Dissolve 0.1 g of Paraformaldehyde in 5 mL of ammonia TS, add 5 mL of silver nitrate TS, shake, and add 3 mL of a solution of sodium hydroxide (1 in 10): a mirror of metallic silver is immediately formed on the sides of the container.

(2) Add a solution of 0.04 g of salicylic acid in 5 mL of sulfuric acid to 0.02 g of Paraformaldehyde, and warm slowly: a persistent, dark red color is produced.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Paraformaldehyde in 10 mL of ammonia TS: the solution is clear and colorless.

(2) Acidity or alkalinity—To 0.5 g of Paraformaldehyde add 10 mL of water, shake vigorously for 1 minute, and filter: the filtrate is neutral.

(3) Chloride <1.03>—Dissolve 1.5 g of Paraformaldehyde in 75 mL of water and 7.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize with diluted nitric acid (3 in 10), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 7.5 mL of sodium carbonate TS, a volume of diluted nitric acid (3 in 10) required for neutralization of the sample, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.006%).

(4) Sulfate <1.14>—Dissolve 1.5 g of Paraformaldehyde in 45 mL of water and 4.5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite at about 500°C. Dissolve the residue in 15 mL of water, filter, if necessary, neutralize the diluted hydrochloric acid (3 in 5), and boil for 5 minutes. After cooling, add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 4.5 mL of sodium carbonate TS add an equal volume of diluted hydrochloric acid (3 in 5) for the neutralization of the sample and 15 mL of water, and boil for 5 minutes. After cooling, add 0.35 mL of 0.005 mol/L sulfuric acid VS, 1 mL of dilute hydrochloric acid and water to make

50 mL (not more than 0.011%).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 50 mg of Paraformaldehyde, accurately weighed, in 10 mL of potassium hydroxide TS in an iodine flask. Add 40 mL of water and an exactly measured 50 mL of 0.05 mol/L iodine VS, stopper, and allow to stand for 5 minutes. Then add 5 mL of dilute hydrochloric acid, stopper immediately, allow to stand for 15 minutes, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 1.501 mg of CH₂O

Containers and storage Containers—Tight containers.

Dental Paraformaldehyde Paste

歯科用パラホルムパスタ

Method of preparation

Paraformaldehyde, finely powdered	35 g
Procaine Hydrochloride, finely powdered	35 g
Hydrous Lanolin	a sufficient quantity
To make 100 g	

Prepare as directed under Ointments, with the above ingredients.

Description Dental Paraformaldehyde Paste is yellowish white in color. It has a characteristic odor.

Identification (1) To 0.15 g of Dental Paraformaldehyde Paste add 20 mL of diethyl ether and 20 mL of 0.5 mol/L sodium hydroxide TS, shake well, separate the water layer, and dilute with water to make 100 mL. To 1 mL of this solution add 10 mL of acetylacetone TS, and heat on a water bath for 10 minutes: a yellow color is produced (paraformaldehyde).

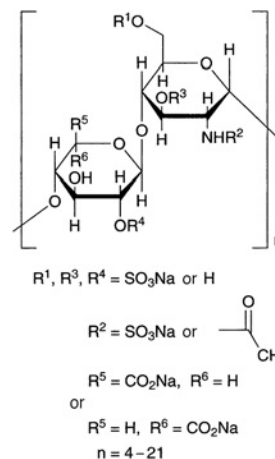
(2) To the diethyl ether layer obtained in (1) add 5 mL of dilute hydrochloric acid and 20 mL of water, shake well, and separate the water layer: the solution responds to Qualitative Tests <1.09> for primary aromatic amines (procaine hydrochloride).

(3) To 0.15 g of Dental Paraformaldehyde Paste add 25 mL of diethyl ether and 25 mL of water, shake, separate the water layer, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of procaine hydrochloride in 5 mL of water, and use this solution as standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots from the sample solution and standard solution show the same *R_f* value.

Containers and storage Containers—Tight containers.

Parnaparin Sodium

パルナパリンナトリウム



Parnaparin Sodium is a low-molecular heparin sodium obtained by depolymerization, with hydrogen peroxide and with copper (II) acetate, of heparins sodium from the healthy edible porcine intestinal mucosa. The mass-average molecular mass ranges between 4500 and 6400.

The potency is not less than 70 low-molecular-mass-heparin units and not more than 95 low-molecular-mass-heparin units of anti-factor Xa activity per milligram calculated with reference of the dried substance.

Description Parnaparin Sodium occurs as a white or light yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Mix 0.1 mL of a solution of Parnaparin Sodium (1 in 20) and 10 mL of a solution of tritoluidine blue O (1 in 100,000), and shake the mixture: the blue color of solution immediately changes to purple.

(2) A solution of Parnaparin Sodium (1 in 20) responds to Qualitative Tests <1.09> for sodium salt.

pH <2.54> Dissolve 0.1 g of Parnaparin Sodium in 10 mL of water: the pH of this solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Parnaparin Sodium in 10 mL of water: the solution is clear and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Parnaparin Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 8.0% (0.2 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours)

Molecular mass Calculate the molecular mass of Parnaparin Sodium by the following methods: The mass-average molecular mass ranges between 4500 and 6400.

(i) Creation of calibration curve Weigh 20 mg of low-molecular mass heparin for calibration of molecular mass, and dissolve it in 2.0 mL of the mobile phase as the standard

solution. Perform the test with 50 μL of the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak height, H_{UV} , in chromatogram obtained by the ultraviolet absorption photometer, and determine the peak height, H_{RI} , in chromatogram obtained by the differential refractometer. Calculate the ratio of H_{UV} to H_{RI} , H_{RI}/H_{UV} , at each peak. Assume the molecular mass in the 4th peak from the low molecular mass in chromatogram obtained by the ultraviolet absorption photometer as 2400, and make the calculation of the standard coefficient from dividing 2400 by the H_{RI}/H_{UV} at the corresponding peak. Make the calculation to multiply the H_{RI}/H_{UV} at each peak by the standard coefficient, and determine the molecular mass of each peak by the calculation. Prepare the calculation curve by plotting the logarithm of molecular masses at each peak on the vertical axis and the retention time on the chromatogram obtained by the differential refractometer on the horizontal axis.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 234 nm) and a differential refractometer.

Column: Connect two stainless steel columns which are 7.5 mm in inside diameter and 30 cm in length, and are packed with porous silica gel for liquid chromatography; one column, the molecular mass of limited size exclusion is about 500,000; the other, the molecular mass of limited size exclusion is about 100,000. Connect a pump, the about 500,000-molecular mass of limited size exclusion column, the about 100,000-molecular mass of limited size exclusion column, the ultraviolet absorption photometer and the differential refractometer in this order.

Column temperature; A constant temperature of about 40°C.

Mobile phase: Dissolve 28.4 g of sodium sulfate anhydride in 1000 mL of water, and 5.0 with 0.05 mol/L sulfuric acid TS.

Flow rate: 0.5 mL/min

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, confirm that more than ten peaks in chromatogram obtained as directed under either the Ultraviolet-visible Spectrophotometry, or the Differential Refractometry are observed.

System repeatability: When the tests repeated 6 times with 50 μL of the standard solution under the above operating conditions, relative standard deviation of the 4th peak height in chromatogram (H_{UV} and H_{RI}) is not more than 3.0%.

(ii) **Determination of molecular mass** Dissolve the 20 mg of Parnaparin Sodium with 2.0 mL of mobile phase, and use this solution as the sample solution. Perform the test with 50 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Divide the main peak observed between 30 min and 45 min to 30 sec-interval fractions, and determine the strength of differential refractometer of each 30 sec-interval fraction. Determine the molecular mass of each fraction using the calibration curve and the retention time of each fraction. Determine the mean of molecular mass in the entire peak using the strength of differential refractometer and the molecular mass in every fractions.

Mean molecular mass of parnaparin sodium

$$= \Sigma(n_i \cdot M_i) / \Sigma n_i$$

n_i : The differential refractometer strength of fraction i in the main peak of chromatogram

M_i : Molecular mass of fraction i in main peak

Σn_i : Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

Operating conditions—

Detector: A differential refractometer.

Column, column temperature, mobile phase, and flow rate: Proceed as directed in (i) Creation of calibration curve.

System suitability—

Proceed as directed in (i) Creation of calibration curve.

Distribution of molecular mass The molecular mass of Parnaparin Sodium is calculated as directed in the determination of molecular mass and the distribution of molecular mass is determined by the following equation: the molecular mass of not less than 80% parnaparin sodium is between 1500 and 10,000.

Distribution of molecular mass (%)

$$= (\Sigma n_j / \Sigma n_i) \times 100$$

n_i : The differential refractometer strength of fraction i in the main peak of chromatogram

Σn_j : Sum of differential refractometer strength in the each fraction between 1500 and 10,000 molecular mass in the main peak

The degree of sulfate ester Dissolve 0.5 g of Parnaparin Sodium with 10 mL water. Treat the solution with 5 mL of a strongly basic ion exchange resin, and subsequently with 10 mL of a strongly acidic ion exchange resin. Dilute the solution with water to 50 mL, and titrate <2.50> with 0.1 mol/L Sodium hydroxide VS (potentiometric titration). Determine the degree of sulfate ester of Parnaparin Sodium from the equivalence point by the following equation; it is between 2.0 and 2.4.

The degree of sulfate ester

$$= \frac{\text{the first equivalence point (mL)}}{[\text{the second equivalence point (mL)} - \text{first equivalence point (mL)}]}$$

Total nitrogen Weigh accurately about 0.10 g of Parnaparin Sodium which is dried, and perform the test as directed under Nitrogen Determination <1.08>: it contains not less than 1.9% and not more than 2.3% of nitrogen (N:14.01).

Anti-factor IIa activity When the potency of anti-factor IIa activity is determined according to the following method, it contains not less than 35 and not more than 60 low-molecular-mass-heparin unit per milligram calculated with reference to the dried substance.

(i) **Standard solution** Dissolve low-molecular weight heparin standard with isotonic sodium chloride solution to make solutions which contain 0.1, 0.2 and 0.3 low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL, respectively.

(ii) **Sample solution** Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it with isotonic sodium chloride solution to adjust the solution which contains 4 μg parnaparin sodium in 1 mL.

(iii) **Procedure** To each plastic tube add 0.10 mL of the sample solution and the standard solution, separately. To

each tube add 0.10 mL of human normal plasma and mix it, and incubate at $37 \pm 1^\circ\text{C}$ accurately for 1 min. Next, to each test tube add 0.10 mL of activated thromboplastin-time assay solution, which is pre-warmed at $37 \pm 1^\circ\text{C}$, and after the mixing incubate accurately for 5 min at $37 \pm 1^\circ\text{C}$. Then, to each tube add 0.10 mL of sodium calcium solution (277 in 100,000) which is pre-warmed at $37 \pm 1^\circ\text{C}$, mix it, start a stop watch simultaneously, and permit to stand at the same temperature. Determine the time for the first appearance of fibrin clot.

(iv) Calculation Determine the low-molecular-mass-heparin unit (anti-factor IIa activity) of the sample solution from calibration curve obtained plots of clotting times for each standard solution; calculate the low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium as following equation.

The low-molecular-mass-heparin unit (anti-factor IIa activity) for 1 mg of parnaparin sodium

= the low-molecular-mass-heparin unit (anti-factor IIa activity) in 1 mL of sample solution $\times (b/a)$

a: Amount (mg) of Parnaparin Sodium taken

b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution.

The ratio of anti-factor Xa activity to anti-factor IIa activity

Divide the anti-factor Xa activity, obtained in the Assay, by the anti-factor IIa activity which has been obtained from the test according to the method of anti-factor IIa activity; the ratio of anti-factor Xa activity to anti-factor IIa activity is between 1.5 and 2.5.

Assay

(i) Standard solution Dissolve low-molecular-mass-heparin for calculation of molecular mass in isotonic sodium chloride solution to make solutions which contain 0.4, 0.6 and 0.8 low-molecular-mass-heparin units, (anti-factor Xa activity) in 1 mL, respectively.

(ii) Sample solution Weigh accurately about 50 mg of Parnaparin Sodium, and dissolve it in isotonic sodium chloride solution to make a solution which contains 7 μg parnaparin sodium in 1 mL.

(iii) Procedure To each plastic tube add 0.10 mL of either the sample solution or the standard solution, separately. Subsequently to the every tubes add 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin III solution, and 0.10 mL of normal human plasma, and mix them. To another plastic tube transfer 0.20 mL of these solutions, separately, and incubate for accurate 3 minutes at $37 \pm 1^\circ\text{C}$. Next, to each tube add 0.10 mL of factor Xa TS and mix it, permit to stand $37 \pm 1^\circ\text{C}$ accurately for 30 seconds, and immediately add 0.20 mL of chromogenic synthetic substrate solution (3 in 4000) and mix it, and subsequently incubate accurately for 3 min at $37 \pm 1^\circ\text{C}$. To each test tube add 0.30 mL of diluted acetic acid (100) solution (1 in 2) to stop the reaction. Separately, to plastic tube add 0.10 mL of isotonic sodium chloride solution, 0.70 mL of Tris-buffered solution (pH 8.4), 0.10 mL of anti-thrombin solution, and 0.10 mL of normal human plasma to every tubes, and mix well. To another plastic tube transfer 0.2 mL of the solution, separately, and add both 0.30 mL of water and 0.30 mL of diluted acetic acid (100) (1 in 2). Determine the absorbance of both the sample

solution and the standard solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained from this solution as the blank.

(iv) Calculation method Determine the low-molecular-mass unit (anti-factor Xa activity) of the sample solution using the calibration curve prepared from the absorbance of the standard solutions and their logarithmic concentrations, and calculate the low-molecular-mass unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium.

Low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mg of Parnaparin Sodium

= the low-molecular-mass-heparin unit (anti-factor Xa activity) in 1 mL of the sample solution $\times (b/a)$

a: Amount (mg) of Parnaparin Sodium taken

b: The total volume (mL) in which Parnaparin Sodium has been dissolved with isotonic sodium chloride solution for the preparation of sample solution.

Container and Storage

Container—Well-closed containers.

Peanut Oil

Oleum Arachidis

ラッカセイ油

Peanut Oil is the fixed oil obtained from the seeds of *Arachis hypogaea* Linné (*Leguminosae*).

Description Peanut Oil is a pale yellow, clear oil. It is odorless or has a slight odor. It has a mild taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25} : 0.909 – 0.916

Congealing point of the fatty acids: 22 – 33°C

Identification Saponify 5 g of Peanut Oil by boiling with 2.5 mL of sodium hydroxide solution (3 in 10) and 12.5 mL of ethanol (95). Evaporate the ethanol, dissolve the residue in 50 mL of hot water, and add dilute hydrochloric acid in excess until the free fatty acids separate as an oily layer. Cool the mixture, remove the separated fatty acids, and dissolve them in 75 mL of diethyl ether. To the diethyl ether solution add a solution of 4 g of lead (II) acetate trihydrate in 40 mL of ethanol (95), and allow the mixture to stand for 18 hours. Filter the supernatant liquid, transfer the precipitate to the filter with the aid of diethyl ether, and filter by suction. Place the precipitate in a beaker, heat it with 40 mL of dilute hydrochloric acid and 20 mL of water until the oily layer is entirely clear, cool, and decant the water layer. Boil the fatty acids with 50 mL of diluted hydrochloric acid (1 in 100). When the solution prepared by dissolving 0.1 g of the fatty acids in 10 mL of ethanol (95) is not darkened by the addition of 2 drops of sodium sulfide TS, allow the fatty acids to solidify, and press them between dry filter papers to exclude moisture. Dissolve the solid fatty acid in 25 mL of diluted ethanol (9 in 10) with the aid of gentle heat, and then cool to 15°C to crystallize the fatty acids. Recrystallize them from diluted ethanol (9 in 10) and dry in a desiccator (phosphorus (V) oxide, in vacuum) for 4 hours; the melting point <1.13> of the dried crystals is between 73°C and 76°C.

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 188 – 196

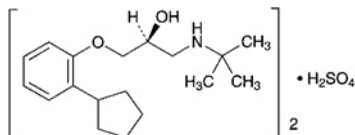
Unsaponifiable matters <1.13> Not more than 1.5%.

Iodine value <1.13> 84 – 103

Containers and storage Containers—Tight containers.

Penbutolol Sulfate

ペンブトロール硫酸塩



(C₁₈H₂₉NO₂)₂·H₂SO₄; 680.94
(2*S*)-3-(2-Cyclopentylphenoxy)-1-(1,1-dimethylethyl)aminopropan-2-ol hemisulfate
[38363-32-5]

Penbutolol Sulfate, when dried, contains not less than 98.5% of (C₁₈H₂₉NO₂)₂·H₂SO₄.

Description Penbutolol Sulfate occurs as a white crystalline powder.

It is very soluble in acetic acid (100), freely soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in water, and practically insoluble in acetic anhydride and in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Penbutolol Sulfate in methanol (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Penbutolol Sulfate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 0.1 g of Penbutolol Sulfate in 25 mL of water by warming, and cool: this solution responds to Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49> [α]_D²⁰: −23 – −25° (after drying, 0.2 g, methanol, 20 mL, 100 mm).

Melting point <2.60> 213 – 217°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Penbutolol Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Penbutolol Sulfate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.8 g of Penbutolol Sulfate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add

methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 2-propanol, ethanol (95) and ammonia solution (28) (85:12:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

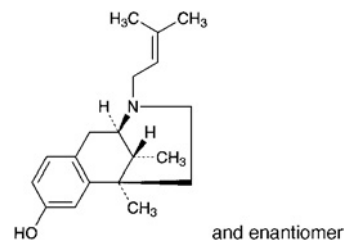
Assay Weigh accurately about 0.8 g of Penbutolol Sulfate, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 68.09 mg of (C₁₈H₂₉NO₂)₂·H₂SO₄

Containers and storage Containers—Well-closed containers.

Pentazocine

ペンタゾシン



C₁₉H₂₇NO; 285.42
(2*RS*,6*RS*,11*RS*)-6,11-Dimethyl-3-(3-methylbut-2-en-1-yl)-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol
[359-83-1]

Pentazocine, when dried, contains not less than 99.0% of C₁₉H₂₇NO.

Description Pentazocine occurs as a white to pale yellowish white, crystalline powder. It is odorless.

It is freely soluble in acetic acid (100) and in chloroform, soluble in ethanol (95), sparingly soluble in diethyl ether and practically insoluble in water.

Identification (1) To 1 mg of Pentazocine add 0.5 mL of formaldehyde-sulfuric acid TS: a deep red color is produced, and it changes to grayish brown immediately.

(2) Dissolve 5 mg of Pentazocine in 5 mL of sulfuric acid, add 1 drop of iron (III) chloride TS, and heat in a water bath for 2 minutes: the color of the solution changes from light yellow to deep yellow. Shake the solution with 1 drop of nitric acid: the solution remains yellow in color.

(3) Determine the absorption spectrum of a solution of Pentazocine in 0.01 mol/L hydrochloric acid TS (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (278 nm): 67.5 – 71.5 (after drying, 0.1 g, 0.01 mol/L hydrochloric acid TS, 1000 mL).

Melting point <2.60> 150 – 158°C

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Pentazocine in 20 mL of 0.1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pentazocine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pentazocine according to Method 3, and perform the test with a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentazocine in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and isopropylamine (94:3:3) to a distance of about 13 cm, and air-dry the plate. Allow to stand for 5 minutes in iodine vapor: any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 5 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

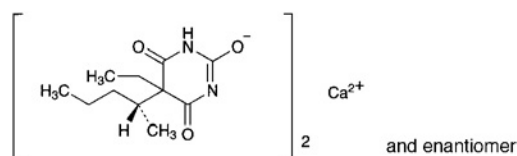
Assay Weigh accurately about 0.5 g of Pentazocine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.54 mg of $\text{C}_{19}\text{H}_{27}\text{NO}$

Containers and storage Containers—Well-closed containers.

Pentobarbital Calcium

ペントバルビタールカルシウム



$\text{C}_{22}\text{H}_{34}\text{CaN}_4\text{O}_6$: 490.61
Monocalcium bis[5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-

dioxo-1,4,5,6-tetrahydropyrimidin-2-olate]
[76-74-4, Pentobarbital]

Pentobarbital Calcium contains not less than 98.0% and not more than 102.0% of $\text{C}_{22}\text{H}_{34}\text{CaN}_4\text{O}_6$, calculated on the dried basis.

Description Pentobarbital Calcium occurs as a white powder.

It is sparingly soluble in water, slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

A solution of Pentobarbital Calcium (1 in 100) shows no optical rotation.

Identification (1) Determine the infrared absorption spectrum of Pentobarbital Calcium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, shake with 5 mL of dilute hydrochloric acid and 10 mL of water, allow to cool, and filter. To the filtrate add 1 drop of methyl red TS, and add ammonia TS until a slight yellow color develops: the solution responds to Qualitative Tests <1.09> (1), (2) and (3) for calcium salt.

Purity (1) Chloride <1.03>—To 1.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 2.5 mL of dilute nitric acid, dissolve by warming with shaking, cool, add water to make 50 mL, shake well, and filter. Discard the first 10 mL of the filtrate, and to the subsequent 15 mL add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 1.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.035%).

(2) Heavy metals <1.07>—To 2.0 g of Pentobarbital Calcium add 5 mL of ethanol (95) and 5 mL of dilute hydrochloric acid, dissolve by warming with shaking, cool, add water to make 80 mL, shake well, and filter. Discard the first 10 mL of the filtrate, to the subsequent 40 mL add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: To 2.5 mL of ethanol (95) add 2.5 mL of dilute hydrochloric acid and water to make 30 mL. Add 1 drop of phenolphthalein TS, add dropwise ammonia TS until a pale red color develops, then add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pentobarbital Calcium in 100 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of any peak other than the peak of pentobarbital from the sample solution is not bigger than 3/10 of the peak area of pentobarbital from the standard so-

lution, and the total of these peak area is not bigger than the peak area of pentobarbital from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of pentobarbital beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 2 mL of the standard solution, add water to make exactly 20 mL, and confirm that the peak area of pentobarbital obtained from 20 μ L of this solution is equivalent to 5 to 15% of that of pentobarbital obtained from 20 μ L of the standard solution.

System performance: Proceed as directed in the system performance in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of pentobarbital is not more than 5%.

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 5 hours).

Assay Weigh accurately about 20 mg of Pentobarbital Calcium, dissolve in 5 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 18 mg of Pentobarbital Reference Standard, previously dried at 105°C for 2 hours, dissolve in 10 mL of acetonitrile, add exactly 5 mL of the internal standard solution and water to make 50 mL. To 5 mL of this solution add water to make 20 mL. To 2 mL of this solution add water to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of pentobarbital to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{22}H_{34}CaN_4O_6 \\ = W_S \times (Q_T / Q_S) \times 1.0841 \end{aligned}$$

W_S : Amount (mg) of Pentobarbital Reference Standard

Internal standard solution—Dissolve 0.2 g of isopropyl parahydroxybenzoate in 20 mL of acetonitrile, and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.36 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust to pH 4.0 with diluted phosphoric acid (1 in 10). To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pentobarbital is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, pentobarbital and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pentobarbital to that of the internal standard is not more than 1.0%.

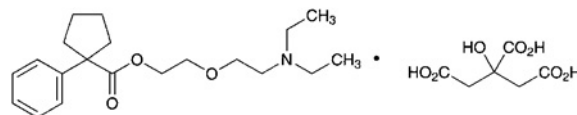
Containers and storage Containers—Well-closed containers.

Pentoxiverine Citrate

Carbetapentane Citrate

Carbetapentene Citrate

ペントキシベリンクエン酸塩



$C_{20}H_{31}NO_3 \cdot C_6H_8O_7$: 525.59

2-[2-(Diethylamino)ethoxy]ethyl
1-phenylcyclopentanecarboxylate monocitrate
[23142-01-0]

Pentoxiverine Citrate, when dried, contains not less than 98.5% of $C_{20}H_{31}NO_3 \cdot C_6H_8O_7$.

Description Pentoxiverine Citrate occurs as a white, crystalline powder.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.1 g of Pentoxiverine Citrate in 10 mL of water, and add 10 mL of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Pentoxiverine Citrate, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pentoxiverine Citrate (1 in 10) responds to Qualitative Tests <1.09> (1) and (2) for citrate.

Melting point <2.60> 92 – 95°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pentoxiverine Citrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pentoxiverine Citrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pentoxiverine Citrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Pentoxiverine Citrate in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 15 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Immediately after air-drying, develop the plate with a mixture of chloroform, methanol, ethyl acetate and ammonia solution (28) (25:10:10:1) to a distance of about 10 cm, and air-dry the plate. Allow to stand in iodine vapor for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

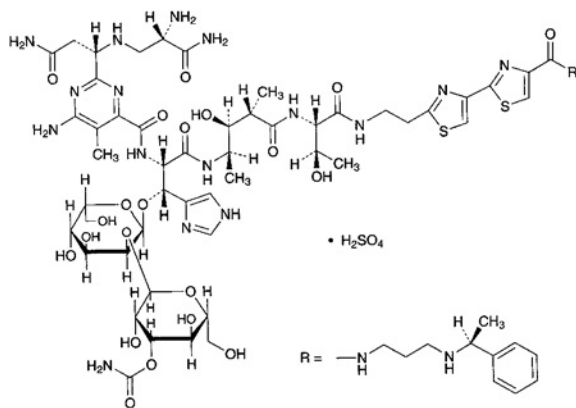
Assay Weigh accurately about 0.5 g of Pentoxiverine Citrate, previously dried, dissolve in 30 mL of acetic acid (100), add 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L of perchloric acid VS until the color of the solution changes from purple through blue-green to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 52.56 mg of $C_{20}H_{31}NO_3 \cdot C_6H_8O_7$

Containers and storage Containers—Well-closed containers.

Peplomycin Sulfate

ペプロマイシン硫酸塩



$C_{61}H_{88}N_{18}O_{21}S_2 \cdot H_2SO_4$: 1571.67
 N^1 -{3-[(1*S*)-(1-Phenylethyl)amino]propyl} bleomycinamide monosulfate [70384-29-1]

Peplomycin Sulfate is the sulfate of a substance having antitumor activity produced by the growth of *Streptomyces verticillus*.

It contains not less than 865 μ g (potency) and not more than 1010 μ g (potency) per mg, calculated on the dried basis. The potency of Peplomycin Sulfate is

expressed as mass (potency) of peplomycin ($C_{61}H_{88}N_{18}O_{21}S_2$: 1473.59).

Description Peplomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) To 4 mg of Peplomycin Sulfate add 5 μ L of copper (II) sulfate TS, and dissolve in water to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 10 mg each of Peplomycin Sulfate and Peplomycin Sulfate Reference Standard in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions: the retention time of the principal peak obtained from the sample solution is the same as that from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase stock solution, mobile phase A, mobile phase B, flowing of the mobile phase, and flow rate: Proceed as directed in the operating conditions in the Purity (3).

(3) A solution of Peplomycin Sulfate (1 in 200) responds to Qualitative Tests <1.09> (1) and (2) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-2 - -5^\circ$ (0.1 g calculated on the dried basis, 0.1 mol/L phosphate buffer solution, pH 5.3, 10 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Peplomycin Sulfate in 20 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 80 mg of Peplomycin Sulfate in 4 mL of water: the solution is clear and colorless.

(2) Copper—Dissolve exactly 75 mg of Peplomycin Sulfate in exactly 10 mL of diluted nitric acid (1 in 100), and use this solution as the sample solution. Separately, to 5.0 mL of Standard Copper Stock Solution add diluted nitric acid (1 in 100) to make exactly 100 mL. To 3.0 mL of this solution add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is not more than that of the standard solution (not more than 200 ppm).

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: Copper hollow cathode lamp

Wavelength: 324.8 nm

(3) Related substances—Dissolve about 10 mg of Peplomycin Sulfate in 6 mL of water, add 0.5 mL of a solution of copper (II) sulfate pentahydrate (1 in 125), and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chro-

matography <2.01> according to the following conditions. Determine the areas of the peaks, appeared after the peak of copper sulfate, by the automatic integration method, and calculate the amounts of them by the area percentage method: the total amount of the peaks other than peplomycin is not more than 7.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (7 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase stock solution: Dissolve 0.96 g of sodium 1-pentanesulfonate and 1.86 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 1000 mL of water and 5 mL of acetic acid (100), and adjust the pH to 4.3 with ammonia TS.

Mobile phase A: A mixture of mobile phase stock solution and methanol (9:1).

Mobile phase B: A mixture of mobile phase stock solution and methanol (3:2).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 60	100 → 0	0 → 100
60 – 75	0	100

Flow rate: 1.2 mL per minute.

Time span of measurement: As long as 20 minutes after elution of peplomycin beginning after the peak of copper sulfate.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add water to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add water to make exactly 10 mL. Confirm that the peak area of peplomycin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the solution for system suitability test.

System performance: When the procedure is run with 10 μ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of peplomycin are not less than 30,000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of peplomycin is not more than 2.0%.

Loss on drying <2.41> Not more than 3.0% (60 mg, in vacuum, phosphorus (V) oxide, 60°C, 3 hours). Handle the sample avoiding absorption of moisture.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Mycobacterium smegmatis* ATCC

607

(ii) Agar media for seed and base layer, and for transferring test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Agar	15.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iii) Liquid medium for suspending test organism

Glycerin	10.0 g
Peptone	10.0 g
Meat extract	10.0 g
Sodium chloride	3.0 g
Water	1000 mL

Mix all the ingredients and adjust the pH of the solution with sodium hydroxide TS so that it will be 6.9 to 7.1 after sterilization.

(iv) Preparation of agar medium of seeded layer—Inoculate the test organism onto the slant of the agar medium for transferring test organism, and incubate the slant at 27°C for 40 to 48 hours. Inoculate the subcultured test organism into 100 mL of the liquid medium for suspending test organism, incubate at 25 to 27°C for 5 days while shaking, and use this suspension as the suspension of the test organism. Keep the suspension of the test organism at a temperature of not exceeding 5°C and use within 14 days. Add 0.5 mL of the suspension of the test organism in 100 mL of the Agar medium for seed layer previously kept at 48°C, mix thoroughly, and use this as the agar medium of seeded layer.

(v) Preparation of cylinder-agar plate—Proceed as directed in 7. Preparation of cylinder-agar plates under the Microbial Assay for Antibiotics with the exception of the amounts of the agar medium for base layer and the agar medium of seeded layer to put in the Petri dish, which are 5.0 mL and 8.0 mL, respectively.

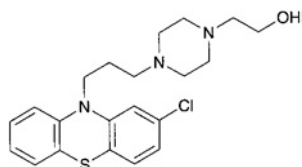
(vi) Standard solutions—Weigh accurately an amount of Peplomycin Sulfate Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C, and use within 15 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(vii) Sample solutions—Weigh accurately an amount of Peplomycin Sulfate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 6.8 to make exactly 100 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 6.8 to make solutions so that each mL contains 4 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers

Perphenazine

ペルフェナジン



$C_{21}H_{26}ClN_3OS$: 403.97

2-[4-[3-(2-Chloro-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl] ethanol [58-39-9]

Perphenazine, when dried, contains not less than 98.5% of $C_{21}H_{26}ClN_3OS$.

Description Perphenazine occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in methanol and in ethanol (95), soluble in acetic acid (100), sparingly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Identification (1) Dissolve 5 mg of Perphenazine in 5 mL of sulfuric acid: a red color, changing to deep red-purple upon warming, is produced.

(2) Dissolve 0.2 g of Perphenazine in 2 mL of methanol, add this solution to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and allow to stand for 4 hours. Collect the crystals, wash with a small volume of methanol, and dry at 105°C for 1 hour: the crystals so obtained melt <2.60> between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1 or the spectrum of a solution of Perphenazine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 10 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2 or the spectrum of a solution of Perphenazine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine as directed under Flame Coloration Test <1.04> (2): a green color appears.

Melting point <2.60> 95 – 100°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Perphenazine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Perform the test in the current of nitrogen in light-resistant containers under the protection from sunlight. Dissolve 0.10 g of Perphenazine in 10 mL of

ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and 1 mol/L ammonia TS (5:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): any spot other than the principal spot from the sample solution is not more intense than that from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 65°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Perphenazine, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue-purple to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.20 mg of $C_{21}H_{26}ClN_3OS$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Perphenazine Tablets

ペルフェナジン錠

Perphenazine Tablets contain not less than 90% and not more than 110% of the labeled amount of perphenazine ($C_{21}H_{26}ClN_3OS$: 403.97).

Method of preparation Prepare as directed under Tablets, with Perphenazine.

Identification (1) Shake well a quantity of powdered Perphenazine Tablets, equivalent to 25 mg of Perphenazine according to the labeled amount, with 10 mL of methanol, and filter. Evaporate 2 mL of the filtrate on a water bath to dryness. With the residue, proceed as directed in the Identification (1) under Perphenazine.

(2) Add 5 mL of the filtrate obtained in the Identification (1) to 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), and proceed as directed in the Identification (2) under Perphenazine.

(3) Determine the absorption spectrum of the filtrate obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 309 nm and 313 nm. Add 30 mL of methanol to another 10 mL of the filtrate, and determine the absorption spectrum: it exhibits a maximum between 256 nm and 260 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 Perphenazine Tablet by shaking with 5 mL of water, shake well with 70 mL of methanol, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet x mL of the supernatant liquid, add methanol to make exactly V mL of a solution containing about $4 \mu\text{g}$ of perphenazine ($\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{OS}$) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of perphenazine for assay, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of perphenazine (C}_{21}\text{H}_{26}\text{ClN}_3\text{OS)} \\ = W_S \times (A_T/A_S) \times (V/25) \times (1/x) \end{aligned}$$

W_S : Amount (mg) of perphenazine for assay

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Perphenazine Tablets at 100 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Take 30 mL or more of the dissolved solution 90 minutes after start of the test, and filter through a membrane filter with pore size of not more than $0.8 \mu\text{m}$. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 10 mg of Perphenazine Reference Standard, previously dried in vacuum with phosphorus (V) oxide at 65°C for 4 hours, dissolve in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 2nd fluid for dissolution test to make exactly 250 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Perphenazine Tablets in 90 minutes is not less than 70%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to the} \\ \text{labeled amount of perphenazine (C}_{21}\text{H}_{26}\text{ClN}_3\text{OS)} \\ = W_S \times (A_T/A_S) \times (1/C) \times 36 \end{aligned}$$

W_S : Amount (mg) of Perphenazine Reference Standard
C: Labeled amount (mg) of perphenazine ($\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{OS}$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Perphenazine Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of perphenazine ($\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{OS}$), add 70 mL of methanol, shake well, and add methanol to make exactly 100 mL. Filter the solution, and discard the first 20 mL of the filtrate. Pipet 5 mL of the subsequent filtrate, add methanol to make exactly 50 mL, and use this solution as the sample solution. Weigh accurately about 10 mg of perphenazine for assay, previously dried in vacuum over phosphorus (V) oxide at 65°C for 4 hours, and dissolve in methanol to make exactly 250 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 258 nm as directed under Ultraviolet-visible Spec-

trophotometry <2.24>.

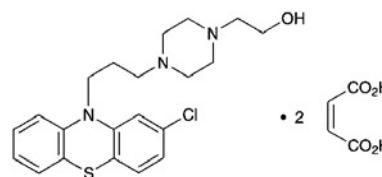
$$\begin{aligned} \text{Amount (mg) of perphenazine (C}_{21}\text{H}_{26}\text{ClN}_3\text{OS)} \\ = W_S \times (A_T/A_S) \times (2/5) \end{aligned}$$

W_S : Amount (mg) of perphenazine for assay

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Perphenazine Maleate

ペルフェナジンマレイン酸塩



$\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{OS} \cdot 2\text{C}_4\text{H}_4\text{O}_4$: 636.11

2-[4-[3-(2-Chlorophenothiazin-10-yl)propyl]piperazin-1-yl] ethanol dimaleate [58-39-9, Perphenazine]

Perphenazine Maleate, when dried, contains not less than 98.0% of $\text{C}_{21}\text{H}_{26}\text{ClN}_3\text{OS} \cdot 2\text{C}_4\text{H}_4\text{O}_4$.

Description Perphenazine Maleate occurs as a white to light yellow powder. It is odorless.

It is sparingly soluble in acetic acid (100), slightly soluble in water and in ethanol (95), and practically insoluble in chloroform.

It dissolves in dilute hydrochloric acid.

It is gradually colored by light.

Melting point: about 175°C (with decomposition).

Identification (1) Dissolve 8 mg of Perphenazine Maleate in 5 mL of sulfuric acid: a red color is produced, which becomes deep red-purple on warming.

(2) Dissolve 0.3 g of Perphenazine Maleate in 3 mL of dilute hydrochloric acid, add 2 mL of water and 3 mL of ammonia solution (28), shake, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (5)]. Evaporate the combined chloroform extracts on a water bath to dryness, dissolve the residue in 20 mL of methanol, and pour into 10 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25). Allow to stand for 4 hours, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 237°C and 244°C (with decomposition).

(3) Determine the absorption spectrum of a solution of Perphenazine Maleate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, to 10 mL of the solution add 30 mL of water. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Perform the test with Perphenazine Maleate as directed under Flame Coloration Test <1.04> (2): a green color ap-

pears.

(5) Evaporate the aqueous layer reserved in (2) to dryness. To the residue add 1 mL of dilute sulfuric acid and 5 mL of water, and extract with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate in a water bath at about 35°C with the aid of a current of air: the residue melts <2.60> between 128°C and 136°C.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of perphenazine maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Perphenazine Maleate according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Perphenazine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 31.81 \text{ mg of } C_{21}H_{26}ClN_3OS.2C_4H_4O_4 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Perphenazine Maleate Tablets

ペルフェナジンマレイン酸塩錠

Perphenazine Maleate Tablets contain not less than 93% and not more than 107% of the labeled amount of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$; 636.11).

Method of preparation Prepare as directed under Tablets, with Perphenazine Maleate.

Identification (1) Shake a quantity of powdered Perphenazine Maleate Tablets, equivalent to 0.04 g of Perphenazine Maleate according to the labeled amount, with 3 mL of dilute hydrochloric acid and 30 mL of water, centrifuge, filter the supernatant solution, add 3 mL of ammonia solution (28) to the filtrate, and extract with three 10-mL portions of chloroform. [Reserve the aqueous layer, and use for test (4).] Wash the combined chloroform extracts with two 5-mL portions of water, and separate the chloroform layer. Evaporate 6 mL of the chloroform solution on a water bath to dryness. Proceed with the residue as directed in the Identification (1) under Perphenazine Maleate.

(2) Evaporate 20 mL of the chloroform solution obtained in (1) on a water bath to dryness, dissolve the residue in 20 mL of methanol, and filter, if necessary. Warm the filtrate, add 5 mL of a warm solution of 2,4,6-trinitrophenol in methanol (1 in 25), allow to stand for 4 hours, and proceed as directed in the Identification (2) under Perphenazine Maleate.

(3) To 2 mL of the filtrate obtained in the Assay add water to make 50 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 253 nm and 257 nm and between 303 nm and 313 nm.

(4) Filter, if necessary, the aqueous layer reserved in (1), evaporate the filtrate to make about 5 mL, add 2 mL of dilute sulfuric acid, and extract with two 10-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate on a water bath to dryness, dissolve the residue in 5 mL of sulfuric acid TS, and add 1 to 2 drops of potassium permanganate TS: the red color of potassium permanganate TS fades immediately.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Disintegrate 1 tablet of Perphenazine Maleate Tablets by shaking with 15 mL of 0.1 mol/L hydrochloric acid TS, shake vigorously with 50 mL of methanol, add water to make exactly 100 mL, and centrifuge. Pipet x mL of the supernatant liquid, add water to make exactly V mL of a solution containing about 6 μ g of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$) in each mL, and use this solution as the sample solution. Separately, weigh accurately 30 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in 15 mL of 0.1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add 3 mL of 0.1 mol/L hydrochloric acid TS, 10 mL of methanol and water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\begin{aligned} \text{Amount (mg) of perphenazine maleate} \\ (C_{21}H_{26}ClN_3OS.2C_4H_4O_4) \\ = W_S \times (A_T/A_S) \times (V'/V) \times (1/50) \end{aligned}$$

W_S : Amount (mg) of perphenazine maleate for assay

Assay Weigh accurately and powder not less than 20 Perphenazine Maleate Tablets. Weigh accurately a portion of the powder, equivalent to about 40 mg of perphenazine maleate ($C_{21}H_{26}ClN_3OS.2C_4H_4O_4$), shake well with 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, measure exactly 5 mL of the subsequent filtrate, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of perphenazine maleate for assay, previously dried at 105°C for 3 hours, dissolve in a mixture of 15 mL of 1 mol/L hydrochloric acid TS and 50 mL of methanol, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 255 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\begin{aligned} \text{Amount (mg) of perphenazine maleate} \\ (C_{21}H_{26}ClN_3OS.2C_4H_4O_4) \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of perphenazine maleate for assay

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Adsorbed Purified Pertussis Vaccine

沈降精製百日せきワクチン

Adsorbed Purified Pertussis Vaccine is a liquid for injection prepared by adding an aluminum salt to a liquid containing the protective antigen of *Bordetella pertussis* to make the antigen insoluble.

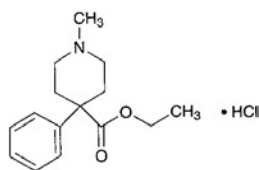
It conforms to the requirements of Adsorbed Purified Pertussis Vaccine in the Minimum Requirements for Biological Products.

Description Adsorbed Purified Pertussis Vaccine forms a homogeneous, white turbidity on shaking.

Pethidine Hydrochloride

Operidine

ペチジン塩酸塩



$C_{15}H_{21}NO_2 \cdot HCl$: 283.79

Ethyl 1-methyl-4-phenylpiperidine-4-carboxylate monohydrochloride [50-13-5]

Pethidine Hydrochloride, when dried, contains not less than 98.0% of $C_{15}H_{21}NO_2 \cdot HCl$.

Description Pethidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in acetic acid (100), freely soluble in ethanol (95), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution dissolved 1.0 g of Pethidine Hydrochloride in 20 mL of water is between 3.8 and 5.8.

Identification (1) Determine the absorption spectrum of a solution of Pethidine Hydrochloride (1 in 2000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pethidine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pethidine Hydrochloride (1 in 50) responds to Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 187 – 189°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pethidine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.20 g of Pethidine Hydrochloride. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.240%).

(3) Related substances—Dissolve 0.05 g of Pethidine Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from both solutions by the automatic integration method: the total area of the peaks other than that of pethidine from the sample solution is not larger than the peak area of pethidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

Time span of measurement: About 2 times as long as the retention time of pethidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of pethidine obtained from 20 μ L of this solution is equivalent to 5 to 15% of that of pethidine obtained from 20 μ L of the standard solution.

System performance: To 2 mL each of the sample solution and a solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 50,000) add the mobile phase to make 10 mL. When the procedure is run with 20 μ L of this solution according to the above operating conditions, pethidine and isoamyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pethidine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.5 g of Pethidine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.38 mg of $C_{15}H_{21}NO_2 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pethidine Hydrochloride Injection

Operidine Injection

ペチジン塩酸塩注射液

Pethidine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$: 283.79).

Method of preparation Prepare as directed under Injections, with Pethidine Hydrochloride.

Description Pethidine Hydrochloride Injection is a clear, colorless liquid.

It is affected by light.

pH 4.0 – 6.0

Identification Take a volume of Pethidine Hydrochloride Injection equivalent to 0.1 g of Pethidine Hydrochloride according to the labeled amount, and add water to make 200 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 250 nm and 254 nm, between 255 nm and 259 nm, and between 261 nm and 265 nm.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Pethidine Hydrochloride Injection, equivalent to about 0.1 g of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$) according to the labeled amount, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of pethidine hydrochloride for assay, previously dried at 105°C for 3 hours, add exactly 10 mL of the internal standard solution, and add the mobile phase to make 50 mL. To 5 mL of this solution add the mobile phase to make 20 mL, and use this solution as the standard solution. Perform the test with 20 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of pethidine to that of the internal standard.

Amount (mg) of pethidine hydrochloride ($C_{15}H_{21}NO_2 \cdot HCl$)
= $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of pethidine hydrochloride for assay

Internal standard solution—A solution of isoamyl parahydroxybenzoate in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 257 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.0 g of sodium lauryl sulfate in 1000 mL of diluted phosphoric acid (1 in 1000), adjust the pH to 3.0 with sodium hydroxide TS, and to 550 mL of this solution add 450 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pethidine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, pethidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pethidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Hydrophilic Petrolatum

親水ワセリン

Method of preparation

White Beeswax	80 g
Stearyl Alcohol or Cetanol	30 g
Cholesterol	30 g
White Petrolatum	a sufficient quantity
To make 1000 g	

Melt and mix Stearyl Alcohol or Cetanol, White Beeswax and White Petrolatum on a water bath. Add Cholesterol, and melt completely by stirring. Stop warming, and stir until the mixture congeals.

Description Hydrophilic Petrolatum is white in color. It has a slight, characteristic odor.

When mixed with an equal volume of water, it retains the consistency of ointment.

Containers and storage Containers—Tight containers.

White Petrolatum

白色ワセリン

White Petrolatum is a decolorized and purified mixture of hydrocarbons obtained from petroleum.

Description White Petrolatum is a white to pale yellow, homogeneous, unctuous mass. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95) and in ethanol (99.5).

It dissolves in diethyl ether making a clear liquid or producing slight insoluble substances.

It becomes a clear liquid when warmed.

Melting point <2.60> 38 – 60°C (Method 3).

Purity (1) Color—Melt White Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: Add 3.4 mL of water to 1.6 mL of Ferric Chloride Colorimetric Stock Solution.

(2) Acidity or alkalinity—To 35.0 g of White Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the White Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of White Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of White Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of White Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of White Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of White Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Yellow Petrolatum

黄色ワセリン

Yellow Petrolatum is a purified mixture of hydrocarbons obtained from petroleum.

Description Yellow Petrolatum occurs as a yellow, homogeneous, unctuous mass, It is odorless and tasteless.

It is slightly soluble in ethanol (95), and practically insoluble in water.

It dissolves in diethyl ether, in petroleum benzine and in turpentine oil, making a clear liquid or producing slight insoluble substances.

It becomes a yellow, clear liquid with slight fluorescence when warmed.

Melting point <2.60> 38 – 60°C (Method 3).

Purity (1) Color—Melt Yellow Petrolatum by warming, and pour 5 mL of it into a test tube, and keep the content in a liquid condition: the liquid has no more color than the following control solution, when observed transversely from side against a white background.

Control solution: To 3.8 mL of Ferric Chloride Stock CS add 1.2 mL of Cobaltous Chloride Stock CS.

(2) Acidity or alkalinity—To 35.0 g of Yellow Petrolatum add 100 mL of hot water, shake vigorously for 5 minutes, and then draw off the aqueous layer. Treat the Yellow Petrolatum layer in the same manner using two 50-mL portions of hot water. To the combined aqueous layer add 1 drop of phenolphthalein TS, and boil: no red color is produced. Further add 2 drops of methyl orange TS: no red color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Yellow Petrolatum according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Yellow Petrolatum, according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 2 ppm).

(5) Sulfur compound—To 4.0 g of Yellow Petrolatum add 2 mL of ethanol (99.5) and 2 drops of sodium hydroxide solution (1 in 5) saturated with lead (II) oxide, warm the mixture for 10 minutes at about 70°C with frequent shaking, and allow to cool: no dark color is produced.

(6) Organic acids—To 100 mL of dilute ethanol add 1 drop of phenolphthalein TS, and titrate with 0.01 mol/L sodium hydroxide VS, until the color of the solution changes to light red. Mix this solution with 20.0 g of Yellow Petrolatum, and boil for 10 minutes under a reflux condenser. Add 2 to 3 drops of phenolphthalein TS to the mixture and 0.40 mL of 0.1 mol/L sodium hydroxide VS with vigorous shaking: the color of the solution remains red.

(7) Fats and fatty oils or resins—To 10.0 g of Yellow Petrolatum add 50 mL of sodium hydroxide solution (1 in 5), and boil for 30 minutes under a reflux condenser. Cool the mixture, separate the aqueous layer, and filter, if necessary. To the aqueous layer add 200 mL of dilute sulfuric acid: neither oily matter nor precipitate is produced.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Tight containers.

Petroleum Benzin

石油ベンジン

Petroleum Benzin is a mixture of low-boiling point

hydrocarbons from petroleum.

Description Petroleum Benzin occurs as a colorless, clear, volatile liquid. It shows no fluorescence. It has a characteristic odor.

It is miscible with ethanol (99.5) and with diethyl ether.

It is practically insoluble in water.

It is very flammable.

Specific gravity d_{20}^{20} : 0.65 – 0.71

Purity (1) Acid—Shake vigorously 10 mL of Petroleum Benzin with 5 mL of water for 2 minutes, and allow to stand: the separated aqueous layer does not change moistened blue litmus paper to red.

(2) Sulfur compounds and reducing substances—To 10 mL of Petroleum Benzin add 2.5 mL of ammonia-ethanol TS and 2 to 3 drops of silver nitrate TS, and warm the mixture at about 50°C for 5 minutes, protected from light: no brown color develops.

(3) Fatty oil and sulfur compounds—Drop and evaporate 10 mL of Petroleum Benzin in small portions on odorless filter paper spread on a previously warmed glass plate: no spot or no foreign odor is perceptible.

(4) Benzene—Warm 5 drops of Petroleum Benzin with 2 mL of sulfuric acid and 0.5 mL of nitric acid for about 10 minutes, allow to stand for 30 minutes, transfer the mixture to a porcelain dish, and dilute with water: no odor of nitrobenzene is perceptible.

(5) Residue on evaporation—Evaporate 140 mL of Petroleum Benzin on a water bath to dryness, and heat the residue at 105°C to constant mass: the mass is not more than 1 mg.

(6) Readily carbonizable substances—Shake vigorously 5 mL of Petroleum Benzin with 5 mL of sulfuric acid for readily carbonizable substances for 5 minutes in a Nessler tube, and allow to stand: the sulfuric acid layer has no more color than Matching Fluid A.

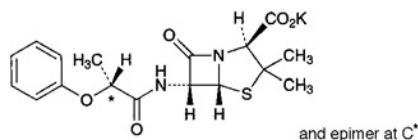
Distilling range <2.57> 50 – 80°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Remote from fire, and not exceeding 30°C.

Phenethicillin Potassium

フェネチシリンカリウム



$C_{17}H_{19}KN_2O_5S$: 402.51

Monopotassium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*RS*)-2-phenoxypropanoylamino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [I32-93-4]

Phenethicillin Potassium contains not less than 1400 units and not more than 1480 units per mg, calculated on the dried basis. The potency of Phenethicillin Potassium is expressed as unit based on the amount of phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$). One unit of Phenethicillin Potassium is equivalent to 0.68 μ g of

phenethicillin potassium ($C_{17}H_{19}KN_2O_5S$).

Description Phenethicillin Potassium occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Phenethicillin Potassium (1 in 5000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phenethicillin Potassium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Phenethicillin Potassium responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +217 – +244° (1 g calculated on the dried basis, phosphate TS, 100 mL, 100 mm).

L- α -Phenethicillin potassium Dissolve about 50 mg of Phenethicillin Potassium in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_D and A_L , of D- α -phenethicillin and L- α -phenethicillin by the automatic integration method: $A_L/(A_D + A_L)$ is between 0.50 and 0.70.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Adjust the pH of a mixture of a solution of diammonium hydrogen phosphate (1 in 150) and acetonitrile (41:10) to 7.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of L- α -phenethicillin is about 25 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the sample solution under the above operating conditions, D- α -phenethicillin and L- α -phenethicillin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the sample solution under the above operating conditions, the relative standard deviation of the peak area of L- α -phenethicillin is not more than 2.0%.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Phenethicillin Potassium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Phenethicillin Potassium according to Method 4 and, perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Phenethicillin

Potassium in 50 mL of the mobile, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total of the peak areas other than D- α -phenethicillin and L- α -phenethicillin obtained from the sample solution is not more than 5 times the total of the peak areas of D- α -phenethicillin and L- α -phenethicillin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the L- α -Phenethicillin potassium.

Time span of measurement: About 1.5 times as long as the retention time of L- α -phenethicillin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of L- α -phenethicillin obtained from 10 μ L of this solution is equivalent to 14 to 26% of that from 10 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the L- α -Phenethicillin potassium.

Loss on drying <2.41> Not more than 1.0% (0.1 g, in vacuum, 60°C, 3 hours).

Assay Weigh accurately an amount of Phenethicillin Potassium and dried L-Phenethicillin Potassium Reference Standard, equivalent to about 40,000 units, dissolve each in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use these solutions as the sample solution and standard solution, respectively. Pipet 2 mL each of these solutions in 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS to them, and allow to stand for exactly 15 minutes. To them add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, and allow them to stand for exactly 15 minutes. Add 0.2–0.5 mL of starch TS, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution disappears. Separately, to exactly 2 mL each of the sample solution and standard solution add exactly 10 mL of 0.005 mol/L iodine VS, then proceed in the same manner as above without allowing to stand for 15 minutes as a blank determination, and make any necessary correction. Determine the volumes, V_T and V_S , of 0.005 mol/L iodine VS consumed in the sample solution and standard solution.

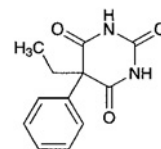
$$\text{Amount (unit) of } C_{17}H_{19}KN_2O_5S = W_S \times (V_T/V_S)$$

W_S : Amount (unit) of L-Phenethicillin Potassium Reference Standard

Containers and storage Containers—Well-closed containers.

Phenobarbital

フェノバルビタール



$C_{12}H_{12}N_2O_3$: 232.24

5-Ethyl-5-phenylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione
[50-06-6]

Phenobarbital, when dried, contains not less than 99.0% of $C_{12}H_{12}N_2O_3$.

Description Phenobarbital occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in *N,N*-dimethylformamide, freely soluble in ethanol (95), in acetone and in pyridine, soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

The pH of a saturated solution of Phenobarbital is between 5.0 and 6.0.

Identification (1) Boil 0.2 g of Phenobarbital with 10 mL of sodium hydroxide TS: the gas evolved changes moistened red litmus paper to blue.

(2) Dissolve 0.1 g of Phenobarbital in 5 mL of diluted pyridine (1 in 10), shake the solution with 0.3 mL of copper (II) sulfate TS, and allow to stand for 5 minutes: a light red-purple precipitate is produced. Shake the mixture with 5 mL of chloroform: the chloroform layer remains colorless. Dissolve 0.1 g of Phenobarbital in a mixture of 2 to 3 drops of ammonia-ammonium chloride buffer solution, pH 10.7, and 5 mL of diluted pyridine (1 in 10), then add 5 mL of chloroform and 0.3 mL of copper (II) sulfate TS: a light red-purple precipitate is produced in the water layer. Shake again: the chloroform layer remains colorless.

(3) Shake 0.4 g of Phenobarbital with 0.1 g of anhydrous sodium carbonate and 4 mL of water, and add a solution of 0.3 g of 4-nitrobenzyl chloride in 7 mL of ethanol (95). Heat on a water bath for 30 minutes under a reflux condenser, and allow to stand for 1 hour. Filter the crystals, wash with 7 mL of sodium hydroxide TS, then with a small amount of water, recrystallize from a mixture of ethanol (95) and chloroform (1:1), and dry at 105°C for 30 minutes: the crystals melt <2.60> between 181°C and 185°C.

(4) Dissolve 0.1 g of Phenobarbital in 2 mL of sulfuric acid, shake the solution with 5 to 6 mg of potassium nitrate, and allow to stand for 10 minutes: a yellow to yellow-brown color develops.

Melting point <2.60> 175–179°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Phenobarbital in 5 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.30 g of Phenobarbital in 20 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take

0.30 mL of 0.01 mol/L hydrochloric acid VS, 20 mL of acetone and 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.035%).

(3) Sulfate <1.14>—Dissolve 0.40 g of Phenobarbital in 20 mL of acetone, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: take 0.40 mL of 0.005 mol/L sulfuric acid VS, 20 mL of acetone, and 1 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Phenobarbital according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead solution (not more than 20 ppm).

(5) Phenylbarbituric acid—Boil 1.0 g of Phenobarbital with 5 mL of ethanol (95) for 3 minutes: the solution is clear.

(6) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Phenobarbital. The solution has not more color than Matching Fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenobarbital, previously dried, dissolve in 50 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution change from yellow to yellow-green (indicator: 1 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination using a mixture of 50 mL of *N,N*-dimethylformamide and 22 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Well-closed containers.

10% Phenobarbital Powder

Phenobarbital Powder

フェノバルビタール散 10%

10% Phenobarbital Powder contains not less than 9.3% and not more than 10.7% of phenobarbital ($C_{12}H_{12}N_2O_3$: 232.24).

Method of preparation

Phenobarbital	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification Shake thoroughly 5 g of 10% Phenobarbital Powder with 20 mL of hexane, and filter. Collect the residue, and dry on a water bath, then extract with four 30-mL portions of chloroform. Filter the combined chloroform ex-

tracts, and evaporate the filtrate to dryness. Dry the residue at 105°C for 1 hour: the residue so obtained melts <2.60> between 174°C and 179°C. With the residue, proceed as directed in the Identification (1) and (2) under Phenobarbital.

Assay Weigh accurately about 10 g of 10% Phenobarbital Powder, transfer to a glass-stoppered flask, and add exactly 100 mL of a mixture of chloroform and ethanol (95) (10:1). Stopper tightly, shake, and allow to stand for 30 minutes. Transfer the mixture to a glass-stoppered centrifuge tube, and centrifuge. Measure exactly 50 mL of the supernatant liquid, evaporate on a water bath to dryness, dissolve the residue in 50 mL of *N,N*-dimethylformamide, and proceed as directed in the Assay under Phenobarbital.

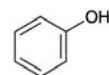
Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 23.22 mg of $C_{12}H_{12}N_2O_3$

Containers and storage Containers—Well-closed containers.

Phenol

Carbolic Acid

フェノール



C_6H_6O : 94.11
Phenol [108-95-2]

Phenol contains not less than 98.0% of C_6H_6O .

Description Phenol occurs as colorless to slightly red crystals or crystalline masses. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and soluble in water.

Phenol (10 g) is liquefied by addition of 1 mL of water.

The color changes gradually through red to dark red by light or air.

It cauterizes the skin, turning it white.

Congealing point: about 40°C

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Purity (1) Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay Dissolve about 1.5 g of Phenol, accurately weighed, in water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05

mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at once stopper the flask, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Phenol for Disinfection

Carbolic Acid for Disinfection

消毒用フェノール

Phenol for Disinfection contains not less than 95.0% of phenol (C₆H₆O: 94.11).

Description Phenol for Disinfection occurs as colorless to slightly red crystals, crystalline masses, or liquid containing these crystals. It has a characteristic odor.

It is very soluble in ethanol (95) and in diethyl ether, and freely soluble in water.

Phenol for Disinfection (10 g) is liquefied by addition of 1 mL of water.

It cauterizes the skin, turning it white.

Congealing point: about 30°C

Identification (1) To 10 mL of a solution of Phenol for Disinfection (1 in 100) add 1 drop of iron (III) chloride TS: a blue-purple color is produced.

(2) To 5 mL of a solution of Phenol for Disinfection (1 in 10,000) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Purity (1) Clarity of solution—Dissolve 1.0 g of Phenol for Disinfection in 15 mL of water: the solution is clear.

(2) Residue on evaporation—Weigh accurately about 5 g of Phenol for Disinfection, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.10% of the mass of the sample.

Assay Dissolve about 1 g of Phenol for Disinfection, accurately weighed, in water to make exactly 1000 mL. Pipet 25 mL of the solution into an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper immediately, shake well, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Liquefied Phenol

Liquefied Carbolic Acid

液状フェノール

Liquefied Phenol is Phenol maintained in a liquid condition by the presence of 10% of Water or Purified Water.

It contains not less than 88.0% of phenol (C₆H₆O: 94.11)

Description Liquefied Phenol is a colorless or slightly reddish liquid. It has a characteristic odor.

It is miscible with ethanol (95), with diethyl ether and with glycerin.

A mixture of equal volumes of Liquefied Phenol and glycerin is miscible with water.

The color changes gradually to dark red on exposure to light or air.

It cauterizes the skin, turning it white.

Specific gravity d_{20}^{20} : about 1.065

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of a solution of Liquefied Phenol (1 in 100): a blue-purple color develops.

(2) Add bromine TS dropwise to 5 mL of a solution of Liquefied Phenol (1 in 10,000): a white precipitate is produced, which at first dissolves with shaking, but becomes permanent as excess of the reagent is added.

Boiling point <2.57> Not more than 182°C.

Purity (1) Clarity and color of solution and acidity or alkalinity—Dissolve 1.0 g of Liquefied Phenol in 15 mL of water: the solution is clear, and neutral or only faintly acid. Add 2 drops of methyl orange TS: no red color develops.

(2) Residue on evaporation—Weigh accurately about 5 g of Liquefied Phenol, evaporate on a water bath, and dry the residue at 105°C for 1 hour: the mass is not more than 0.05% of the mass of the sample.

Assay Dissolve about 1.7 g of Liquefied Phenol, accurately weighed, in a water to make exactly 1000 mL. Transfer exactly 25 mL of this solution to an iodine flask, add exactly 30 mL of 0.05 mol/L bromine VS, then 5 mL of hydrochloric acid, and immediately stopper the flask. Shake the flask repeatedly for 30 minutes, allow to stand for 15 minutes, then add 7 mL of potassium iodide TS, at one stopper the flask tightly, and shake well. Add 1 mL of chloroform, stopper the flask, and shake thoroughly. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dental Phenol with Camphor

歯科用フェノール・カンフル

Method of preparation

Phenol	35 g
<i>d</i> - or <i>dl</i> -Camphor	65 g
To make 100 g	

Melt Phenol by warming, add *d*-Camphor or *dl*-Camphor, and mix.

Description Dental Phenol with Camphor is a colorless or light red liquid. It has a characteristic odor.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Phenol and Zinc Oxide Liniment

フェノール・亜鉛華リニメント

Method of preparation

Liquefied Phenol	22 mL
Powdered Tragacanth	20 g
Carmellose Sodium	30 g
Glycerin	30 mL
Zinc Oxide	100 g
Purified Water	a sufficient quantity
To make 1000 g	

Mix Liquefied Phenol, Glycerin and Purified Water, add Powdered Tragacanth in small portions by stirring, and allow the mixture to stand overnight. To the mixture add Carmellose Sodium in small portions by stirring to make a pasty mass, add Zinc Oxide in small portions, and prepare the liniment as directed under Liniments. Less than 5 g of Powdered Tragacanth or Carmellose Sodium can be replaced by each other to make 50 g in total.

Description Phenol and Zinc Oxide Liniment is a white, pasty mass. It has a slight odor of phenol.

Identification (1) Shake well 1 g of Phenol and Zinc Oxide Liniment with 10 mL of diethyl ether, and filter. To the filtrate add 10 mL of dilute sodium hydroxide TS, shake well, and separate the water layer. To 1 mL of the water layer add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and add 3 mL of sodium hydroxide TS: a yellow color develops (phenol).

(2) Place 1 g of Phenol and Zinc Oxide Liniment in a porcelain crucible, heat gradually raising the temperature until the content is charred, and then ignite it strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is produced (zinc oxide).

(3) Shake 0.5 g of Phenol and Zinc Oxide Liniment with 1 mL of water and 5 mL of chloroform, separate the chlo-

roform layer, and use this solution as the sample solution. Separately, dissolve 0.01 g of phenol in 5 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia solution (28) (50:5:1) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor: the spots obtained from the sample solution and the standard solution show the same *R*_f value.

Containers and storage Containers—Tight containers.

Phenolated Water

フェノール水

Phenolated Water contains not less than 1.8 w/v% and not more than 2.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Liquefied Phenol	22 mL
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Mix the above ingredients.

Description Phenolated Water is a colorless, clear liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water: a blue-purple color develops.

(2) To 5 mL of a solution of Phenolated Water (1 in 200) add bromine TS dropwise: a white precipitate is formed, and it dissolves at first upon shaking but becomes permanent as excess of the reagent is added.

Assay Take exactly 2 mL of Phenolated Water into an iodine flask, add 25 mL of water, then add exactly 40 mL of 0.05 mol/L bromine VS and 5 mL of hydrochloric acid, stopper immediately, shake for 30 minutes, and allow to stand for 15 minutes. Add 7 mL of potassium iodide TS, stopper tightly at once, shake well, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C₆H₆O

Containers and storage Containers—Tight containers.

Phenolated Water for Disinfection

消毒用フェノール水

Phenolated Water for Disinfection contains not less than 2.8 w/v% and not more than 3.3 w/v% of phenol (C₆H₆O: 94.11).

Method of preparation

Phenol for Disinfection	31 g
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Mix the above ingredients.

Description Phenolated Water for Disinfection is a clear, colorless liquid, having the odor of phenol.

Identification (1) Add 1 drop of iron (III) chloride TS to 10 mL of Phenolated Water for Disinfection: a blue-purple color develops.

(2) Proceed with 5 mL of a solution of Phenolated Water for Disinfection (1 in 200) as directed in the Identification (2) under Phenol for Disinfection.

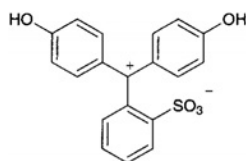
Assay Take exactly 5 mL of Phenolated Water for Disinfection, add water to make exactly 100 mL, then pipet 25 mL of the solution into an iodine flask, and proceed as directed in the Assay under Phenol for Disinfection.

Each mL of 0.05 mol/L bromine VS
= 1.569 mg of C_6H_6O

Containers and storage Containers—Tight containers.

Phenolsulfonphthalein

フェノールスルホンフタレイン



$C_{19}H_{14}O_5S$: 354.38
2-[Bis(4-hydroxyphenyl)methyl]benzenesulfonate
[143-74-8]

Phenolsulfonphthalein, when dried, contains not less than 98.0% of $C_{19}H_{14}O_5S$.

Description Phenolsulfonphthalein occurs as a vivid red to dark red, crystalline powder.

It is very slightly soluble in water and in ethanol (95).

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 5 mg of Phenolsulfonphthalein in 2 to 3 drops of sodium hydroxide TS, add 2 mL of 0.05 mol/L bromine VS and 1 mL of dilute sulfuric acid, shake well, and allow to stand for 5 minutes. Render the solution alkaline with sodium hydroxide TS: a deep blue-purple color develops.

(2) Dissolve 0.01 g of Phenolsulfonphthalein in diluted sodium carbonate TS (1 in 10) to make 200 mL. To 5 mL of this solution add diluted sodium carbonate TS (1 in 10) to make 100 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Insoluble substances—To about 1 g of Phenolsulfonphthalein, accurately weighed, add 20 mL of a solution

of sodium hydrogen carbonate (1 in 40). Allow the mixture to stand for 1 hour with frequent shaking, dilute with water to 100 mL, and allow to stand for 24 hours. Collect the insoluble substances using a tared glass filter (G4), wash with 25 mL of a solution of sodium hydrogen carbonate (1 in 100) and with five 5-mL portions of water, and dry at 105°C for 1 hour: the mass of the residue is not more than 0.2%.

(2) Related substances—Dissolve 0.10 g of Phenolsulfonphthalein in 5 mL of dilute sodium hydroxide TS, and use this solution as the sample solution. Pipet 0.5 mL of this solution, add dilute sodium hydroxide TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of *t*-amyl alcohol, acetic acid (100) and water (4:1:1) to a distance of about 15 cm, and air-dry the plate. After allowing the plate to stand in an ammonia vapor, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.15 g of Phenolsulfonphthalein, previously dried, transfer to an iodine flask, dissolve in 30 mL of a solution of sodium hydroxide (1 in 250), and add water to make 200 mL. Add exactly measured 50 mL of 0.05 mol/L bromine VS, add 10 mL of hydrochloric acid to the solution quickly, and stopper immediately. Allow the mixture to stand for 5 minutes with occasional shaking, add 7 mL of potassium iodide TS, stopper again immediately, and shake gently for 1 minute. Titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 4.430 mg of $C_{19}H_{14}O_5S$

Containers and storage Containers—Well-closed containers.

Phenolsulfonphthalein Injection

フェノールスルホンフタレイン注射液

Phenolsulfonphthalein Injection is an aqueous solution for injection.

It contains not less than 0.54 w/v% and not more than 0.63 w/v% of phenolsulfonphthalein ($C_{19}H_{14}O_5S$: 354.38).

Method of preparation

Phenolsulfonphthalein	6 g
Sodium Chloride	9 g
Sodium Bicarbonate (or Sodium Hydroxide)	1.43 g (0.68 g)
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description Phenolsulfonphthalein Injection is a clear, orange-yellow to red liquid.

Identification To 1 mL of Phenolsulfonphthalein Injection add 2 to 3 drops of sodium hydroxide TS, and proceed as directed in the Identification (1) under Phenolsulfonphthalein.

pH <2.54> 6.0 – 7.6

Extractable volume <6.05> It meets the requirement.

Sensitivity To 1.0 mL of Phenolsulfonphthalein Injection add 5 mL of water. To 0.20 mL of this solution add 50 mL of freshly boiled and cooled water and 0.40 mL of 0.01 mol/L sodium hydroxide VS: a deep red-purple color develops, and it changes to light yellow on the addition of 0.40 mL of 0.005 mol/L sulfuric acid VS.

Assay Pipet 5 mL of Phenolsulfonphthalein Injection, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of phenolsulfonphthalein for assay, previously dried in a desiccator (silica gel) for 4 hours, and dissolve in a solution of anhydrous sodium carbonate (1 in 100) to make exactly 250 mL. Pipet 5 mL of this solution, add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 559 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

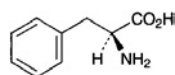
$$\begin{aligned} \text{Amount (mg) of phenolsulfonphthalein (C}_{19}\text{H}_{14}\text{O}_5\text{S)} \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of phenolsulfonphthalein for assay

Containers and storage Containers—Hermetic containers.

L-Phenylalanine

L-フェニルアラニン



$\text{C}_9\text{H}_{11}\text{NO}_2$: 165.19

(2S)-2-Amino-3-phenylpropanoic acid [63-91-2]

L-Phenylalanine, when dried, contains not less than 98.5% of $\text{C}_9\text{H}_{11}\text{NO}_2$.

Description L-Phenylalanine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly bitter taste.

It is freely soluble in formic acid, sparingly soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Phenylalanine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-33.0 - -35.5^\circ$ (after drying, 0.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of L-Phenylalanine in 20 mL of water: the pH of this solution is between 5.3 and 6.3.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Phenylalanine in 10 mL of 1 mol/L hydrochloric acid TS: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Phenylalanine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Phenylalanine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Phenylalanine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Dissolve 1.0 g of L-Phenylalanine in 40 mL of water and 2 mL of dilute acetic acid by warming, cool, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of L-Phenylalanine in 5 mL of dilute hydrochloric acid and 15 mL of water, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Phenylalanine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

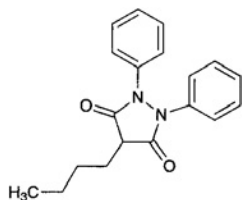
Assay Weigh accurately about 0.17 g of L-Phenylalanine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 16.52 \text{ mg of C}_9\text{H}_{11}\text{NO}_2 \end{aligned}$$

Containers and storage Containers—Tight containers.

Phenylbutazone

フェニルブタゾン



$C_{19}H_{20}N_2O_2$: 308.37

4-Butyl-1,2-diphenylpyrazolidine-3,5-dione [50-33-9]

Phenylbutazone, when dried, contains not less than 99.0% of $C_{19}H_{20}N_2O_2$.

Description Phenylbutazone occurs as a white to slightly yellowish white, crystalline powder. It is odorless, and is at first tasteless but leaves a slightly bitter aftertaste.

It is freely soluble in acetone, soluble in ethanol (95) and in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) To 0.1 g of Phenylbutazone add 1 mL of acetic acid (100) and 1 mL of hydrochloric acid, and heat on a water bath under a reflux condenser for 30 minutes. Add 10 mL of water, and cool with ice water. Filter, and to the filtrate add 3 to 4 drops of sodium nitrite TS. To 1 mL of this solution add 1 mL of 2-naphthol TS and 3 mL of chloroform, and shake: a deep red color develops in the chloroform layer.

(2) Dissolve 1 mg of Phenylbutazone in 10 mL of dilute sodium hydroxide TS, and dilute with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 104 – 107°C

Purity (1) Clarity of solution—Dissolve 1.0 g of Phenylbutazone in 20 mL of sodium hydroxide solution (2 in 25), and allow to stand at $25 \pm 1^\circ\text{C}$ for 3 hours: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.05.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Phenylbutazone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of phenylbutazone, according to Method 3, and perform the test (not more than 2 ppm).

(4) Readily carbonizable substances—Dissolve 1.0 g of Phenylbutazone in 20 mL of sulfuric acid, and allow to stand at $25 \pm 1^\circ\text{C}$ for exactly 30 minutes: the solution is clear. Determine the absorbance of this solution at 420 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.10.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum,

silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

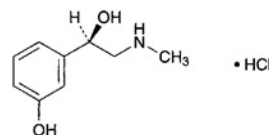
Assay Weigh accurately about 0.5 g of Phenylbutazone, previously dried, dissolve in 25 mL of acetone, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the solution shows a blue color which persists for 15 seconds (indicator: 5 drops of bromothymol blue TS). Perform a blank determination with a mixture of 25 mL of acetone and 16 mL of water, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 30.84 mg of $C_{19}H_{20}N_2O_2$

Containers and storage Containers—Tight containers.

Phenylephrine Hydrochloride

フェニレフリン塩酸塩



$C_9H_{13}NO_2 \cdot HCl$: 203.67

(1*R*)-1-(3-Hydroxyphenyl)-2-methylaminoethanol monohydrochloride [61-76-7]

Phenylephrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_9H_{13}NO_2 \cdot HCl$.

Description Phenylephrine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Phenylephrine Hydrochloride (1 in 100) is between 4.5 and 5.5.

Identification (1) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of copper (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue color is produced. To the solution so obtained add 1 mL of diethyl ether, and shake vigorously: no blue color develops in the diethyl ether layer.

(2) To 1 mL of a solution of Phenylephrine Hydrochloride (1 in 100) add 1 drop of iron (III) chloride TS: a persistent purple color is produced.

(3) Dissolve 0.3 g of Phenylephrine Hydrochloride in 3 mL of water, add 1 mL of ammonia TS, and rub the inner side of the test tube with a glass rod: a precipitate is produced. Collect the precipitate, wash with a few drops of ice-cold water, and dry at 105°C for 2 hours: it melts <2.60> between 170°C and 177°C.

(4) A solution of Phenylephrine Hydrochloride (1 in 100) responds to Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-42.0 - -47.5^\circ$ (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point <2.60> 140 – 145°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Phenylephrine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Take 0.5 g of Phenylephrine Hydrochloride, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Ketone—Dissolve 0.20 g of Phenylephrine Hydrochloride in 1 mL of water, and add 2 drops of sodium pentacyanonitrosylferrate (III) TS, 1 mL of sodium hydroxide TS and then 0.6 mL of acetic acid (100): the solution has no more color than the following control solution.

Control solution: Prepare as directed above without Phenylephrine Hydrochloride.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g of Phenylephrine Hydrochloride, previously dried, dissolve in 40 mL of water contained in an iodine flask, add exactly measured 50 mL of 0.05 mol/L bromine VS, then add 5 mL of hydrochloric acid, and immediately stopper tightly. Shake the mixture, and allow to stand for 15 minutes. To this solution add 10 mL of potassium iodide TS carefully, stopper tightly immediately, shake thoroughly, allow to stand for 5 minutes, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.05 mol/L bromine VS} \\ = 3.395 \text{ mg of } C_9H_{13}NO_2 \cdot HCl \end{aligned}$$

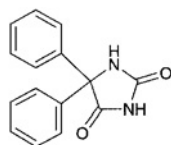
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Phenytoin

Diphenylhydantoin

フェニトイン



$C_{15}H_{12}N_2O_2$; 252.27

5,5-Diphenylimidazolidine-2,4-dione [57-41-0]

Phenytoin, when dried, contains not less than 99.0% of $C_{15}H_{12}N_2O_2$.

Description Phenytoin occurs as a white, crystalline powder or granules. It is odorless and tasteless.

It is sparingly soluble in ethanol (95) and in acetone, slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 296°C (with decomposition).

Identification (1) Dissolve 0.02 g of Phenytoin in 2 mL of ammonia TS, and add 5 mL of silver nitrate TS: a white precipitate is produced.

(2) Boil a mixture of 0.01 g of Phenytoin, 1 mL of ammonia TS and 1 mL of water, and add dropwise 2 mL of a

mixture prepared from 50 mL of a solution of copper (II) sulfate pentahydrate (1 in 20) and 10 mL of ammonia TS: a red, crystalline precipitate is produced.

(3) Heat 0.1 g of Phenytoin with 0.2 g of sodium hydroxide, and fuse: the gas evolved turns moistened red litmus paper blue.

(4) Add 3 mL of chlorinated lime TS to 0.1 g of phenytoin, shake for 5 minutes, and dissolve the oily precipitate in 15 mL of hot water. After cooling, add 1 mL of dilute hydrochloric acid dropwise, then add 4 mL of water. Filter the white precipitate thus obtained, wash with water, and press it with dry filter paper to remove the accompanying water. Dissolve the precipitate with 1 mL of chloroform, add 5 mL of diluted ethanol (9 in 10), and rub the inner surface of the flask to produce a white, crystalline precipitate. Collect the precipitate, wash with ethanol (95), and dry: the melting point <2.60> is between 165°C and 169°C.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Phenytoin in 10 mL of 0.2 mol/L sodium hydroxide VS: the solution is clear and colorless. Then heat the solution: no turbidity is produced. Cool, and mix the solution with 5 mL of acetone: the solution is clear and colorless.

(2) Acidity or alkalinity—Shake 2.0 g of phenytoin with 40 mL of water for 1 minute, filter, and perform the following tests using this filtrate as the sample solution.

(i) To 10 mL of the sample solution add 2 drops of phenolphthalein TS: no color develops. Then add 0.15 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(ii) To 10 mL of the sample solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and 5 drops of methyl red TS: a red to orange color develops.

(3) Chloride <1.03>—Dissolve 0.30 g of Phenytoin in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution from 30 mL of acetone, 0.60 mL of 0.01 mol/L hydrochloric acid VS and 6 mL of dilute nitric acid, and add water to 50 mL (not more than 0.071%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Phenytoin, previously dried, dissolve in 40 mL of ethanol (95) with the aid of gentle heating, add 0.5 mL of thymolphthalein TS immediately, and titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops. Then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 25 mL of silver nitrate TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light red color, which persists for 1 minute, develops.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 25.23 \text{ mg of } C_{15}H_{12}N_2O_2 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Phenytoin Powder

Diphenylhydantoin Powder

フェニトイン散

Phenytoin Powder contains not less than 95% and not more than 105% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Method of preparation Prepare as directed under Powders, with Phenytoin.

Identification Weigh a portion of Phenytoin Powder, equivalent to 0.3 g of Phenytoin according to the labeled amount, stir well with two 100-mL portions of diethyl ether, and extract. Combine the diethyl ether extracts, and filter. Evaporate the filtrate on a water bath to dryness, and proceed with the residue as directed in the Identification under Phenytoin.

Assay Weigh accurately Phenytoin Powder, equivalent to about 0.5 g of phenytoin ($C_{15}H_{12}N_2O_2$), add exactly 100 mL of ethanol (95), stir for 30 minutes, and centrifuge. Pipet 50 mL of the supernatant liquid, add 0.5 mL of thymolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS until a light blue color develops, then add 1 mL of pyridine, 5 drops of phenolphthalein TS and 12.5 mL of silver nitrate TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light red color persists for 1 minute.

Each mL of 0.1 mol/L sodium hydroxide VS
= 25.23 mg of $C_{15}H_{12}N_2O_2$

Containers and storage Containers—Well-closed containers.

Phenytoin Tablets

Diphenylhydantoin Tablets

フェニトイン錠

Phenytoin Tablets contain not less than 95% and not more than 105% of the labeled amount of phenytoin ($C_{15}H_{12}N_2O_2$: 252.27).

Method of preparation Prepare as directed under Tablets, with Phenytoin.

Identification Proceed with the residue obtained in the Assay as directed in the Identification under Phenytoin.

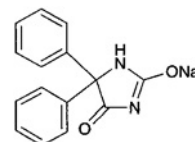
Assay Weigh accurately and powder not less than 20 Phenytoin Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of phenytoin ($C_{15}H_{12}N_2O_2$), transfer to a separator, and add 1 mL of dilute hydrochloric acid and 10 mL of water. Extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin ($C_{15}H_{12}N_2O_2$).

Containers and storage Containers—Well-closed containers.

Phenytoin Sodium for Injection

Diphenylhydantoin Sodium for Injection

注射用フェニトインナトリウム



$C_{15}H_{11}N_2NaO_2$: 274.25
Monosodium 5,5-diphenyl-4-oximidazolidin-2-olate
[630-93-3]

Phenytoin Sodium for Injection is a preparation for injection which is dissolved before use.

When dried, it contains not less than 98.5% of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$), and contains not less than 92.5% and not more than 107.5% of the labeled amount of phenytoin sodium ($C_{15}H_{11}N_2NaO_2$).

Method of preparation Prepare as directed under Injections.

Description Phenytoin Sodium for Injection occurs as white crystals or crystalline powder. It is odorless.

It is soluble in water and in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

The pH of a solution of Phenytoin Sodium for Injection (1 in 20) is about 12.

It is hygroscopic.

A solution of Phenytoin Sodium for Injection absorbs carbon dioxide gradually when exposed to air, and a crystalline precipitate of phenytoin is produced.

Identification (1) With the residue obtained in the Assay, proceed as directed in the Identification under Phenytoin.

(2) Ignite 0.5 g of Phenytoin Sodium for Injection, cool, and dissolve the residue in 10 mL of water: the solution changes red litmus paper to blue, and responds to Qualitative Tests <1.09> (1) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Phenytoin Sodium for Injection in 20 mL of freshly boiled and cooled water in a glass-stoppered test tube: the solution is clear and colorless. If any turbidity is produced, add 4.0 mL of 0.1 mol/L sodium hydroxide VS: the solution becomes clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Phenytoin Sodium for Injection according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 2.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately the content of not less than 10 preparations of Phenytoin Sodium for Injection, transfer about 0.3 g of the content, previously dried and accurately weighed, to a separator, dissolve in 50 mL of water, add 10

mL of dilute hydrochloric acid, and extract with 100 mL of diethyl ether, then with four 25-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate on a water bath. Dry the residue at 105°C for 2 hours, and weigh it as the mass of phenytoin (C₁₅H₁₂N₂O₂: 252.27).

Amount (mg) of phenytoin sodium (C₁₅H₁₁N₂NaO₂)
= amount (mg) of phenytoin (C₁₅H₁₂N₂O₂) × 1.0871

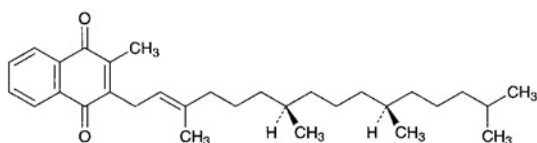
Containers and storage Containers—Hermetic containers.

Phytonadione

Phytomenadione

Vitamin K₁

フィトナジオン



C₃₁H₄₆O₂: 450.70

2-Methyl-3-[(2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-yl]-1,4-naphthoquinone [84-80-0]

Phytonadione contains not less than 97.0% and not more than 102.0% of C₃₁H₄₆O₂.

Description Phytonadione is a clear yellow to orange-yellow, viscous liquid.

It is miscible with isooctane.

It is soluble in ethanol (99.5), and practically insoluble in water.

It decomposes gradually and changes to a red-brown by light.

Specific gravity d_{20}^{20} : about 0.967

Identification (1) Determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 1: both spectra exhibit similar intensities of absorption at the same wavelengths. Separately, determine the absorption spectrum of a solution of Phytonadione in isooctane (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum 2: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Phytonadione as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Refractive index <2.45> n_D^{20} : 1.525 – 1.529

Purity (1) Ratio of absorbances—Determine the absorbances, A_1 , A_2 and A_3 , of a solution of Phytonadione in isooctane (1 in 100,000) at 248.5 nm, 253.5 nm and 269.5 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>: the ratio A_2/A_1 is between 0.69 and 0.73, and the ratio A_2/A_3 is between 0.74 and 0.78. Deter-

mine the absorbances, A_4 and A_5 , of a solution of Phytonadione in isooctane (1 in 10,000) at 284.5 nm and 326 nm, respectively: the ratio A_4/A_5 is between 0.28 and 0.34.

(2) Heavy metals <1.07>—Carbonize 1.0 g of Phytonadione by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Menadione—Dissolve 20 mg of Phytonadione in 0.5 mL of a mixture of water and ethanol (95) (1:1), add 1 drop of a solution of 3-methyl-1-phenyl-5-pyrazolone in ethanol (95) (1 in 20) and 1 drop of ammonia solution (28), and allow to stand for 2 hours: no blue-purple color develops.

Isomer ratio Conduct this procedure rapidly and without exposure to light. Dissolve 30 mg of Phytonadione in 50 mL of the mobile phase. To 4 mL of this solution add the mobile phase to make 25 mL. To 10 mL of this solution add the mobile phase to make 25 mL, and use this solution as the sample solution. Perform the test with 50 μ L of the sample solution as directed under Liquid Chromatograph <2.01> according to the following conditions, and determine the peak areas of *Z*-isomer and *E*-isomer, A_{TZ} and A_{TE} : $A_{TZ}/(A_{TZ} + A_{TE})$ is between 0.05 and 0.18.

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: When the procedure is run with 50 μ L of the sample solution under the above operating conditions, *Z*-isomer and *E*-isomer are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 50 μ L of the sample solution under the above operating conditions, the relative standard deviation of the total area of the peaks of *Z*-isomer and *E*-isomer is not more than 2.0%.

Assay Conduct this procedure rapidly and without exposure to light. Weigh accurately about 30 mg each of Phytonadione and Phytonadione Reference Standard, and dissolve each in the mobile phase to make exactly 50 mL. Pipet 4 mL each of these solutions, and add the mobile phase to make exactly 25 mL. To exactly 10 mL each of these solution add exactly 7 mL of the internal standard solution and the mobile phase to make 25 mL, and use these as the sample solution and standard solution, respectively. Perform the test with 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard.

Amount (mg) of C₃₁H₄₆O₂ = $W_S \times (Q_T/Q_S)$

W_S : Amount (mg) of Phytonadione Reference Standard

Internal standard solution—A solution of cholesterol benzoate in the mobile phase (1 in 400).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with porous silica gel for

liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of hexane and *n*-amyl alcohol (4000 : 3).

Flow rate: Adjust the flow rate so that the retention time of the peak of *E*-isomer of phytonadione is about 25 minutes.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the internal standard, *Z*-isomer and *E*-isomer are eluted in this order with the resolution between the peaks of *Z*-isomer and *E*-isomer being not less than 1.5.

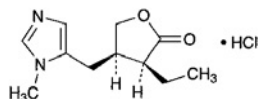
System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the total area of the peaks of *Z*-isomer and *E*-isomer to the peak area of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at a cold place.

Pilocarpine Hydrochloride

ピロカルピン塩酸塩



$\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2 \cdot \text{HCl}$: 244.72

(3*S*,4*R*)-3-Ethyl-4-(1-methyl-1*H*-imidazol-5-ylmethyl)-4,5-dihydrofuran-2(3*H*)-one monohydrochloride [54-71-7]

Pilocarpine Hydrochloride, when dried, contains not less than 99.0% of $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2 \cdot \text{HCl}$.

Description Pilocarpine Hydrochloride occurs as colorless crystals or white powder. It is odorless, and has a slightly bitter taste.

It is very soluble in acetic acid (100), freely soluble in water, in methanol and in ethanol (95), soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Pilocarpine Hydrochloride (1 in 10) is between 3.5 and 4.5.

It is hygroscopic.

It is affected by light.

Identification (1) Dissolve 0.1 g of Pilocarpine Hydrochloride in 5 mL of water, add 1 drop of dilute nitric acid, 1 mL of hydrogen peroxide TS, 1 mL of chloroform and 1 drop of a potassium dichromate solution (1 in 300), and shake the mixture vigorously: a violet color develops in the chloroform layer while no color or a light yellow color is produced in the aqueous layer.

(2) To 1 mL of a solution of Pilocarpine Hydrochloride (1 in 20) add 1 mL of dilute nitric acid and 2 to 3 drops of silver nitrate TS: a white precipitate or opalescence is produced.

Melting point <2.60> 200 – 203°C

Purity (1) Sulfate—Dissolve 0.5 g of Pilocarpine Hydrochloride in 20 mL of water, and use this solution as the

sample solution. To 5.0 mL of the sample solution add 1 mL of dilute hydrochloric acid and 0.5 mL of barium chloride TS: no turbidity is produced.

(2) Nitrate—To 2.0 mL of the sample solution obtained in (1) add 2 mL of iron (II) sulfate TS, and superimpose the mixture upon 4 mL of sulfuric acid: no dark brown color develops at the zone of contact.

(3) Related substances—Dissolve 0.3 g of Pilocarpine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia TS (85:14:2) to a distance of about 13 cm, and dry the plate at 105°C for 10 minutes. Cool, and spray evenly bismuth potassium iodide TS on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(4) Readily carbonizable substances <1.15>—Take 0.25 g of Pilocarpine Hydrochloride, and perform the test: the solution has no more color than Matching Fluid B.

Loss on drying <2.41> Not more than 3.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.5% (0.1 g).

Assay Weigh accurately about 0.5 g of Pilocarpine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.47 mg of $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_2 \cdot \text{HCl}$

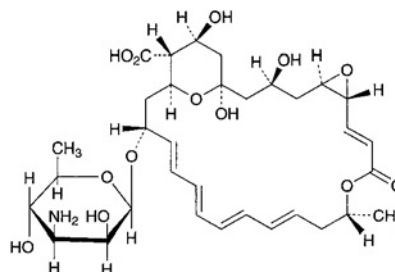
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Pimaricin

Natamycin

ピマリシン



$\text{C}_{33}\text{H}_{47}\text{NO}_{13}$: 665.73

(1*R**,3*S**,5*R**,7*R**,8*E*,12*R**,14*E*,16*E*,18*E*,20*E*,22*R**,24*S**,25*R**,26*S**)-22-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.0^{5,7}]octacosane-8,14,16,18,20-

pentaene-25-carboxylic acid [7681-93-8]

Pimaricin is a polyene macrolide substance having antifungal activity produced by the growth of *Streptomyces natalensis*.

It contains not less than 900 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the anhydrous basis. The potency of Pimaricin is expressed as mass (potency) of pimaricin ($\text{C}_{33}\text{H}_{47}\text{NO}_{13}$).

Description Pimaricin occurs as white to yellowish white crystalline powder.

It is slightly soluble in methanol and in acetic acid (100), and practically insoluble in water and in ethanol (99.5).

Identification (1) To 3 mg of Pimaricin add 1 mL of hydrochloric acid, and mix: a blue-purple color appears.

(2) Dissolve 5 mg of Pimaricin in a solution of acetic acid (100) in methanol (1 in 100) to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pimaricin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> $[\alpha]_D^{20}$: +243 – +259° (0.1 g, acetic acid (100), 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pimaricin according to Method 4, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 20 mg of Pimaricin in methanol to make 100 mL, and use this solution as the sample solution. Perform the test with 10 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the total area of the peaks other than pimaricin by the automatic integration method: not more than 4.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 303 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.0 g of ammonium acetate in 1000 mL of a mixture of water, methanol and tetrahydrofuran (47:44:2).

Flow rate: Adjust the flow rate so that the retention time of pimaricin is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pimaricin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add methanol to make exactly 10 mL. Confirm that the peak area of pimaricin obtained from 10 μL of this solution is equivalent to 7 to 13% of that from 10 μL of the solution for system suitability test.

System performance: When the procedure is run with 10 μL of the solution for system suitability test under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pimaricin are not less than 1500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of pimaricin is not more than 2.0%.

Water <2.48> Between 6.0% and 9.0% (0.2 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pimaricin and Pimaricin Reference Standard, equivalent to about 25 mg (potency), and dissolve each in methanol to make exactly 100 mL. Pipet 2 mL each of these solution, add a solution of acetic acid (100) in methanol (1 in 100) to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances at 295.5 nm, A_{T1} and A_{S1} , at 303 nm, A_{T2} and A_{S2} , and at 311 nm, A_{T3} and A_{S3} , of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} & \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{33}\text{H}_{47}\text{NO}_{13} \\ &= W_S \times \frac{A_{T2} - \frac{A_{T1} + A_{T3}}{2}}{A_{S2} - \frac{A_{S1} + A_{S3}}{2}} \times 1000 \end{aligned}$$

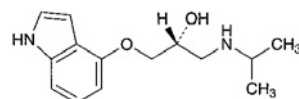
W_S : Amount [mg (potency)] of Pimaricin Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light resistant.

Pindolol

ピンドロール



and enantiomer

$\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_2$: 248.32

(2*RS*)-1-(1*H*-Indol-4-yloxy)-

3-(1-methylethyl)aminopropan-2-ol [13523-86-9]

Pindolol, when dried, contains not less than 98.5% of $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_2$.

Description Pindolol occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It dissolves in dilute sulfuric acid and in acetic acid (100).

Identification (1) To 1 mL of a solution of Pindolol in methanol (1 in 10,000) add 1 mL of a solution of 1-(4-pyridyl)-pyridinium chloride hydrochloride (1 in 1000) and 1 mL of sodium hydroxide TS, then add 1 mL of hydrochloric acid: a blue to blue-purple color, changing to red-purple, is produced.

(2) Dissolve 0.05 g of Pindolol in 1 mL of dilute sulfuric acid, and add 1 mL of Reinecke salt TS: a light red precipitate is produced.

(3) Determine the absorption spectrum of a solution of Pindolol in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pindolol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (264 nm): 333 – 350 (10 mg, methanol, 500 mL).

Melting point <2.60> 169 – 173°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Pindolol in 10 mL of acetic acid (100), and observe immediately: the solution is clear, and has no more color than the following control solution.

Control solution: Measure accurately 4 mL of Matching Fluid A, add exactly 6 mL of water, and mix.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pindolol according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pindolol according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pindolol in 10 mL of methanol, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and isopropylamine (5:4:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (3 in 5) and a sodium nitrite solution (1 in 50) on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

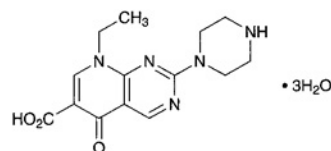
Assay Weigh accurately about 0.5 g of Pindolol, previously dried, dissolve in 80 mL of methanol, and titrate <2.50> with 0.1 mol/L hydrochloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L hydrochloric acid VS} \\ = 24.83 \text{ mg of } \text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_2 \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pipemic Acid Hydrate

ピペミド酸水和物



$\text{C}_{14}\text{H}_{17}\text{N}_5\text{O}_3 \cdot 3\text{H}_2\text{O}$: 357.36
8-Ethyl-5-oxo-2-(piperazin-1-yl)-
5,8-dihydropyrido[2,3-*d*]pyrimidine-
6-carboxylic acid trihydrate
[51940-44-4, anhydride]

Pipemic Acid Hydrate, when dried, contains not less than 98.5% and not more than 101.0% of pipemic acid ($\text{C}_{14}\text{H}_{17}\text{N}_5\text{O}_3$: 303.32).

Description Pipemic Acid Hydrate occurs as a pale yellow, crystalline powder.

It is freely soluble in acetic acid (100), very slightly soluble in water and in ethanol (99.5).

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.1 g of Pipemic Acid Hydrate in 20 mL of sodium hydroxide TS, and dilute with water to make 200 mL. To 1 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pipemic Acid Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Pipemic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute nitric acid, and filter through a glass filter (G3). To 30 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 5 mL of sodium hydroxide TS, 13.5 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Pipemic Acid Hydrate in 35 mL of water and 10 mL of sodium hydroxide TS, shake well with 15 mL of dilute hydrochloric acid, and filter through a glass filter (G3). To 30 mL of the filtrate add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 5 mL of sodium hydroxide TS, 7.5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Pipemidic Acid Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pipemidic Acid Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Pipemidic Acid Hydrate in 10 mL of diluted acetic acid (100) (1 in 20), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted acetic acid (100) (1 in 20) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, formic acid and triethylamine (25:15:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 14.5 – 16.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Pipemidic Acid Hydrate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

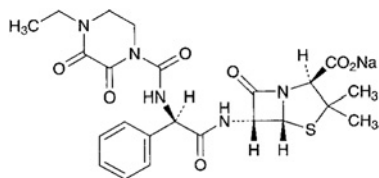
Each mL of 0.1 mol/L perchloric acid VS
= 30.33 mg of $C_{14}H_{17}N_5O_3$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piperacillin Sodium

ピペラシリンナトリウム



$C_{23}H_{26}N_5NaO_7S$: 539.54

Monosodium (2*S*,5*R*,6*R*)-6-[(2*R*)-2-[(4-ethyl-2,3-dioxopiperazine-1-carbonyl)amino]-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [59703-84-3]

Piperacillin Sodium contains not less than 863 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Piperacillin Sodium is expressed as mass (potency) of piperacillin ($C_{23}H_{27}N_5O_7S$: 517.55).

Description Piperacillin Sodium occurs as a white powder or mass.

It is very soluble in water, freely soluble in methanol and in ethanol (95), and practically insoluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Piperacillin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Piperacillin Sodium responds to Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +175 – +190° (0.8 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Piperacillin Sodium in 4 mL of water: the pH of the solution is between 5.0 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Piperacillin Sodium in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Piperacillin Sodium according to Method 4, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 0.1 g of Piperacillin Sodium in 50 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the area of the peak of ampicillin appeared at the retention time of about 7 minutes from the sample solution is not larger than 1/2 of that of piperacillin from the standard solution, the total area of related compounds 1 appeared at the retention times of about 17 minutes and about 21 minutes is not larger than 2 times of the peak area of piperacillin from the standard solution, the peak area of related compound 2 appeared at the retention time of about 56 minutes is not larger than that of piperacillin from the standard solution, and the total area of the peaks other than piperacillin is not larger than 5 times of the peak area of piperacillin from the standard solution. The peak areas of ampicillin, related compounds 1 and related compound 2 are used after multiplying by their relative response factor, 1.39, 1.32 and 1.11, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water, acetonitrile and 0.2 mol/L potassium dihydrogenphosphate (45:4:1).

Mobile phase B: A mixture of acetonitrile, water and 0.2 mol/L potassium dihydrogenphosphate (25:24:1).

Flowing of the mobile phase: Control the gradient by mix-

ing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 7	100	0
7 - 13	100→83	0→17
13 - 41	83	17
41 - 56	83→20	17→80
56 - 60	20	80

Flow rate: 1.0 mL per minute. The retention time of piperacillin is about 33 minutes.

Time span of measurement: About 1.8 times as long as the retention time of piperacillin beginning after the solvent peak.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piperacillin are not less than 15,000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of piperacillin is not more than 2.0%.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Piperacillin Sodium, equivalent to about 0.1 g (potency), and dissolve in water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately an amount of Piperacillin Reference Standard, equivalent to about 0.1 g (potency), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of piperacillin to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of piperacillin } (\text{C}_{23}\text{H}_{27}\text{N}_5\text{O}_7\text{S}) \\ &= W_S \times (Q_T / Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Piperacillin Reference Standard

Internal standard solution—A solution of acetanilide in the mobile phase (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 60.1 g of acetic acid (100) and 101.0 g of

triethylamine add water to make exactly 1000 mL. To 25 mL of this solution add 25 mL of dilute acetic acid and 210 mL of acetonitrile, and add water to make exactly 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of piperacillin is about 5 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the internal standard and piperacillin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of piperacillin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Piperacillin Sodium for Injection

注射用ピペラシリンナトリウム

Piperacillin Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of piperacillin ($\text{C}_{23}\text{H}_{27}\text{N}_5\text{O}_7\text{S}$; 517.55).

Method of preparation Prepare as directed under Injections, with Piperacillin Sodium.

Description Piperacillin Sodium for Injection is a white powder or masses.

Identification Proceed as directed in the Identification under Piperacillin Sodium.

pH <2.54> The pH of a solution prepared by dissolving an amount of Piperacillin Sodium for Injection, equivalent to 1.0 g (potency) of Piperacillin Sodium, in 4 mL of water is 5.0 - 7.0.

Purity (1) Clarity and color of solution—Dissolve an amount of Piperacillin Sodium for Injection, equivalent to 4.0 g (potency) of Piperacillin Sodium according to the labeled amount, in 17 mL of water: the solution is clear and colorless.

(2) Related substances—Proceed as directed in the Purity (4) under Piperacillin Sodium.

Water <2.48> Not more than 1.0% (3 g, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.04 EU/mg (potency).

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane

filtration method: it meets the requirement.

Assay Weigh accurately the mass of the contents of not less than 10 Piperacillin Sodium for Injection. Weigh accurately an amount of the contents, equivalent to about 20 mg (potency) of Piperacillin Sodium according to the labeled amount, dissolve in water to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Piperacillin Reference Standard, and dissolve in the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Proceed as directed in the Assay under Piperacillin Sodium.

Amount [mg (potency)] of piperacillin ($C_{23}H_{27}N_5O_7S$)
 $= W_S \times (Q_T/Q_S)$

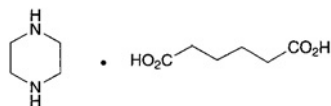
W_S : Amount [mg (potency)] of Piperacillin Reference Standard

Internal standard solution—A solution of acetonitrile in the mobile phase (1 in 5000).

Containers and storage Containers—Hermetic containers. Polyethylene or polypropylene containers for aqueous injections may be used.

Piperazine Adipate

ピペラジンアジピン酸塩



$C_4H_{10}N_2 \cdot C_6H_{10}O_4$: 232.28

Piperazine hexanedioate [142-88-1]

Piperazine Adipate, when dried, contains not less than 98.5% of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$.

Description Piperazine Adipate occurs as a white, crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in water and in acetic acid (100), and practically insoluble in ethanol (95), in acetone and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 0.5 g of Piperazine Adipate in 10 mL of water, add 1 mL of hydrochloric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, evaporate to dryness on a water bath, and dry the residue at 105°C for 1 hour: the melting point <2.60> is between 152°C and 155°C.

(2) To 3 mL of a solution of Piperazine Adipate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the infrared absorption spectrum of Piperazine Adipate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> The pH of a solution of Piperazine Adipate (1 in

20) is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Piperazine Adipate in 30 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Piperazine Adipate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Piperazine Adipate, previously dried, dissolve in a mixture of 20 mL of acetic acid for nonaqueous titration and 40 mL of acetone for nonaqueous titration, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the red-purple color of the solution changes to blue-purple (indicator: 6 drops of bromocresol green-methylrosaniline chloride TS). Perform a blank determination, and make any necessary correction.

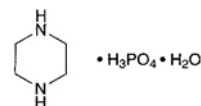
Each mL of 0.1 mol/L perchloric acid VS

= 11.61 mg of $C_4H_{10}N_2 \cdot C_6H_{10}O_4$

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Hydrate

ピペラジンリン酸塩水和物



$C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$: 202.15

Piperazine monophosphate monohydrate [18534-18-4]

Piperazine Phosphate Hydrate contains not less than 98.5% of piperazine phosphate ($C_4H_{10}N_2 \cdot H_3PO_4$: 184.13), calculated on the anhydrous basis.

Description Piperazine Phosphate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a slightly acid taste.

It is soluble in formic acid, sparingly soluble in water, very slightly soluble in acetic acid (100), and practically insoluble in methanol, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid.

Melting point: about 222°C (with decomposition).

Identification (1) To 3 mL of a solution of Piperazine Phosphate Hydrate (1 in 100) add 3 drops of Reinecke salt TS: a light red precipitate is formed.

(2) Determine the infrared absorption spectrum of Piperazine Phosphate Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Piperazine Phosphate Hydrate (1 in 100) responds to Qualitative Tests <1.09> (1) and (3) for phos-

phate.

pH <2.54> Dissolve 1.0 g of Piperazine Phosphate Hydrate in 100 mL of water: the pH of the solution is between 6.0 and 6.5.

Purity (1) Chloride <1.03>—To 0.5 g of Piperazine Phosphate Hydrate add 6 mL of dilute nitric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid (not more than 0.018%).

(2) Heavy metals <1.07>—To 2.0 g of Piperazine Phosphate Hydrate add 5 mL of dilute hydrochloric acid, 30 mL of water and 2 mL of dilute acetic acid, and dissolve. Add sodium hydroxide TS, adjust the pH of the solution to 3.3, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Dissolve 2.0 g of Piperazine Phosphate Hydrate in 5 mL of dilute hydrochloric acid, and use this solution as the test solution. Perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Piperazine Phosphate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, ammonia solution (28) and ethanol (99.5) (8:3:3:2) to a distance of about 13 cm, and air-dry the plate. Spray evenly 4-dimethylaminocinnamaldehyde TS, and allow to stand for 15 minutes: the spots other than the principal spot and the spot on the starting line from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 8.0 – 9.5% (0.3 g, direct titration).

Assay Weigh accurately about 0.15 g of Piperazine Phosphate Hydrate, dissolve in 10 mL of formic acid, add 60 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.207 mg of $C_4H_{10}N_2 \cdot H_3PO_4$

Containers and storage Containers—Well-closed containers.

Piperazine Phosphate Tablets

ピペラジンリン酸塩錠

Piperazine Phosphate Tablets contain not less than 95% and not more than 105% of the labeled amount of piperazine phosphate hydrate ($C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$: 202.15).

Method of preparation Prepare as directed under Tablets, with Piperazine Phosphate Hydrate.

Identification Take a quantity of Piperazine Phosphate

Tablets equivalent to 0.1 g of Piperazine Phosphate Hydrate according to the labeled amount, previously powdered, add 10 mL of water, shake while warming for 10 minutes, allow to cool, and filter. To 3 mL of the filtrate add 3 drops of Reinecke salt TS: a light red precipitate is formed.

Disintegration <6.09> It meets the requirement. The time limit of the test is 10 minutes.

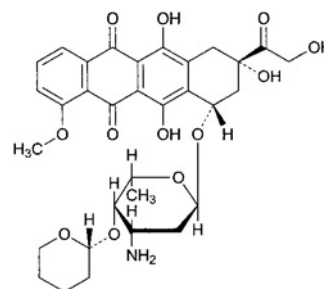
Assay Weigh accurately not less than 20 Piperazine Phosphate Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of piperazine phosphate hydrate ($C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$) according to the labeled amount. Add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. To the residue add 5 mL of formic acid, shake for 5 minutes, centrifuge, and collect the supernatant liquid. Repeat twice the same procedure with 5 mL each of acetic acid (100), combine all the supernatant liquids, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 10.107 mg of $C_4H_{10}N_2 \cdot H_3PO_4 \cdot H_2O$

Containers and storage Containers—Tight containers.

Pirarubicin

ピラルビシン



$C_{32}H_{37}NO_{12}$: 627.64
(2*S*,4*S*)-4- {3-Amino-2,3,6-trideoxy-4-*O*-[(2*R*)-3,4,5,6-tetrahydro-2*H*-pyran-2-yl]- α -*L*-lyxo-hexopyranosyloxy}-2,5,12-trihydroxy-2-hydroxyacetyl-7-methoxy-1,2,3,4-tetrahydrotetracene-6,11-dione [72496-41-4]

Pirarubicin is a derivative of daunorubicin.

It contains not less than 950 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Pirarubicin is expressed as mass (potency) of pirarubicin ($C_{32}H_{37}NO_{12}$).

Description Pirarubicin occurs as a red-orange crystalline powder.

It is soluble in chloroform, very slightly soluble in acetonitrile, in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Dissolve 10 mg of Pirarubicin in 80 mL of methanol and 6 mL of diluted hydrochloric acid (1 in 5000), and add water to make 100 mL. To 10 mL of this solution add diluted methanol (4 in 5) to make 100 mL. Deter-

mine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pirarubicin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Dissolve 5 mg each of Pirarubicin and Pirarubicin Reference Standard in 5 mL of chloroform, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (5:1) to a distance of about 10 cm, and air-dry the plate. Examine the spots with the naked eye: the principal spot obtained from the sample solution and the spot from the standard solution show a red-orange color and the same *R_f* value.

Optical rotation <2.49> $[\alpha]_D^{20}$: +195 – +215° (10 mg, chloroform, 10 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 10 mg of Pirarubicin in 10 mL of 0.01 mol/L hydrochloric acid TS: the solution is clear and red.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pirarubicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 10 mg of Pirarubicin in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak area of doxorubicin, having the relative retention time of about 0.45 with respect to pirarubicin, and the area of the peak, having the relative retention time of about 1.2 with respect to pirarubicin, obtained from the sample solution are not more than the peak area of pirarubicin from the standard solution, respectively, and the sum of the areas of the peaks, having the relative retention times of about 1.9 and about 2.0 with respect to pirarubicin, from the sample solution is not more than 5 times the peak area of pirarubicin from the standard solution. For these calculations, use the peak area for doxorubicin after multiplying by the relative response factor 0.94 and the area for the two peaks, having the relative retention times of about 1.9 and about 2.0, after multiplying by their relative response factors, 1.09, respectively.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of pirarubicin.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mobile phase to make exactly 10 mL. Confirm that the peak area of pirarubicin obtained from 20 μ L of this solution is equivalent to 14 to 26%

of that from 20 μ L of the standard solution.

System performance, and system repeatability: Proceed as directed in the system suitability in the Assay.

Water <2.48> Not more than 2.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Pirarubicin and Pirarubicin Reference Standard, equivalent to about 10 mg (potency), and dissolve in the mobile phase to make exactly 10 mL. Pipet 5 mL of these solutions, add exactly 5 mL of the internal standard solution, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of pirarubicin to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{32}\text{H}_{37}\text{NO}_{12} \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Pirarubicin Reference Standard

Internal standard solution—A solution of 2-naphthol in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of 0.05 mol/L ammonium formate buffer solution, pH 4.0 and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of pirarubicin is about 7 minutes.

System suitability—

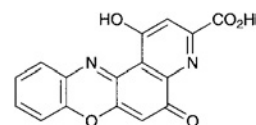
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, pirarubicin and the internal standard are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pirarubicin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Pirenoxine

ピレノキシシ



$\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_5$: 308.25

1-Hydroxy-5-oxo-5*H*-pyrido[3,2-*a*]phenoxazine-3-carboxylic acid [1043-21-6]

Pirenoxine, when dried, contains not less than 98.0% of $C_{16}H_8N_2O_5$.

Description Pirenoxine occurs as a yellow-brown powder. It is odorless, and has a slightly bitter taste.

It is very slightly soluble in dimethylsulfoxide, and practically insoluble in water, in acetonitrile, in ethanol (95), in tetrahydrofuran and in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 2 mg of Pirenoxine in 10 mL of phosphate buffer solution, pH 6.5, add 5 mL of a solution of L-ascorbic acid (1 in 50), and shake vigorously: a dark purple precipitate is formed.

(2) Determine the absorption spectrum of a solution of Pirenoxine in phosphate buffer solution, pH 6.5 (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Pirenoxine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pirenoxine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 10 mg of Pirenoxine in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than pirenoxine is not larger than the peak area of pirenoxine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 1.39 g of tetra *n*-butylammonium chloride and 4.5 g of disodium hydrogen phosphate dodecahydrate in 1000 mL of water, and adjust the pH to 6.5 with phosphoric acid. To 700 mL of this solution add 200 mL of acetonitrile and 30 mL of tetrahydrofuran, and mix.

Flow rate: Adjust the flow rate so that the retention time of pirenoxine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of pirenoxine.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 30 mL. Confirm that the peak area of pirenoxine obtained from 5 μ L

of this solution is equivalent to 5 to 8% of that of pirenoxine obtained from 5 μ L of the standard solution.

System performance: Dissolve 3 mg of Pirenoxine and 16 mg of methyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 5 μ L of this solution under the above operating conditions, pirenoxine and methyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenoxine is not more than 1.0%.

Loss on drying <2.41> Not more than 1.5% (0.5 g, in vacuum, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

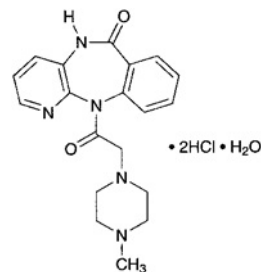
Assay Weigh accurately about 0.1 g of Pirenoxine, previously dried, dissolve in 140 mL of dimethylsulfoxide by heating on a water bath. After cooling, add 30 mL of water, and titrate <2.50> immediately with 0.02 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L sodium hydroxide VS
= 6.165 mg of $C_{16}H_8N_2O_5$

Containers and storage Containers—Tight containers.

Pirenzepine Hydrochloride Hydrate

ピレンゼピン塩酸塩水和物



$C_{19}H_{21}N_5O_2 \cdot 2HCl \cdot H_2O$: 442.34

11-[(4-Methylpiperazin-1-yl)acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one dihydrochloride monohydrate [29868-97-1, anhydride]

Pirenzepine Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of pirenzepine hydrochloride ($C_{19}H_{21}N_5O_2 \cdot 2HCl$: 424.32), calculated on the anhydrous basis.

Description Pirenzepine Hydrochloride Hydrate occurs as a white to pale yellow crystalline powder.

It is freely soluble in water and in formic acid, slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

The pH of a solution by dissolving 1 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is between 1.0 and 2.0.

Melting point: about 245°C (with decomposition).

It is gradually colored by light.

Identification (1) Determine the absorption spectrum of a

solution of Pirenzepine Hydrochloride Hydrate (1 in 40,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pirenzepine Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pirenzepine Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water is clear and not more color than that of the following control solution.

Control solution: To 1.2 mL of Matching fluid for color F add 8.8 mL of diluted hydrochloric acid (1 in 40).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pirenzepine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.3 g of Pirenzepine Hydrochloride Hydrate in 10 mL of water. To 1 mL of this solution add 5 mL of methanol and the mobile phase A to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add 5 mL of methanol and the mobile phase A to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pirenzepine is not more than 3/10 times the peak area of pirenzepine from the standard solution, and the total area of the peaks other than pirenzepine is not more than 3/5 times the peak area of pirenzepine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Dissolve 2 g of sodium lauryl sulfate in 900 mL of water, adjust the pH to 3.2 with acetic acid (100), and add water to make 1000 mL.

Mobile phase B: Methanol

Mobile phase C: Acetonitrile

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A, B and C as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)	Mobile phase C (vol%)
0 - 15	55 → 25	30	15 → 45
15 -	25	30	45

Flow rate: Adjust the flow rate so that the retention time of pirenzepine is about 8 minutes.

Time span of measurement: About 2 times as long as the retention time of pirenzepine beginning after the solvent peak.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add 5 mL of methanol and the mobile phase A to make exactly 10 mL. Confirm that the peak area of pirenzepine obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the standard solution.

System performance: Dissolve 0.1 g of phenylpiperazine hydrochloride in 10 mL of methanol. Mix 1 mL of this solution and 1 mL of the sample solution, and add 5 mL of methanol and the mobile phase A to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, pirenzepine and phenylpiperazine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pirenzepine is not more than 2.0%.

Water <2.48> Not less than 3.5% and not more than 5.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pirenzepine Hydrochloride Hydrate, dissolve in 2 mL of formic acid, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

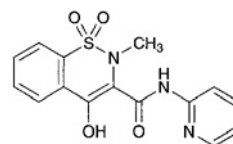
Each mL of 0.1 mol/L perchloric acid VS
= 14.14 mg of C₁₉H₂₁N₅O₂·2HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Piroxicam

ピロキシカム



C₁₅H₁₃N₃O₄S: 331.35

4-Hydroxy-2-methyl-N-(pyridin-2-yl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide [36322-90-4]

Piroxicam contains not less than 98.5% and not

more than 101.0% of $C_{15}H_{13}N_3O_4S$, calculated on the dried basis.

Description Piroxicam occurs as a white to pale yellow crystalline powder.

It is sparingly soluble in acetic anhydride, slightly soluble in acetonitrile, in methanol and in ethanol (99.5), very slightly soluble in acetic acid (100), and practically insoluble in water.

Melting point: about 200°C (with decomposition).

Identification (1) Dissolve 0.1 g of Piroxicam in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 200 mL. To 1 mL of this solution add the mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Piroxicam as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve the sample with dichloromethane, evaporate the solvent, dry the residue on a water bath, and perform the test.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Piroxicam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 75 mg of Piroxicam in 50 mL of acetonitrile for liquid chromatography, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetonitrile for liquid chromatography to make exactly 10 mL. Pipet 1 mL of this solution, add acetonitrile for liquid chromatography to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than piroxicam is not larger than the peak area of piroxicam with the standard solution, and the total area of the peaks other than piroxicam is not larger than 2 times the peak area of piroxicam with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and acetonitrile for liquid chromatography (3:2).

Flow rate: Adjust the flow rate so that the retention time of piroxicam is about 10 minutes.

Time span of measurement: About 5 times as long as the retention time of piroxicam beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add acetonitrile for liquid chromatography to make exactly 20 mL. Confirm that the peak area of piroxicam obtained with 20 μ L of this solution is equivalent to 17.5 to 32.5% of that with 20 μ L of the standard solution.

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of piroxicam are not less than 6000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of piroxicam is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

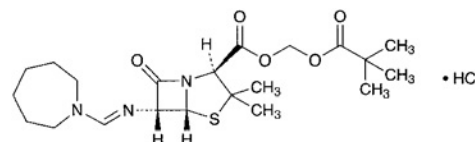
Assay Weigh accurately about 0.25 g of Piroxicam, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 33.14 mg of $C_{15}H_{13}N_3O_4S$

Containers and storage Containers—Tight containers.

Pivmecillinam Hydrochloride

ピブメシリナム塩酸塩



$C_{21}H_{33}N_3O_5S \cdot HCl$: 476.03

2,2-Dimethylpropanoyloxymethyl (2*S*,5*R*,6*R*)-6-[(azepan-1-ylmethylene)amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [32887-03-9]

Pivmecillinam Hydrochloride contains not less than 630 μ g (potency) and not more than 710 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Pivmecillinam Hydrochloride is expressed as mass (potency) of mecillinam ($C_{15}H_{23}N_3O_3S$: 325.43).

Description Pivmecillinam Hydrochloride occurs as a white to yellowish white crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in water and in ethanol (99.5), and soluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Pivmecillinam Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the

Reference Spectrum or the spectrum of Pivmecillinam Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Dissolve 0.5 g of Pivmecillinam Hydrochloride in 10 mL of water, and add 1 mL of dilute nitric acid and 1 drop of silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +200 – +220° (1 g calculated on the anhydrous basis, water, 100 mL, 100 mm).

Purity (1) Heavy metals <1.07>—To 1.0 g of Pivmecillinam Hydrochloride in a crucible add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), fire the ethanol to burn, and heat gradually to incinerate. If a carbonized substance remains, moisten with a small amount of nitric acid, and ignite to incinerate. Cool, add 3 mL of hydrochloric acid to the residue, dissolve by warming on a water bath, and heat to dryness. To the residue add 10 mL of water, and dissolve by warming on a water bath. After cooling, adjust the pH to 3 to 4 with ammonia TS, add 2 mL of dilute acetic acid, filter if necessary, and wash the crucible and the filter with 10 mL of water. Put the filtrate and the washings to a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution in the same manner as the test solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pivmecillinam Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Pivmecillinam Hydrochloride in 4.0 mL of a mixture of acetonitrile and acetic acid (100) (97:3), and use this solution as the sample solution. Separately, dissolve 2.0 mg of Pivmecillinam Hydrochloride Reference Standard in 4.0 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L of the standard solution on a plate of silica gel for thin-layer chromatography, allow to stand for 30 minutes, then spot 2 μ L of the sample solution on the plate. Immediately, develop the plate with a mixture of acetone, water and acetic acid (100) (10:1:1) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand for 10 minutes in iodine vapor: the spot from the sample solution appeared at the position corresponding to the spot obtained from the standard solution is not larger and not more intense than the spot from the standard solution, and any spot other than the principal spot and the above spot is not observable.

Water <2.48> Not more than 1.0% (0.25 g, coulometric titration).

Assay Weigh accurately an amount of Pivmecillinam Hydrochloride and Pivmecillinam Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution and the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of pivmecillinam to that of the internal standard.

Amount [μ g (potency)] of mecillinam ($C_{15}H_{23}N_3O_3S$)
 $= W_S \times (Q_T/Q_S) \times 1000$

W_S : Amount [mg (potency)] of Pivmecillinam Hydrochloride Reference Standard

Internal standard solution—A solution of diphenyl in the mobile phase (1 in 12,500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.771 g of ammonium acetate in about 900 mL of water, adjust the pH to 3.5 with acetic acid (100), and add water to make 1000 mL. To 400 mL of this solution add 600 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pivmecillinam is about 6.5 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, pivmecillinam and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pivmecillinam to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Live Oral Poliomyelitis Vaccine

経口生ポリオワクチン

Live Oral Poliomyelitis Vaccine contains live attenuated poliovirus of type I, II and III.

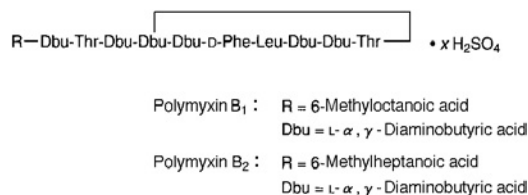
Monovalent or bivalent product may be prepared, if necessary.

Live Oral Poliomyelitis Vaccine conforms to the requirements of Live Oral Poliomyelitis Vaccine in the Minimum Requirements for Biological Products.

Description Live Oral Poliomyelitis Vaccine is a light yellow-red to light red, clear liquid.

Polymixin B Sulfate

ポリミキシン B 硫酸塩



Polymixin B Sulfate is the sulfate of a mixture of peptide substances having antibacterial activity produced by the growth of *Bacillus polymyxa*.

It contains not less than 6500 units per mg, calculated on the dried basis. The potency of Polymixin B Sulfate is expressed as mass unit of polymixin B (C₅₅₋₅₆H₉₆₋₉₈N₁₆O₁₃). One unit of Polymixin B Sulfate is equivalent to 0.129 μg of polymixin B sulfate (C₅₅₋₅₆H₉₆₋₉₈N₁₆O₁₃·1.2H₂SO₄).

Description Polymixin B Sulfate occurs as a white to yellow-brown powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) To 5 mL of a solution of Polymixin B Sulfate (1 in 10) add 5 mL of a solution of sodium hydroxide (1 in 10), add 5 drops of a solution of copper (II) sulfate pentahydrate (1 in 100) while shaking: a purple color develops.

(2) Transfer 5 mg each of Polymixin B Sulfate and Polymixin B Sulfate Reference Standard separately into two glass stoppered test tubes, add 1 mL of diluted hydrochloric acid (1 in 2), stopper the tube, heat at 135°C for 5 hours, then heat to dryness on a water bath, and keep the heating until no more hydrochloric acid odor is evolved. Dissolve the residue in 0.5 mL of water, and use these solutions as the sample solution and standard solution (1). Separately, dissolve 20 mg each of L-leucine, L-threonine, phenylalanine and L-serine separately in 10 mL of water, and use these solutions as the standard solutions (2), (3), (4) and (5), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 3 μL each of the sample solution, the standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography, and expose the plate to a saturated vapor of the developing solvent for 15 hours. Develop the plate with a mixture of phenol and water (3:1) to a distance of about 13 cm while without exposure to light, and dry the plate at 110°C for 5 minutes. Spray evenly ninhydrin-acetic acid TS on the plate, and heat at 110°C for 5 minutes: *R_f* value of each spot obtained from the sample solution is the same with *R_f* value of the corresponding spots from the standard solution (1). Each of the spots from the sample solution appears at the position corresponding to each of the spots from the standard solutions (2), (3) and (4), but not appears at the position corresponding to the spot from the standard solution (5).

(3) A solution of Polymixin B Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: -78 - -90° (0.5 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Polymixin B Sulfate in 50 mL of water is between 5.0 and 7.0.

Phenylalanine Weigh accurately about 0.375 g of Polymixin B Sulfate, dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Determine absorbances, *A*₁, *A*₂, *A*₃, *A*₄ and *A*₅, of this solution at 252 nm, at 258 nm, at 264 nm, at 280 nm and at 300 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, and calculate the amount of phenylalanine by the following equation: the amount of phenylalanine calculated on the dried basis is not less than 9.0% and not more than 12.0%.

$$\text{Amount (\% of phenylalanine)} = \{(A_2 - 0.5A_1 + 0.5A_3 - 1.8A_4 + 0.8A_5) / W_T\} \times 9.4787$$

W_T: Amount (g) of the sample, calculated on the dried basis

Purity Heavy metals <1.07>—Proceed with 1.0 g of Polymixin B Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 6.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.75% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- (i) Test organism—*Escherichia coli* NIHJ
- (ii) Agar media for seed and base layer

Peptone	10.0 g
Meat extract	3.0 g
Sodium chloride	30.0 g
Agar	20.0 g
Water	1000 mL

Mix all the ingredients, and sterilize. Adjust the pH <2.54> of the solution so that it will be 6.5 to 6.6 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Polymixin B Sulfate Reference Standard, equivalent to about 200,000 units, dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Polymixin B Sulfate, equivalent to about 200,000 units, and dissolve in phosphate buffer solution, pH 6.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4000 units and 1000 units, and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Polyoxyl 40 Stearate

ステアリン酸ポリオキシシル 40

Polyoxyl 40 Stearate is the monostearate of condensation polymers of ethylene oxide represented by the formula $H(OCH_2CH_2)_nOCOC_{17}H_{35}$, in which n is approximately 40.

Description Polyoxyl 40 Stearate occurs as a white to light yellow, waxy solid or powder. It is odorless or has a faint fat-like odor.

It is soluble in water, in ethanol (95) and in diethyl ether.

Congealing point <2.42> 39.0 – 44.0°C

Congealing point of the fatty acid <1.13> Not below 53°C.

Acid value <1.13> Not more than 1.

Saponification value <1.13> 25 – 35

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Polyoxyl 40 Stearate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Polyoxyl 40 Stearate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.67 g of Polyoxyl 40 Stearate, according to Method 3, and perform the test (not more than 3 ppm).

Residue on ignition <2.44> Not more than 0.1% (1 g)

Containers and storage Containers—Tight containers.

Polysorbate 80

ポリソルベート 80

Polysorbate 80 is a polyoxyethylene ether of anhydrous sorbitol, partially esterified with oleic acid.

Description Polysorbate 80 is a colorless or orange-yellow, viscous liquid, having a faint, characteristic odor and a warm, slightly bitter taste.

It is miscible with methanol, with ethanol (95), with warm ethanol (95), with pyridine and with chloroform.

It is freely soluble in water and slightly soluble in diethyl ether.

The pH of a solution of Polysorbate 80 (1 in 20) is between 5.5 and 7.5.

Identification (1) To 5 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of sodium hydroxide TS, boil for 5 minutes, cool, and acidify with dilute hydrochloric acid: the solution is opalescent.

(2) To 5 mL of a solution of polysorbate 80 (1 in 20) add 2 to 3 drops of bromine TS: the color of the test solution is discharged.

(3) Mix 6 mL of Polysorbate 80 with 4 mL of water at an ordinary, or lower than ordinary, temperature: a jelly-like

mass is produced.

(4) To 10 mL of a solution of Polysorbate 80 (1 in 20) add 5 mL of ammonium thiocyanate-cobaltous nitrate TS, shake well, add 5 mL of chloroform, shake, and allow to stand: a blue color develops in the chloroform layer.

Viscosity <2.53> 345 – 445 mm²/s (Method 1, 25°C).

Specific gravity <1.13> d_{20}^{20} : 1.065 – 1.095

Acid value <1.13> Not more than 2.0.

Saponification value <1.13> 45 – 55

Iodine value <1.13> 19 – 24 Use chloroform instead of cyclohexane, and titrate without using an indicator, until the yellow color of iodine disappears.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Polysorbate 80 according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Polysorbate 80 according to Method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 3.0% (1 g, back titration).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Containers and storage Containers—Tight containers.

Potash Soap

カリ石ケン

Potash Soap contains not less than 40.0% as fatty acids.

Method of preparation

Fixed oil	470 mL
Potassium Hydroxide	a sufficient quantity
Water or Purified Water	a sufficient quantity
To make 1000 g	

Dissolve Potassium Hydroxide, in required quantity for saponification, in Water or Purified Water, add this solution to fixed oil, previously warmed, add a sufficient quantity of Ethanol if necessary, stir thoroughly, heat in a water bath, and continue the saponification. After complete saponification, add Water or Purified Water to make 1000 g.

Description Potash Soap occurs as a yellow-brown, transparent, unctuous, soft mass, having a characteristic odor.

It is freely soluble in water and in ethanol (95).

Purity Silicic acid and alkalinity—Dissolve 10 g of Potash Soap in 30 mL of ethanol (95), and add 0.50 mL of 1 mol/L hydrochloric acid VS: no turbidity is produced. Add 1 drop of phenolphthalein TS to this solution: no red color develops.

Assay Weigh accurately about 5 g of Potash Soap, dissolve in 100 mL of hot water, and transfer to a separator. Acidify the mixture with dilute sulfuric acid, and cool. Extract the solution with 50-mL, 40-mL, and 30-mL portions of diethyl ether. Wash the combined diethyl ether extracts with 10-mL portions of water until the washing contains no acid. Trans-

fer the diethyl ether solution to a tared flask, evaporate diethyl ether on a water bath at a temperature as low as possible. Dry the residue at 80°C to constant mass, and weigh as fatty acids.

Containers and storage Containers—Tight containers.

Potassium Bromide

臭化カリウム

KBr: 119.00

Potassium Bromide, when dried, contains not less than 99.0% of KBr.

Description Potassium Bromide occurs as colorless or white crystals, granules or crystalline powder. It is odorless.

It is freely soluble in water and in glycerin, soluble in hot ethanol (95), and slightly soluble in ethanol (95).

Identification A solution of Potassium Bromide (1 in 10) responds to Qualitative Tests <1.09> for potassium salt and for bromide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Bromide in 10 mL of water, add 0.10 mL of 0.05 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boiling, and cool: no color develops.

(3) Chloride—Make a calculation from the result obtained in the Assay: not more than 84.5 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Potassium Bromide.

(4) Sulfate <1.14>—Proceed with 2.0 g of Potassium Bromide, and perform the test. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple to purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Potassium Bromide in 10 mL of freshly boiled and cooled water, and add 0.1 mL of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Potassium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Bromide according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.4 g of Potassium Bromide,

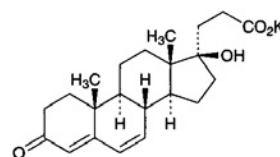
previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and exactly measured 50 mL of 0.1 mol/L silver nitrate VS, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 11.90 mg of KBr

Containers and storage Containers—Tight containers.

Potassium Canrenoate

カンレノ酸カリウム



C₂₂H₂₉KO₄: 396.56

Monopotassium 17-hydroxy-3-oxo-17 α -pregna-4,6-diene-21-carboxylate [2181-04-6]

Potassium Canrenoate, when dried, contains not less than 98.0% and not more than 102.0% of C₂₂H₂₉KO₄.

Description Potassium Canrenoate occurs as a pale yellowish white to pale yellow-brown, crystalline powder.

It is freely soluble in water, soluble in methanol, sparingly soluble in ethanol (95), and practically insoluble in chloroform and in diethyl ether.

Identification (1) Dissolve 2 mg of Potassium Canrenoate in 2 drops of sulfuric acid: an orange color develops. Observe under ultraviolet light (main wavelength: 365 nm): the solution shows a yellow-green fluorescence. Add 1 drop of acetic anhydride to this solution: the color of the solution changes to red.

(2) Determine the absorption spectrum of a solution of Potassium Canrenoate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Potassium Canrenoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) The solution of Potassium Canrenoate (1 in 10) responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: -71 - -76° (after drying, 0.2 g, methanol, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Potassium Canrenoate in 20 mL of water: the pH of this solution is between 8.4 and 9.4.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Potassium Canrenoate in 5 mL of water: the solution is clear,

and shows a pale yellow to light yellow color.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Canrenoate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Canrenoate according to Method 3, and perform the test (not more than 2 ppm).

(4) Canrenone—Place 0.40 g of Potassium Canrenoate in a glass-stoppered centrifuge tube, cool in ice-water to a temperature not higher than 5°C, add 6 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, being cooled to a temperature not higher than 5°C to dissolve, and add 8 mL of water being cooled to a temperature not higher than 5°C. Add exactly 10 mL of chloroform, allow to stand for 3 minutes at a temperature not higher than 5°C, shake vigorously for 2 minutes, and centrifuge. Drain off the water layer, collect 5 mL of the chloroform layer, transfer to a glass-stoppered centrifuge tube containing 3 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 10.0, cooled to a temperature not higher than 5°C, and 4 mL of water cooled to a temperature not higher than 5°C, shake for 1 minute, and centrifuge. Drain off the water layer, pipet 2 mL of the chloroform layer, and add chloroform to make exactly 10 mL. Determine the absorbance of this solution at 283 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: it is not more than 0.67.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.2 g of Potassium Canrenoate, previously dried, dissolve in 75 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Use a solution of saturated potassium chloride-acetic acid (100) as the internal liquid. Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.66 mg of C₂₂H₂₉KO₄

Containers and storage Containers—Tight containers.

Potassium Carbonate

炭酸カリウム

K₂CO₃: 138.21

Potassium Carbonate, when dried, contains not less than 99.0% of K₂CO₃.

Description Potassium Carbonate occurs as white granules or powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (95).

A solution of Potassium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Potassium Carbonate (1 in 10) responds to Qualitative Tests <1.09> for potassium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Potassium Carbonate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Potassium Carbonate in 2 mL of water and 6 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 6 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution to dryness, and dilute with water to 50 mL (not more than 20 ppm).

(3) Sodium—Dissolve 1.0 g of Potassium Carbonate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1): no persisting yellow color is produced.

(4) Arsenic <1.11>—Prepare the test solution with 0.5 g of Potassium Carbonate, according to Method 1, and perform the test (not more than 4 ppm).

Loss on drying <2.41> Not more than 1.0% (3 g, 180°C, 4 hours).

Assay Dissolve about 1.5 g of Potassium Carbonate, previously dried and accurately weighed, in 25 mL of water, titrate with 0.5 mol/L sulfuric acid VS until the blue color of the solution changes to yellow-green, boil cautiously, then cool, and titrate <2.50> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 69.10 mg of K₂CO₃

Containers and storage Containers—Tight containers.

Potassium Chloride

塩化カリウム

KCl: 74.55

Potassium Chloride, when dried, contains not less than 99% of KCl.

Description Potassium Chloride occurs as colorless or white crystals or crystalline powder. It is odorless, and has a saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Potassium Chloride (1 in 10) is neutral.

Identification A solution of Potassium Chloride (1 in 50) responds to Qualitative Tests <1.09> for potassium salt and for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Chloride in 5 mL of water: the solution is clear and colorless.

(2) Acidity and alkalinity—Dissolve 5.0 g of Potassium Chloride in 50 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: no red color develops. Then add 0.50 mL of 0.01 mol/L sodium hydroxide VS: a red color develops.

(3) Bromide—Dissolve 1.0 g of Potassium Chloride in

water to make 100 mL. To 5 mL of the solution add 3 drops of dilute hydrochloric acid and 1 mL of chloroform, and add 3 drops of sodium toluensulfonchloramide TS dropwise while shaking: no yellow to yellow-red color develops in the chloroform layer.

(4) Iodide—Dissolve 0.5 g of Potassium Chloride in 10 mL of water, add 3 drops of iron (III) chloride TS and 1 mL of chloroform, shake, allow to stand for 30 minutes, and shake again: no red-purple to purple color develops in the chloroform layer.

(5) Heavy metals <1.07>—Proceed with 4.0 g of Potassium Chloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(6) Calcium and magnesium—Dissolve 0.20 g of Potassium Chloride in 20 mL of water, add 2 mL of ammonia TS, 2 mL of ammonium oxalate TS and 2 mL of disodium hydrogenphosphate TS, and then allow to stand for 5 minutes: no turbidity is produced.

(7) Sodium—Dissolve 1.0 g of Potassium Chloride in 20 mL of water, and perform the Flame Coloration Test <1.04> (1): no persistent, yellow color develops.

(8) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Chloride according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 130°C, 2 hours).

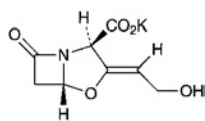
Assay Weigh accurately about 0.2 g of Potassium Chloride, previously dried, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS = 7.455 mg of KCl

Containers and storage Containers—Tight containers.

Potassium Clavulanate

クラブラン酸カリウム



$C_8H_8KNO_5$: 237.25

Monopotassium (2*R*,5*R*)-3-[(1*Z*)-2-hydroxyethylidene]-7-oxo-4-oxa-1-azabicyclo[3.2.0]heptane-2-carboxylate [61177-45-5]

Potassium Clavulanate is the potassium salt of a substance having β -lactamase inhibiting activity produced by the growth of *Streptomyces clavuligerus*.

It contains not less than 810 μ g (potency) and not more than 860 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Potassium Clavulanate is expressed as mass (potency) of clavulanic acid ($C_8H_9NO_5$: 199.16).

Description Potassium Clavulanate occurs as a white to light yellowish white, crystalline powder.

It is very soluble in water, soluble in methanol, and slightly

soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 1 mL of a solution of Potassium Clavulanate (1 in 50,000) add 5 mL of imidazole TS, and warm in a water bath at 30°C for 12 minutes. After cooling, determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Potassium Clavulanate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Potassium Clavulanate responds to Qualitative Tests <1.09> (1) for potassium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +53 – +63° (0.5 g calculated on the anhydrous basis, water, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Clavulanate according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Clavulanate according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Potassium Clavulanate in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of each peak other than clavulanic acid from the sample solution is not more than the peak area of clavulanic acid from the standard solution, and the total area of the peaks other than clavulanic acid from the sample solution is not more than 2 times of the peak area of clavulanic acid from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase A: Adjust the pH of 0.05 mol/L sodium dihydrogen phosphate TS to 4.0 with phosphoric acid.

Mobile phase B: A mixture of the mobile phase A and methanol (1:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 4	100	0
4 - 15	100 → 0	0 → 100
15 - 25	0	100

Flow rate: 1.0 mL per minute.

Time span of measurement: About 6 times as long as the retention time of clavulanic acid.

System suitability—

Test for required detectability: Pipet 1 mL of the standard solution, and add the mobile phase A to make exactly 10 mL. Confirm that the peak area of clavulanic acid obtained from 20 μ L of this solution is equivalent to 7 to 13% of that from 20 μ L of the standard solution.

System performance: Dissolve 10 mg each of Potassium Clavulanate and Amoxicillin in 100 mL of the mobile phase A. When the procedure is run with 20 μ L of this solution under the above operating conditions, clavulanic acid and amoxicillin are eluted in this order with the resolution between these peaks being not less than 8 and the number of theoretical plates of the peak of clavulanic acid is not less than 2500.

System repeatability: When the test is repeated 3 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of clavulanic acid is not more than 2.0%.

Water <2.48> Not more than 1.5% (5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Potassium Clavulanate and Lithium Clavulanate Reference Standard, equivalent to about 12.5 mg (potency), dissolve each in 30 mL of water, add exactly 5 mL of the internal standard solution and water to make 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of clavulanic acid to that of the internal standard.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of clavulanic acid (C}_8\text{H}_9\text{NO}_5) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Lithium Clavulanate Reference Standard

*Internal standard solution—*Dissolve 0.3 g of sulfanilamide in 30 mL of methanol, and add water to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.36 g of sodium acetate trihydrate in 900 mL of water, adjust to pH 4.5 with diluted acetic acid (31) (2 in 5), and add 30 mL of methanol and water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of clavulanic acid is about 6 minutes.

System suitability—

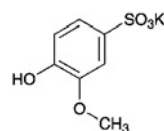
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, clavulanic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of clavulanic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Potassium Guaiacolsulfonate

グアヤコールスルホン酸カリウム



$C_7H_7KO_5S$: 242.29

Monopotassium 4-hydroxy-3-methoxybenzenesulfonate
[I321-14-8]

Potassium Guaiacolsulfonate contains not less than 98.5% of $C_7H_7KO_5S$, calculated on the anhydrous basis.

Description Potassium Guaiacolsulfonate occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in water and in formic acid, soluble in methanol, and practically insoluble in ethanol (95), in acetic anhydride and in diethyl ether.

Identification (1) To 10 mL of a solution of Potassium Guaiacolsulfonate (1 in 100) add 2 drops of iron (III) chloride TS: a blue-purple color develops.

(2) Dissolve 0.25 g of Potassium Guaiacolsulfonate in water to make 500 mL, and to 10 mL of this solution add phosphate buffer solution, pH 7.0, to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) A solution of Potassium Guaiacolsulfonate (1 in 10) responds to Qualitative Tests <1.09> for potassium salt.

pH <2.54> Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the pH of the solution is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Guaiacolsulfonate in 20 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.8 g of Potassium Guaiacolsulfonate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than

0.030%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Potassium Guaiaacolsulfonate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Potassium Guaiaacolsulfonate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Potassium Guaiaacolsulfonate in 200 mL of mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area obtained from these solutions by the automatic integration method: the total area of peaks other than the peak of potassium guaiaacolsulfonate from the sample solution is not larger than the peak area of potassium guaiaacolsulfonate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 279 nm).

Column: A stainless steel column 4 mm in inside diameter and 20 to 25 cm in length, packed with dimethylaminopropylsilanized silica gel, (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogenphosphate VS and methanol (20:1).

Flow rate: Adjust the flow rate so that the retention time of potassium guaiaacolsulfonate is about 10 minutes.

Selection of column: Weigh 50 mg each of potassium guaiaacolsulfonate and guaiacol, and dissolve in 50 mL of the mobile phase. Proceed with 5 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of guaiacol and potassium guaiaacolsulfonate in this order with the resolution of these peaks being not less than 4.

Detection sensitivity: Adjust the sensitivity so that the peak height of potassium guaiaacolsulfonate from 5 μ L of the standard solution is not less than 10 mm.

Time span of measurement: About twice as long as the retention time of potassium guaiaacolsulfonate.

Water <2.48> 3.0 – 4.5% (0.3 g, direct titration).

Assay Weigh accurately about 0.3 g of Potassium Guaiaacolsulfonate, dissolve in 2.0 mL of formic acid, add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.23 mg of C₇H₇KO₅S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Potassium Hydroxide

水酸化カリウム

KOH: 56.11

Potassium Hydroxide contains not less than 85.0% of KOH.

Description Potassium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in the presence of moisture.

Identification (1) A solution of Potassium Hydroxide (1 in 500) is alkaline.

(2) A solution of Potassium Hydroxide (1 in 25) responds to Qualitative Tests <1.09> for potassium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 2.0 g of Potassium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 8 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Potassium Hydroxide in 5 mL of water, add 7 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 30 ppm).

(4) Sodium—Dissolve 0.10 g of Potassium Hydroxide in 10 mL of dilute hydrochloric acid, and perform the test as directed under Flame Coloration Test <1.04> (1): no persistent yellow color develops.

(5) Potassium carbonate—The amount of potassium carbonate (K₂CO₃: 138.21) is not more than 2.0% when calculated by the following equation using *B* (mL) obtained in the Assay.

Amount of potassium carbonate (mg) = 138.21 × *B*

Assay Weigh accurately about 1.5 g of Potassium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein TS, and titrate <2.50> with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount *A* (mL) of 0.5 mol/L sulfuric acid VS consumed, then add 2 drops of methyl orange TS, and titrate <2.50> again with 0.5 mol/L sulfuric acid VS until the solution changes to a persistent light red color. Record the

amount *B* (mL) of 0.5 mol/L sulfuric acid VS consumed.

Calculate the amount KOH from the amount, *A* (mL) – *B* (mL).

Each mL of 0.5 mol/L sulfuric acid VS
= 56.11 mg of KOH

Containers and storage Containers—Tight containers.

Potassium Iodide

ヨウ化カリウム

KI: 166.00

Potassium Iodide, when dried, contains not less than 99.0% of KI.

Description Potassium Iodide occurs as colorless or white crystals, or a white crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It is slightly deliquescent in moist air.

Identification A solution of Potassium Iodide (1 in 20) responds to Qualitative Tests <1.09> for potassium salt and for iodide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Potassium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 0.50 mL of 0.005 mol/L sulfuric acid and 1 drop of phenolphthalein TS: no color develops.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Potassium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for 2 to 3 minutes, and filter. To 10 mL of the filtrate, add 15 mL of dilute nitric acid: no brown color develops. The solution has no more turbidity than that of the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, and 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Potassium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert the absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on it. Heat the test tube carefully on a water bath for 15 minutes: the gas evolved does not turn red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Potassium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, then add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Potassium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops immediately.

(7) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Iodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Potassium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Sodium—Dissolve 1.0 g of Potassium Iodide in 10 mL of water, and perform the Flame Coloration Test (1) <1.04>: a yellow color develops, but does not persist.

(10) Arsenic <1.11>—Prepare the test solution with 0.40 g of Potassium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 1.0% (2 g, 105°C, 4 hours).

Assay Weigh accurately about 0.5 g of Potassium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS with shaking until the red-purple color of the chloroform layer disappears. The end point is reached when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 16.60 mg of KI

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Potassium Permanganate

過マンガン酸カリウム

KMnO₄: 158.03

Potassium Permanganate, when dried, contains not less than 99.0% of KMnO₄.

Description Potassium Permanganate occurs as dark purple crystals and has a metallic luster.

It is soluble in water.

A solution of Potassium Permanganate (1 in 1000) has a slightly sweet, astringent taste.

Identification A solution of Potassium Permanganate (1 in 100) responds to Qualitative Tests <1.09> for permanganate.

Purity (1) Water-insoluble substances—Dissolve 2.0 g of Potassium Permanganate, previously powdered, in 200 mL of water. Filter the insoluble substances through a tared glass filter (G4), wash with water until the last washing shows no color, and dry at 105°C for 2 hours: the mass of the residue is not more than 4 mg.

(2) Arsenic <1.11>—Dissolve 0.40 g of Potassium Permanganate in 10 mL of water, add 1 mL of sulfuric acid, add hydrogen peroxide (30) dropwise until the solution remains colorless, and evaporate on a sand bath nearly to dryness. Dissolve the residue in 5 mL of water, and perform the test with this solution as the test solution: the color produced is not more intense than the following standard color.

Standard color: To 10 mL of water add 1 mL of sulfuric acid and the same volume of hydrogen peroxide (30) as used for the preparation of the test solution. Evaporate the solution on a sand bath nearly to dryness, add 2.0 mL of Standard Arsenic Solution and water to make 5 mL, and carry out

the test with this solution in the same manner as the test solution (not more than 5 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 18 hours).

Assay Weigh accurately about 0.6 g of Potassium Permanganate, previously dried, dissolve in water to make exactly 200 mL, and use this solution as the sample solution. Pipet 25 mL of 0.05 mol/L oxalic acid VS into a 500-mL conical flask, add 200 mL of diluted sulfuric acid (1 in 20), and keep at a temperature between 30°C and 35°C. Transfer the sample solution to a buret. Add quickly 23 mL of the sample solution from the buret to the flask while shaking gently, and then allow the flask to stand until the red color disappears. Warm the mixture to a temperature between 55°C and 60°C, and continue the titration <2.50> slowly until the red color persists for 30 seconds.

$$\begin{aligned} \text{Each mL of 0.05 mol/L oxalic acid VS} \\ = 3.161 \text{ mg of KMnO}_4 \end{aligned}$$

Containers and storage Containers—Tight containers.

Potassium Sulfate

硫酸カリウム

K₂SO₄: 174.26

Potassium Sulfate, when dried, contains not less than 99.0% of K₂SO₄.

Description Potassium Sulfate occurs as colorless crystals or a white, crystalline powder. It has a slightly saline, somewhat bitter taste.

It is soluble in water and practically insoluble in ethanol (95).

Identification A solution of Potassium Sulfate (1 in 20) responds to Qualitative Tests <1.09> for potassium salt and for sulfate.

Purity (1) Clarity and color of solution, and acid or alkali—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water: the solution is clear, colorless and neutral.

(2) Chloride <1.03>—Perform the test with 0.5 g of Potassium Sulfate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Potassium Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Sodium—Dissolve 1.0 g of Potassium Sulfate in 20 mL of water, and perform the test as directed under Flame Coloration Test <1.04> (1): no persistent yellow color develops.

(5) Arsenic <1.11>—Prepare the test solution with 0.40 g of Potassium Sulfate according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.5 g of Potassium Sulfate,

previously dried, boil with 200 mL of water and 1.0 mL of hydrochloric acid, and add gradually 8 mL of boiling barium chloride TS. Heat the mixture on a water bath for 1 hour, collect the precipitate, and wash the precipitate with water until the last washing shows no opalescence on the addition of silver nitrate TS. Dry, heat strongly to constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate (BaSO₄: 233.39).

$$\begin{aligned} \text{Amount (mg) of K}_2\text{SO}_4 \\ = \text{amount (mg) of barium sulfate (BaSO}_4) \times 0.7466 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Potato Starch

Amylum Solani

パレイシヨデンブシ

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Potato Starch consists of starch granules derived from the tuber of *Solanum tuberosum* Linné (*Solanaceae*).

♦**Description** Potato Starch occurs as a white powder.

It is practically insoluble in water and in ethanol (99.5).♦

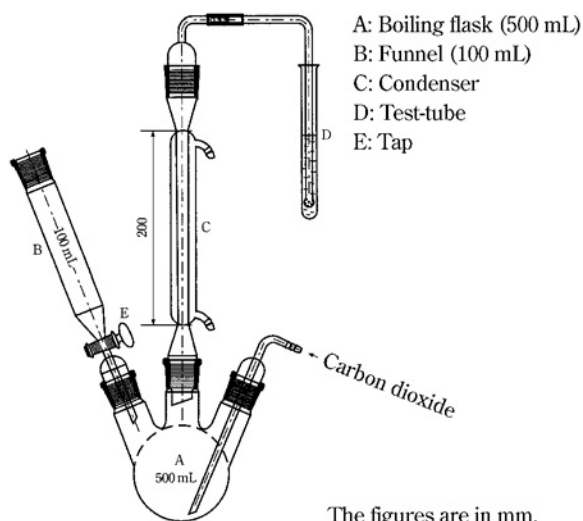
Identification (1) Under a microscope, Potato Starch, preserved in a mixture of water and glycerin (1:1), appears as unevenly ovoid or pyriform simple grains usually 30 – 100 μm, often more than 100 μm in diameter, or spherical simple grains 10 – 35 μm in diameter, rarely 2- to 4-compound grains; ovoid or pyriform simple grains with eccentric hilum, spherical simple grains with non-centric or slightly eccentric hilum; striation distinct in all grains; a black cross, its intersection point on hilum, is observed when grains are put between two nicol prisms fixed at right angle to each other.

(2) To 1 g of Potato Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): an orange-red to deep blue color is formed, and the color disappears by heating.

pH <2.54> Put 5.0 g of Potato Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 5.0 and 8.0.

Purity (1) Iron—To 1.5 g of Potato Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (2 in 10) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test so-



lution is not darker than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Potato Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Potato Starch, accurately weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = (V/W) \times 1000 \times 3.203$$

W : Amount (g) of the sample

V : Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Loss on drying <2.41> Not more than 20.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

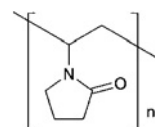
♦**Containers and storage** Containers—Well-closed containers. ♦

Povidone

Polyvidone

Polyvinylpyrrolidone

ポビドン



$(C_6H_9NO)_n$
Poly[(2-oxopyrrolidin-1-yl)ethylene] [9003-39-8]

Povidone is a chain polymer of 1-vinyl-2-pyrrolidone. It contains not less than 11.5% and not more than 12.8% of nitrogen (N: 14.01), calculated on the anhydrous basis.

It has a nominal K-value of not less than 25 and not more than 90. The nominal K-value is shown on the label.

Description Povidone occurs as a white to slightly yellowish fine powder. It is odorless or has a faint, characteristic odor.

It is freely soluble in water, in methanol and in ethanol (95), slightly soluble in acetone, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification Determine the infrared absorption spectrum of Povidone, previously dried at 105°C for 6 hours, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Povidone Reference Standard previously dried at 105°C for 6 hours: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 1.0 g of Povidone in 20 mL of water: the pH of this solution is between 3.0 and 5.0 for Povidone having the nominal K-value of 30 or less, and between 4.0 and 7.0 for Povidone having the nominal K-value exceeding 30.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Povidone in 20 mL of water: the solution is clear and colorless to pale yellow, or pale red.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Povidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Aldehydes—Weigh accurately about 1.0 g of Povidone and dissolve in 0.05 mol/L pyrophosphate buffer solu-

tion, pH 9.0 to make exactly 100 mL. Stopper, heat at 60°C for 60 minutes, allow to cool to room temperature, and use this solution as the sample solution. Separately, dissolve 0.100 g of freshly distilled acetaldehyde in water previously cooled to 4°C to make exactly 100 mL. Allow to stand at 4°C for about 20 hours, pipet 1 mL of this solution, add 0.05 mol/L pyrophosphate buffer solution, pH 9.0 to make exactly 100 mL, and use this solution as the standard solution. Measure 0.5 mL each of the sample solution, standard solution and water (for blank test), transfer to separate cells, add 2.5 mL of 0.05 mol/L pyrophosphate buffer solution, pH 9.0, and 0.2 mL of β -nicotinamide adenine dinucleotide TS to each of these cells, mix and stopper tightly. Allow to stand for 2 to 3 minutes at $22 \pm 2^\circ\text{C}$, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control solution. Determine the absorbances, A_{T1} , A_{S1} and A_{B1} of the subsequent solutions of the sample solution, the standard solution and water at 340 nm. Add 0.05 mL of aldehyde dehydrogenase solution to each of the cells, mix and stopper tightly. Allow to stand for 5 minutes at $22 \pm 2^\circ\text{C}$. Determine the absorbances, A_{T2} , A_{S2} and A_{B2} of these solutions in the same manner as above: the content of aldehydes is not more than 500 ppm (expressed as acetaldehyde).

Content (ppm) of aldehydes expressed as acetaldehyde

$$= \frac{1000}{W} \times \frac{(A_{T2} - A_{T1}) - (A_{B2} - A_{B1})}{(A_{S2} - A_{S1}) - (A_{B2} - A_{B1})}$$

W : Amount (g) of povidone, calculated on the anhydrous basis.

(4) 1-Vinyl-2-pyrrolidone—Weigh accurately about 0.25 g of Povidone, dissolve in diluted methanol (1 in 5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 1-vinyl-2-pyrrolidone in methanol to make exactly 100 mL. Pipet 1 mL of this solution and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of 1-vinyl-2-pyrrolidone in each solution: the content of 1-vinyl-2-pyrrolidone is not more than 10 ppm.

Content (ppm) of 1-vinyl-2-pyrrolidone

$$= (2.5/W) \times (A_T/A_S)$$

W : Amount (g) of Povidone, calculated on the anhydrous basis.

Operating conditions—

Detector: An ultraviolet spectrophotometer (detection wavelength: 254 nm).

Column: Stainless steel columns about 4 mm in inside diameter and about 25 mm in length, and about 4 mm in inside diameter and about 250 mm in length, packed with octylsilylated silica gel for liquid chromatography (5 μm in particle diameter), and use them as a guard column and a separation column, respectively.

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (4:1).

Flow rate: Adjust the flow rate so that the retention time of

1-vinyl-2-pyrrolidone is about 10 minutes.

Selection of column: Dissolve 0.01 g of 1-vinyl-2-pyrrolidone and 0.5 g of vinyl acetate in 100 mL of methanol. To 1 mL of this solution add diluted methanol (1 in 5) to make 100 mL. Proceed with 50 μL of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of 1-vinyl-2-pyrrolidone and vinyl acetate in this order with the resolution between these peaks being not less than 2.0.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of 1-vinyl-2-pyrrolidone obtained from 50 μL of the standard solution is between 10 mm and 15 mm.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of obtained peak areas of 1-vinyl-2-pyrrolidone is not more than 2%.

Washing of the guard column: After each test with the sample solution, wash away the polymeric material of Povidone from the guard column by passing the mobile phase through the column backwards for about 30 minutes at the same flow rate as applied in the test.

(5) Peroxides—Weigh exactly an amount of Povidone, equivalent to 4.0 g calculated on the anhydrous basis, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. To 25 mL of the sample solution add 2 mL of titanium (III) chloride-sulfuric acid TS, and mix. Allow to stand for 30 minutes, and perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by adding 2 mL of 13 % sulfuric acid to 25 mL of the sample solution as a blank: the absorbance of the subsequent solution of the sample solution at 405 nm is not more than 0.35 (not more than 400 ppm, expressed as hydrogen peroxide).

(6) Hydrazine—Transfer 2.5 g of Povidone to a 50-mL centrifuge tube, add 25 mL of water, and stir to dissolve. Add 500 μL of a solution of salicylaldehyde in methanol (1 in 20), stir and warm at 60°C for 15 minutes in a water bath. Allow to cool, add 2.0 mL of toluene, stopper tightly, shake vigorously for 2 minutes, centrifuge, and use the upper layer of the mixture as the sample solution. Separately, dissolve 0.09 g of salicylaldazine in toluene to make exactly 100 mL. Pipet 1 mL of this solution, add toluene to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate coated with a 0.25-mm layer of dimethylsilylated silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and water (2:1) to a distance of about three-fourths of the length of the plate, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): the R_f value of the fluorescent spot from the standard solution is about 0.3, and the fluorescence of the spot from the sample solution corresponding to the spot from the standard solution is not more intense than that of the spot from the standard solution (not more than 1 ppm).

Water <2.48> Not more than 5.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

K-value Weigh accurately an amount of Povidone, equivalent to 1.00 g calculated on the anhydrous basis, and dissolve in water to make exactly 100 mL, allow to stand for 60

minutes, and use this solution as the sample solution. Perform the test with the sample solution and with water at 25°C as directed in Method 1 under Viscosity Determination <2.53>, and calculate the K-value by the following formula.

$$K = \frac{1.5 \log \eta_{\text{rel}} - 1}{0.15 + 0.003 c} + \frac{\sqrt{300 c \log \eta_{\text{rel}} + (c + 1.5 c \log \eta_{\text{rel}})^2}}{0.15 c + 0.003 c^2}$$

c: Mass (g) of Povidone in 100 mL of the solution, calculated on the anhydrous basis.

η_{rel} : Kinematic viscosity of the sample solution relative to that of water.

The K-value of Povidone is not less than 90% and not more than 108% of the nominal K-value.

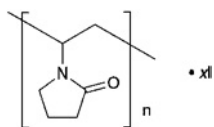
Assay Weigh accurately about 0.1 g of Povidone, and place in a Kjeldahl flask. Add 5 g of a powdered mixture of 33 g of potassium sulfate, 1 g of copper (II) sulfate pentahydrate and 1 g of titanium (IV) oxide, and wash down any adhering sample from the neck of the flask with a small amount of water. Add 7 mL of sulfuric acid allowing to flow down the inside wall of the flask. Heat the flask gradually over a free flame until the solution has a clear, yellow-green color and the inside wall of the flask is free from a carbonaceous material, and then heat for further 45 minutes. After cooling, add cautiously 20 mL of water, cool the solution, and connect the flask to the distillation apparatus previously washed by passing steam through it. To the absorption flask add 30 mL of a solution of boric acid (1 in 25), 3 drops of bromocresol green-methyl red TS and sufficient water to immerse the lower end of the condenser tube. Add 30 mL of a solution of sodium hydroxide (2 in 5) through the funnel, rinse cautiously the funnel with 10 mL of water, immediately close the clamp attached to the rubber tube, then start the distillation with steam to get 80 to 100 mL of the distillate. Remove the absorption flask from the lower end of the condenser tube, rinsing the end part with a small quantity of water, and titrate <2.50> the distillate with 0.025 mol/L sulfuric acid VS until the color of the solution changes from green through pale grayish blue to pale grayish red-purple. Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.025 mol/L sulfuric acid VS
= 0.700 mg of N

Containers and storage Containers—Tight containers.

Povidone-Iodine

ポビドンヨード



$(C_6H_9NO)_n \cdot xI$

Poly[(2-oxopyrrolidin-1-yl)ethylene] iodine [25655-41-8]

Povidone-Iodine is a complex of iodine with 1-vinyl-2-pyrrolidone polymer.

It contains not less than 9.0% and not more than 12.0% of available iodine (I: 126.90), and not less than

9.5% and not more than 11.5% of nitrogen (N: 14.01), calculated on the dried basis.

Description Povidone-Iodine occurs as a dark red-brown powder. It has a faint, characteristic odor.

It is freely soluble in water and in ethanol (99.5).

The pH of a solution obtained by dissolving 1.0 g of Povidone-Iodine in 100 mL of water is between 1.5 and 3.5.

Identification (1) To 10 mL of diluted starch TS (1 in 10) add 1 drop of a solution of Povidone-Iodine (1 in 10): a deep blue color develops.

(2) To 1 mL of a solution of Povidone-Iodine (1 in 100) add 1 mL of sodium thiosulfate TS, and add 1 mL of ammonium thiocyanate-cobaltous nitrate TS and 2 drops of 1 mol/L hydrochloric acid TS: a blue color develops, and a blue precipitate is gradually formed.

Purity (1) Clarity and color of solution—Dissolve 0.30 g of Povidone-Iodine in 100 mL of water: the solution is clear and brown.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Povidone-Iodine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Povidone-Iodine according to Method 4, and perform the test (not more than 2 ppm).

(4) Iodide ion—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 100 mL of water, and add sodium hydrogensulfite TS until the color of iodine completely disappears. To this solution add exactly 25 mL of 0.1 mol/L silver nitrate VS, shake well with 10 mL of nitric acid, titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS until the solution develops a red-brown color, and calculate the total amount of iodine (indicator: 1 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 12.69 mg of I

Obtain the amount of iodide ion, calculated on the dried basis, by deducting the amount (%) of available iodine from the total amount (%) of iodine: it is not more than 6.6%.

Loss on drying <2.41> Not more than 8.0% (1 g, 100°C, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (5 g).

Assay (1) Available iodine—Weigh accurately about 0.5 g of Povidone-Iodine, dissolve in 30 mL of water, and titrate <2.50> with 0.02 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS).

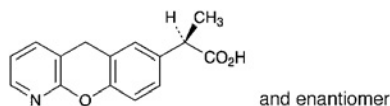
Each mL of 0.02 mol/L sodium thiosulfate VS
= 2.538 mg of I

(2) Nitrogen—Weigh accurately about 20 mg of Povidone-Iodine, and perform the test as directed under Nitrogen Determination <1.08>.

Containers and storage Containers—Tight containers.

Pranopfen

プラノプロフェン



$C_{15}H_{13}NO_3$: 255.27
(2*RS*)-2-(10*H*-9-Oxa-1-azaanthracen-6-yl)propanoic acid
[52549-17-4]

Pranopfen, when dried, contains not less than 98.5% of $C_{15}H_{13}NO_3$.

Description Pranopfen occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in *N,N*-dimethylformamide, soluble in acetic acid (100), sparingly soluble in methanol, slightly soluble in acetonitrile, in ethanol (95) and in acetic anhydride, very slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Pranopfen in *N,N*-dimethylformamide (1 in 30) shows no optical rotation.

Identification (1) Dissolve 0.02 g of Pranopfen in 1 mol/L hydrochloric acid TS to make 100 mL, and dilute 10 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pranopfen as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 186 – 190°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Pranopfen in 40 mL of methanol, and 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.30 mL of 0.01 mol/L hydrochloric acid add 40 mL of methanol, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Pranopfen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of the Standard Lead Solution (not more than 10 ppm).

(3) Related Substances—Dissolve 50 mg of Pranopfen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from both solutions by the automatic integration method: the each area of

the peaks other than the peak of pranopfen from the sample solution is not larger than the peak area of pranopfen from the standard solution, and the total peak area of them is not larger than twice of the peak area of pranopfen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 275 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 7.02 g of sodium perchlorate monohydrate in 1000 mL of water, and adjust the pH to 2.5 with perchloric acid. To 2 volumes of this solution add 1 volume of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of pranopfen is about 10 minutes.

Selection of column: Dissolve 4 mg each of Pranopfen and ethyl parahydroxybenzoate in 200 mL of the mobile phase. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of pranopfen and ethyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 2.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of pranopfen from 10 μ L of the standard solution is between 10 mm and 20 mm.

Time span of measurement: About three times as long as the retention time of pranopfen.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Pranopfen, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

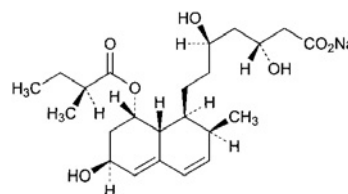
Each mL of 0.1 mol/L perchloric acid VS
= 25.53 mg of $C_{15}H_{13}NO_3$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Pravastatin Sodium

プラバスタチンナトリウム



$C_{23}H_{35}NaO_7$: 446.51
Monosodium (3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8*aR*)-6-hydroxy-2-methyl-

8-[(2S)-2-methylbutanoyloxy]-
1,2,6,7,8,8a-hexahydronaphthalen-1-yl} heptanoate
[81131-70-6]

Pravastatin Sodium contains not less than 98.5% and not more than 101.0% of $C_{23}H_{35}NaO_7$, calculated on the anhydrous basis and corrected on the amount of residual solvent.

Description Pravastatin Sodium occurs as a white to yellowish white, powder or crystalline powder.

It is freely soluble in water and in methanol, and soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Pravastatin Sodium (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pravastatin Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2970 cm^{-1} , 2880 cm^{-1} , 1727 cm^{-1} and 1578 cm^{-1} .

(3) Dissolve 50 mg of Pravastatin Sodium in 5 mL of methanol, and use this solution as the sample solution. Separately, dissolve 24 mg of Pravastatin 1,1,3,3-tetramethylbutylammonium Reference Standard in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot $2\text{ }\mu\text{L}$ each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and acetic acid (100) (80:16:1) to a distance of about 8 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the color tone and the R_f value of the principal spot with the sample solution are not different with them of the spot with the standard solution.

(4) A solution of Pravastatin Sodium (1 in 10) responds to Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49>: $+153 - +159^\circ$ (0.1 g calculated on the anhydrous basis and corrected on the amount of residual solvent, water, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Pravastatin Sodium in 20 mL of freshly boiled and cooled water is between 7.2 and 8.2.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pravastatin Sodium according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.10 g of Pravastatin Sodium in 100 mL of a mixture of water and methanol (11:9), and use this solution as the sample solution. Pipet 10 mL of the sample solution, add the mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 5 mL of this solution, add the mixture of water and methanol (11:9) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $10\text{ }\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of pravastatin to that of the internal standard.

matography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than pravastatin is not larger than 1/5 times the peak area of pravastatin from the standard solution, and the total area of the peaks other than pravastatin is not larger than the peak area of pravastatin from the standard solution. Keep the sample solution and the standard solution at not over than 15°C .

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2.5 times as long as the retention time of pravastatin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add a mixture of water and methanol (11:9) to make exactly 50 mL. Confirm that the peak area of pravastatin obtained with $10\text{ }\mu\text{L}$ of this solution is equivalent to 7 to 13% of that with $10\text{ }\mu\text{L}$ of the standard solution.

System performance: Dissolve 5 mg of Pravastatin Sodium in 50 mL of the mixture of water and methanol (11:9). When the procedure is run with $10\text{ }\mu\text{L}$ of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of pravastatin are not less than 3500 and not more than 1.6, respectively.

System repeatability: When the test is repeated 6 times with $10\text{ }\mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of pravastatin is not more than 2.0%.

(3) Residual solvent—Being specified separately.

Water <2.48> Not more than 4.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.1 g of Pravastatin Sodium, and dissolve in a mixture of water and methanol (11:9) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and the mixture of water and methanol (11:9) to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of Pravastatin 1,1,3,3-tetramethylbutylamine Reference Standard (previously determine the water with 0.5 g by direct titration in volumetric titration) dissolve in the mixture of water and methanol (11:9) to make exactly 25 mL. Proceed with exactly 10 mL of this solution in the same manner for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with $10\text{ }\mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of pravastatin to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of } C_{23}H_{35}NaO_7 \\ &= W_S \times (Q_T/Q_S) \times 4 \times 1.0518 \end{aligned}$$

W_S : Amount (mg) of Pravastatin 1,1,3,3-tetramethylbutylamine Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mixture of water and methanol (11:9) (3 in 4000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water, methanol, acetic acid (100) and triethylamine (550:450:1:1).

Flow rate: Adjust the flow rate so that the retention time of pravastatin is about 21 minutes.

System suitability—

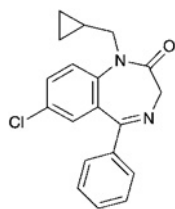
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the internal standard and pravastatin are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of pravastatin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prazepam

プラゼパム



C₁₉H₁₇ClN₂O: 324.80

7-Chloro-1-(cyclopropylmethyl)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one [2955-38-6]

Prazepam, when dried, contains not less than 98.5% of C₁₉H₁₇ClN₂O.

Description Prazepam occurs as white to light yellow crystals or crystalline powder. It is odorless.

It is freely soluble in acetone, soluble in acetic anhydride, sparingly soluble in ethanol (99.5) and in diethyl ether, and practically insoluble in water.

Identification (1) Dissolve 0.01 g of Prazepam in 3 mL of sulfuric acid, and observe under ultraviolet light (main wavelength: 365 nm): the solution shows a grayish blue fluorescence.

(2) Dissolve 0.01 g of Prazepam in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000). Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of

Prazepam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) Perform the Flame Coloration Tests <1.04> (2) with Prazepam: a green color appears.

Melting point <2.60> 145 – 148°C

Purity (1) Chloride <1.03>—To 1.0 g of Prazepam add 50 mL of water, allow to stand for 1 hour with occasional shaking, and filter. To 20 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—To 20 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Prazepam according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Prazepam according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.40 g of Prazepam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add acetone to make exactly 20 mL. Pipet 1 mL of this solution, add acetone to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and acetone (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Prazepam, previously dried, dissolve in 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 32.48 mg of C₁₉H₁₇ClN₂O

Containers and storage Containers—Tight containers.

Prazepam Tablets

プラゼパム錠

Prazepam Tablets contain not less than 93% and not more than 107% of the labeled amount of prazepam ($C_{19}H_{17}ClN_2O$: 324.80).

Method of preparation Prepare as directed under Tablets, with Prazepam.

Identification (1) To a quantity of powdered Prazepam Tablets, equivalent to 0.05 g of Prazepam according to the labeled amount, add 25 mL of acetone, shake well, and filter. Take 5 mL of the filtrate, evaporate on a water bath to dryness, and dissolve the residue in 3 mL of sulfuric acid. With this solution, proceed as directed in the Identification (1) under Prazepam.

(2) To a quantity of powdered Prazepam Tablets, equivalent to 0.02 g of Prazepam according to the labeled amount, add 200 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000), shake well, and filter. To 5 mL of the filtrate add a solution of sulfuric acid in ethanol (99.5) (3 in 1000) to make 50 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 241 nm and 245 nm, between 283 nm and 287 nm and between 363 nm and 367 nm, and minima between 263 nm and 267 nm and between 334 nm and 338 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Proceed with 1 tablet of Prazepam Tablets according to the Basket method, using 900 mL of 0.1 mol/L hydrochloric acid TS as the test solution at 100 rotations per minute. 30 minutes after starting the test, separate 20 mL or more of the dissolved solution, and filter with a membrane filter with pore size not more than 0.8 μ m. Discard the first 10 mL of the filtrate, measure exactly the subsequent V mL of the filtrate, add 0.1 mol/L hydrochloric acid TS to make exactly V' mL so that each mL of this solution might contain about 5 μ g of prazepam ($C_{19}H_{17}ClN_2O$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of prazepam for assay, previously dried at 105°C for 2 hours, add 200 mL of 0.1 mol/L hydrochloric acid TS and dissolve with shaking, or by ultrasonication if necessary, add 0.1 mol/L hydrochloric acid TS to make exactly 1000 mL and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 240 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Prazepam Tablets during 30 minutes is not less than 80%.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ of prazepam} \\ & (C_{19}H_{17}ClN_2O) \text{ to the labeled amount} \\ & = W_S \times (A_T/A_S) \times (V'/V) \times (90/C) \end{aligned}$$

W_S : Amount (mg) of prazepam for assay.

C : Labeled amount (mg) of prazepam ($C_{19}H_{17}ClN_2O$) in each tablet.

Assay Weigh accurately not less than 20 Prazepam Tablets,

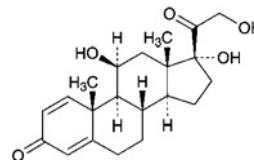
and powder. Weigh accurately a quantity of the powder, equivalent to about 50 mg of prazepam ($C_{19}H_{17}ClN_2O$), add 30 mL of acetone, shake well, centrifuge, and separate the supernatant. Repeat the same procedure twice with 30 mL each of acetone, combine all the supernatants, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.02 mol/L perchloric acid VS} \\ & = 6.496 \text{ mg of } C_{19}H_{17}ClN_2O \end{aligned}$$

Containers and storage Containers—Tight containers.

Prednisolone

プレドニゾロン



$C_{21}H_{28}O_5$: 360.44
11 β ,17,21-Trihydroxypregna-1,4-diene-3,20-dione
[50-24-8]

Prednisolone, when dried, contains not less than 97.0% and not more than 102.0% of $C_{21}H_{28}O_5$.

Description Prednisolone occurs as a white, crystalline powder.

It is soluble in methanol and in ethanol (95), slightly soluble in ethyl acetate and in chloroform, and very slightly soluble in water.

Melting point: about 235°C (with decomposition).

Identification (1) To 2 mg of Prednisolone add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Prednisolone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Prednisolone and Prednisolone Reference Standard in ethyl acetate, respectively, then evaporate the ethyl acetate to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +113 – +119° (after drying, 0.2 g, ethanol (95), 20 mL, 100 mm).

Purity (1) Selenium—To 0.10 g of Prednisolone add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 2 mL of nitric acid, and heat on a water bath until no more brown gas evolves and the solution becomes to be a light yellow.

low clear solution. After cooling, add 4 mL of nitric acid to this solution, then add water to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 3 mL of Standard Selenium Solution, add 0.5 mL of a mixture of perchloric acid and sulfuric acid (1:1) and 6 mL of nitric acid, then add water to make exactly 50 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and determine constant absorbances, A_T and A_S , obtained on a recorder after rapid increasing of the absorption: A_T is smaller than A_S (not more than 30 ppm).

Perform the test by using a hydride generating system and a thermal absorption cell.

Lamp: A selenium hollow cathode lamp

Wavelength: 196.0 nm

Temperature of sample atomizer: When an electric furnace is used, about 1000°C.

Carrier gas: Nitrogen or argon

(2) Related substances—Dissolve 20 mg of Prednisolone in exactly 2 mL of a mixture of methanol and chloroform (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of hydrocortisone and 10 mg of prednisolone acetate each in a mixture of methanol and chloroform (1:1) to make exactly 100 mL, and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, toluene and diethylamine (55:45:2) to a distance of about 15 cm, and air-dry the plate (do not dip the filter paper in the developing vessel). Spray evenly alkaline blue tetrazolium TS on the plate: the spots from the sample solution corresponding to those from the standard solutions (1) and (2) are not more intense than the spots from the standard solutions (1) and (2), and no spots other than the principal spot, hydrocortisone and prednisolone acetate appear from the sample solution.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 25 mg each of Prednisolone and Prednisolone Reference Standard, previously dried and accurately weighed, in 50 mL of methanol, add exactly 25 mL of the internal standard solution to each, and add methanol to make 100 mL. To 1 mL each of these solutions add the mobile phase to make 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of prednisolone to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } C_{21}H_{28}O_5 \\ & = W_S \times (Q_T / Q_S) \end{aligned}$$

W_S : Amount (mg) of Prednisolone Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 247 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with fluorosilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and methanol (13:7).

Flow rate: Adjust the flow rate so that the retention time of prednisolone is about 15 minutes.

System suitability—

System performance: Dissolve 25 mg of Prednisolone and 25 mg of hydrocortisone in 100 mL of methanol. To 1 mL of this solution add the mobile phase to make 10 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, hydrocortisone and prednisolone are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Tablets

プレドニゾロン錠

Prednisolone Tablets contain not less than 90% and not more than 110% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$; 360.44).

Method of preparation Prepare as directed under Tablets, with Prednisolone.

Identification (1) Weigh a quantity of powdered Prednisolone Tablets, equivalent to 0.05 g of Prednisolone according to the labeled amount, add 10 mL of chloroform, shake for 15 minutes, and filter. Evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour, and proceed as directed in the Identification (1) under Prednisolone.

(2) Determine the infrared absorption spectra of the residue obtained in (1) and Prednisolone Reference Standard, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the Reference Standard in ethyl acetate, evaporate to dryness, and repeat the test on the residues.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Transfer 1 tablet of Prednisolone Tablets to a volumetric flask, and shake with 10 mL of water until the tablet is disintegrated. Add 50 mL of methanol, shake for 30 minutes, and add methanol to make exactly 100 mL. Centrifuge this solution, pipet x mL of the supernatant liquid, and add methanol to make exactly V mL to provide a solution that contains about 10 μ g of prednisolone ($C_{21}H_{28}O_5$) per mL, and use this solution as the sample solution. Separately, weigh accurately

about 10 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, dissolve in 10 mL of water and 50 mL of methanol, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of prednisolone (C}_{21}\text{H}_{18}\text{O}_5) \\ = W_S \times (A_T/A_S) \times (V/100) \times (1/x) \end{aligned}$$

W_S : Amount (mg) of Prednisolone Reference Standard

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Prednisolone Tablets at 100 revolutions per minute according to the Paddle method using 900 mL of water as the test solution. Twenty minutes after the start of the test, take 20 mL or more of the dissolved solution, and filter through a membrane filter with pore size of 0.8 μm or less. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 10 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at the maximum wavelength at about 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Prednisolone Tablets after 20 minutes should be not less than 70%.

$$\begin{aligned} \text{Dissolution rate (\%)} \text{ with respect to} \\ \text{the labeled amount of prednisolone (C}_{21}\text{H}_{28}\text{O}_5) \\ = W_S \times (A_T/A_S) \times (45/C) \end{aligned}$$

W_S : Amount (mg) of Prednisolone Reference Standard.

C : Labeled amount (mg) of prednisolone (C₂₁H₂₈O₅) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Prednisolone Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 5 mg of prednisolone (C₂₁H₂₈O₅), add 1 mL of water, and shake gently. Add exactly 5 mL of the internal standard solution and 15 mL of methanol, and shake vigorously for 20 minutes. To 1 mL of this solution add the mobile phase to make 10 mL, and filter through a membrane filter with pore size of 0.45 μm . Discard the first 3 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Reference Standard, previously dried at 105°C for 3 hours, dissolve in 50 mL of methanol, add exactly 25 mL of the internal standard solution, and add methanol to make 100 mL. To 1 mL of this solution add the mobile phase to make 10 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Prednisolone with these solutions.

$$\begin{aligned} \text{Amount (mg) of prednisolone (C}_{21}\text{H}_{28}\text{O}_5) \\ = W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

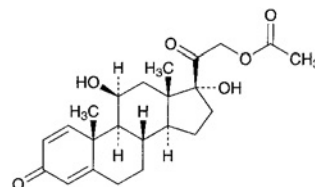
W_S : Amount (mg) of Prednisolone Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (1 in 2000).

Containers and storage Containers—Tight containers.

Prednisolone Acetate

プレドニゾロン酢酸エステル



C₂₃H₃₀O₆: 402.48

11 β ,17,21-Trihydroxypregna-1,4-diene-3,20-dione
21-acetate [52-21-1]

Prednisolone Acetate, when dried, contains not less than 96.0% and not more than 102.0% of C₂₃H₃₀O₆.

Description Prednisolone Acetate occurs as a white, crystalline powder.

It is slightly soluble in methanol, in ethanol (95), in ethanol (99.5), and in chloroform, and practically insoluble in water.

Melting point: about 235°C (with decomposition).

Identification (1) To 2 mg of Prednisolone Acetate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectra of Prednisolone Acetate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum in a range between 4000 cm⁻¹ and 650 cm⁻¹ with the Infrared Reference Spectrum or the spectrum of previously dried Prednisolone Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears, dissolve the sample and the Reference Standard in ethanol (99.5), respectively, evaporate to dryness, and repeat the test on the residues.

Optical rotation <2.49> [α]_D²⁰: +128 – +137° (after drying, 70 mg, methanol, 20 mL, 100 mm).

Purity Related substances—Dissolve 0.20 g of Prednisolone Acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1), and use this solution as the sample solution. Separately, dissolve 20 mg each of prednisolone, cortisone acetate and hydrocortisone acetate in exactly 10 mL of a mixture of chloroform and methanol (9:1). Pipet 1 mL of this solution, add a mixture of chloroform and methanol (9:1) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dichloromethane, diethyl ether, methanol and water (385:75:40:6) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (wavelength: 254 nm): the spots from the sample solution corresponding to those from the standard solution are

not more intense than the spots from the standard solution, and any spot from the sample solution other than the principal spot and the spots from prednisolone, cortisone acetate and hydrocortisone acetate does not appear.

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Dissolve about 10 mg each of Prednisolone Acetate and Prednisolone Acetate Reference Standard, previously dried and accurately weighed, in 60 mL each of methanol, add exactly 2 mL each of the internal standard solution, then add methanol to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of prednisolone acetate to that of the internal standard.

$$\text{Amount (mg) of } C_{23}H_{30}O_6 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Prednisolone Acetate Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in methanol (3 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of prednisolone acetate is about 10 minutes.

System suitability—

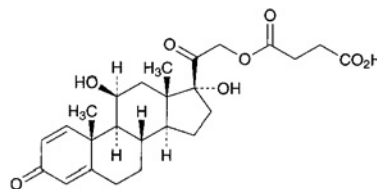
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, prednisolone acetate and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of prednisolone acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Prednisolone Succinate

プレドニゾロンコハク酸エステル



$C_{25}H_{32}O_8$: 460.52

11 β ,17,21-Trihydroxypregna-1,4-diene-3,20-dione
21-(hydrogen succinate) [2920-86-7]

Prednisolone Succinate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{25}H_{32}O_8$.

Description Prednisolone Succinate occurs as a white, fine, crystalline powder. It is odorless.

It is freely soluble in methanol, soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

Melting point: about 205°C (with decomposition).

Identification (1) To 2 mg of Prednisolone Succinate add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Determine the infrared absorption spectrum of Prednisolone Succinate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Prednisolone Succinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +114 – +120° (after drying, 67 mg, methanol, 10 mL, 100 mm).

Purity Related substances—Dissolve 0.10 g of Prednisolone Succinate in methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 30 mg of prednisolone in methanol to make exactly 10 mL. Pipet 1 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg each of Prednisolone

Succinate and Prednisolone Succinate Reference Standard, previously dried, and dissolve each in methanol to make exactly 100 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 242 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

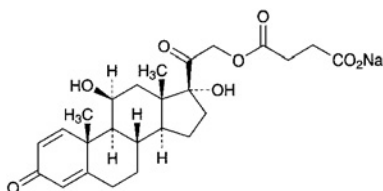
$$\begin{aligned} &\text{Amount (mg) of } C_{25}H_{32}O_8 \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Prednisolone Succinate Reference Standard

Containers and storage Containers—Tight containers.

Prednisolone Sodium Succinate for Injection

注射用プレドニゾロンコハク酸エステルナトリウム



$C_{25}H_{31}NaO_8$: 482.50

Monosodium 11 β ,17,21-trihydroxypregna-1,4-diene-3,20-dione 21-succinate [1715-33-9]

Prednisolone Sodium Succinate for Injection is a preparation for injection which is dissolved before used.

It contains not less than 72.4% and not more than 83.2% of prednisolone sodium succinate ($C_{25}H_{31}NaO_8$), and the equivalent of not less than 90% and not more than 110% of the labeled amount of prednisolone ($C_{21}H_{28}O_5$: 360.44).

The amount should be stated as the amount of prednisolone ($C_{21}H_{28}O_5$).

Method of preparation Prepare as directed under Injections, with Prednisolone Succinate and Dried Sodium Carbonate or Sodium Hydroxide.

It contains a suitable buffer agent.

Description Prednisolone Sodium Succinate for Injection occurs as a white powder or porous, friable mass.

It is freely soluble in water.

It is hygroscopic.

Identification (1) To 2 mg of Prednisolone Sodium Succinate for Injection add 2 mL of sulfuric acid, and allow to stand for 2 to 3 minutes: a deep red color, without fluorescence, develops. To this solution add 10 mL of water cautiously: the color disappears and a gray, flocculent precipitate is formed.

(2) Dissolve 0.01 g of Prednisolone Sodium Succinate for Injection in 1 mL of methanol, add 1 mL of Fehling's TS, and heat: an orange to red precipitate is formed.

(3) Dissolve 0.1 g of Prednisolone Sodium Succinate for

Injection in 2 mL of sodium hydroxide TS, allow to stand for 10 minutes, and filter. Add 1 mL of dilute hydrochloric acid to the filtrate, shake, and filter if necessary. Adjust the solution with diluted ammonia TS (1 in 10) to a pH of about 6, and add 2 to 3 drops of iron (III) chloride TS: a brown precipitate is formed.

(4) Prednisolone Sodium Succinate for Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> Dissolve 1.0 g of Prednisolone Sodium Succinate for Injection in 40 mL of water: the pH of the solution is between 6.5 and 7.2.

Purity Clarity and color of solution—Dissolve 0.25 g of Prednisolone Sodium Succinate for Injection in 10 mL of water: the solution is clear and colorless.

Loss on drying <2.41> Not more than 2.0% (0.15 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Take a quantity of sealed containers of Prednisolone Sodium Succinate for Injection, equivalent to about 0.1 g of prednisolone ($C_{21}H_{28}O_5$), and dissolve the contents in a suitable amount of diluted methanol (1 in 2), and transfer to a 100-mL volumetric flask. Wash each container with diluted methanol (1 in 2), collect the washings in the volumetric flask, and add diluted methanol (1 in 2) to make volume. Pipet 4 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Prednisolone Succinate Reference Standard, previously dried in a desiccator for 6 hours (in vacuum, phosphorus (V) oxide, 60°C), dissolve in methanol to make exactly 25 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with 10 μ L of the sample solution and standard solution as directed under Liquid Chromatography according <2.01> to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of prednisolone succinate to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of prednisolone sodium succinate} \\ &(\text{C}_{25}\text{H}_{31}\text{NaO}_8) \\ &= W_S \times (Q_T/Q_S) \times 5 \times 1.0477 \end{aligned}$$

$$\begin{aligned} &\text{Amount (mg) of prednisolone (C}_{21}\text{H}_{28}\text{O}_5) \\ &= W_S \times (Q_T/Q_S) \times 5 \times 0.7827 \end{aligned}$$

W_S : Amount (mg) of Prednisolone Succinate Reference Standard

Internal standard solution—A solution of propyl parahydroxybenzoate in diluted methanol (1 in 2) (1 in 25,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 0.32 g of tetra *n*-butylammonium bromide, 3.22 g of disodium hydrogen phosphate dodacahy-

drate and 6.94 g of potassium dihydrogen phosphate in 1000 mL of water. To 840 mL of this solution add 1160 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of prednisolone succinate is about 15 minutes.

System suitability—

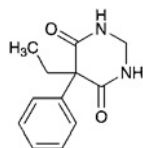
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, prednisolone succinate and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of prednisolone succinate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Primidone

プリミドン



$C_{12}H_{14}N_2O_2$: 218.25

5-Ethyl-5-phenyl-2,3-dihydropyrimidine-4,6(1*H*,5*H*)-dione
[125-33-7]

Primidone, when dried, contains not less than 98.5% of $C_{12}H_{14}N_2O_2$.

Description Primidone occurs as a white, crystalline powder or granules. It is odorless and has a slightly bitter taste.

It is soluble in *N,N*-dimethylformamide, sparingly soluble in pyridine, slightly soluble in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Heat 0.5 g of Primidone with 5 mL of diluted sulfuric acid (1 in 2): the odor of formaldehyde is perceptible.

(2) Mix 0.2 g of Primidone with 0.2 g of anhydrous sodium carbonate, and heat: the gas evolved changes moistened red litmus paper to blue.

Melting point <2.60> 279 – 284°C

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Primidone in 10 mL of *N,N*-dimethylformamide: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Primidone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) 2-Ethyl-2-phenylmalonediamide—Dissolve 0.10 g of Primidone in 2 mL of pyridine, add exactly 2 mL of the internal standard solution, then add 1 mL of bis-trimethyl silyl acetamide, shake well, and heat at 100°C for 5 minutes. Cool, add pyridine to make 10 mL, and use this solution as the sample solution. Separately, dissolve 50 mg of 2-ethyl-2-

phenylmalonediamide in pyridine to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 2 mL of the internal standard solution, proceed in the same manner as Primidone, and use this solution as the standard solution. Perform the test with 2 μ L of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard: Q_T is not more than Q_S .

Internal standard solution—A solution of stearylalcohol in pyridine (1 in 2000).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 150 cm in length, packed with siliceous earth for gas chromatography (125 to 150 μ m in particle diameter) coated with 50% phenyl-methyl silicon polymer for gas chromatography at the ratio of 3%.

Column temperature: A constant temperature of about 195°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of stearylalcohol is about 10 minutes.

System suitability—

System performance: When the procedure is run with 2 μ L of the standard solution under the above operating condition, 2-ethyl-2-phenylmalonediamide and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 5 times with 2 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of 2-ethyl-2-phenylmalonediamide to that of the internal standard is not more than 1.5%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 20 mg each of Primidone and Primidone Reference Standard, previously dried, dissolve each in 20 mL of ethanol (95) by warming, and after cooling, add ethanol (95) to make exactly 25 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absorbance, A_1 , of the sample solution and the standard solution at the wavelength of maximum absorption at about 257 nm, and the absorbances, A_2 and A_3 , at the wavelength of minimum absorption at about 254 nm and at about 261 nm, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank.

$$\begin{aligned} & \text{Amount (mg) of } C_{12}H_{14}N_2O_2 \\ & = W_S \times \{(2A_1 - A_2 - A_3)_T / (2A_1 - A_2 - A_3)_S\} \end{aligned}$$

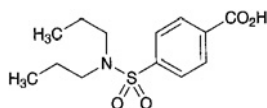
W_S : Amount (mg) of Primidone Reference Standard

where, $(2A_1 - A_2 - A_3)_T$ is the value from the sample solution, and $(2A_1 - A_2 - A_3)_S$ is from the standard solution.

Containers and storage Containers—Tight containers.

Probenecid

プロベネシド



$C_{13}H_{19}NO_4S$: 285.36

4-(Dipropylaminosulfonyl)benzoic acid [57-66-9]

Probenecid, when dried, contains not less than 98.0% of $C_{13}H_{19}NO_4S$.

Description Probenecid occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste, followed by unpleasant bitter.

Probenecid is sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS and in ammonia TS.

Melting point: 198 – 200°C

Identification (1) Heat Probenecid strongly: the odor of sulfur dioxide is perceptible.

(2) Determine the absorption spectrum of a solution of Probenecid in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Probenecid Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Acidity—To 2.0 g of Probenecid add 100 mL of water, heat on a water bath with occasional shaking for 30 minutes, cool, and filter. To the filtrate add 1 drop of phenolphthalein TS and 0.50 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Chloride <1.03>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of nitric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—To 1.0 g of Probenecid add 100 mL of water and 1 mL of hydrochloric acid, and heat on a water bath with occasional shaking for 30 minutes. After cooling, add, if necessary, water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Probenecid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Probenecid according to Method 3, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Probenecid, previously dried, and dissolve in 50 mL of neutralized ethanol. Titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 28.54 mg of $C_{13}H_{19}NO_4S$

Containers and storage Containers—Well-closed containers.

Probenecid Tablets

プロベネシド錠

Probenecid Tablets contain not less than 95% and not more than 105% of the labeled amount of probenecid ($C_{13}H_{19}NO_4S$: 285.36).

Method of preparation Prepare as directed under Tablets, with Probenecid.

Identification (1) Weigh a quantity of powdered Probenecid Tablets, equivalent to 0.5 g of Probenecid according to the labeled amount, add 50 mL of ethanol (95) and 1 mL of 1 mol/L hydrochloric acid TS, shake, and filter. Evaporate the filtrate on a water bath to about 20 mL. After cooling, collect produced crystals, recrystallize with 50 mL of dilute ethanol, and dry at 105°C for 4 hours: it melts <2.60> between 198°C and 200°C. With the crystals so obtained, proceed as directed in the Identification (1) under Probenecid.

(2) Determine the absorption spectrum of a solution of the dried crystals obtained in (1) in ethanol (95) (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 224 nm and 226 nm and between 247 nm and 249 nm, and a minimum between 234 nm and 236 nm.

Dissolution <6.10> Perform the test according to the following method: It meets the requirement.

Perform the test with 1 tablet of Probenecid Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 30 mL or more of the dissolved solution 30 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 14 μ g of probenecid ($C_{13}H_{19}NO_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 70 mg of Probenecid Reference Standard, previously dried at 105°C for 4 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 1 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 244 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Probenecid Tablets in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the

$$\begin{aligned} & \text{labeled amount of procainamide (C}_{13}\text{H}_{19}\text{NO}_4\text{S)} \\ & = W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18 \end{aligned}$$

W_S : Amount (mg) of Procainamide Reference Standard.

C : Labeled amount (mg) of procainamide (C₁₃H₁₉NO₄S) in 1 tablet.

Assay Weigh accurately, and powder not less than 20 Procainamide Tablets. Weigh accurately a portion of the powder, equivalent to about 0.15 g of procainamide (C₁₃H₁₉NO₄S), add 200 mL of ethanol (95) and 5 mL of 1 mol/L hydrochloric acid TS, and heat on a water-bath at 70°C for 30 minutes with occasional shaking. After cooling, add ethanol (95) to make exactly 250 mL, and filter. Discard the first 20 mL of the filtrate. To 5 mL of the subsequent filtrate, exactly measured, add 5 mL of 0.1 mol/L hydrochloric acid TS, dilute with ethanol (95) to exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.15 g of Procainamide Reference Standard, previously dried at 105°C for 4 hours, dissolve in 5 mL of 1 mol/L hydrochloric acid TS, and add ethanol (95) to make exactly 250 mL. Pipet 5 mL of the solution, add 5 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make exactly 250 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 248 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared by mixing 5 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make exactly 250 mL as the blank.

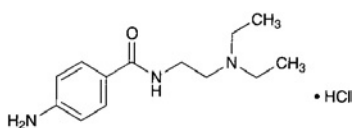
$$\begin{aligned} & \text{Amount (mg) of procainamide (C}_{13}\text{H}_{19}\text{NO}_4\text{S)} \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Procainamide Reference Standard

Containers and storage Containers—Well-closed containers.

Procainamide Hydrochloride

プロカインアミド塩酸塩



C₁₃H₂₁N₃O·HCl: 271.79

4-Amino-*N*-(2-diethylaminoethyl)benzamide monohydrochloride [614-39-1]

Procainamide Hydrochloride, when dried, contains not less than 98.0% of C₁₃H₂₁N₃O·HCl.

Description Procainamide Hydrochloride occurs as a white to light yellow, crystalline powder. It is odorless.

It is very soluble in water, freely soluble in methanol, in acetic acid (100) and in ethanol (95), slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 1 g of Procainamide Hydrochloride in 10 mL of water, add 10 mL of sodium hydroxide TS, and extract with two 10-mL portions of a mixture of diethyl ether and chloroform (1:1). Combine the ex-

tracts, add calcium chloride for drying, and dry the extracts for 30 minutes. Decant the solution into a small flask, add 5 mL of pyridine, and slowly add dropwise 1 mL of benzoyl chloride. Heat the mixture on a water bath for 30 minutes, add 20 mL of a mixture of diethyl ether and chloroform (1:1), shake, and pour the mixture into 100 mL of sodium hydroxide TS, then shake. Separate the organic solvent layer, wash it with 20 mL of water, cool to 10°C, and allow the crystals to separate. Collect the separated crystals, recrystallize from 10 mL of dilute ethanol, and dry at 105°C for 1 hour: the crystals so obtained melt <2.60> between 180°C and 187°C.

(2) Dissolve 0.01 g of Procainamide Hydrochloride in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) A solution of Procainamide Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the pH of this solution is between 5.0 and 6.5.

Melting point <2.60> 165 – 169°C

Purity (1) Clarity of solution—Dissolve 1.0 g of Procainamide Hydrochloride in 10 mL of water: the solution is clear.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procainamide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Procainamide Hydrochloride according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Procainamide Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Then spot 10 μL each of a solution of ammonia solution (28) in methanol (11 in 50) on each of the above spots. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (700:300:7) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spray on the plate: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Assay Weigh accurately about 0.5 g of Procainamide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L perchloric acid VS} \\ & = 27.18 \text{ mg of C}_{13}\text{H}_{21}\text{N}_3\text{O}\cdot\text{HCl} \end{aligned}$$

Containers and storage Containers—Tight containers.

Procainamide Hydrochloride Injection

プロカインアミド塩酸塩注射液

Procainamide Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$; 271.79).

Method of preparation Prepare as directed under Injections, with Procainamide Hydrochloride.

Description Procainamide Hydrochloride Injection is a clear, colorless or light yellow liquid.

pH: 4.0 – 6.0

Identification (1) Proceed with a volume of Procainamide Hydrochloride Injection, equivalent to 1 g of Procainamide Hydrochloride according to the labeled amount, as directed in the Identification (1) under Procainamide Hydrochloride.

(2) Dilute a volume of Procainamide Hydrochloride Injection, equivalent to 0.01 g of Procainamide Hydrochloride according to the labeled amount, with 1 mL of dilute hydrochloric acid and water to 5 mL: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Procainamide Hydrochloride Injection responds to the Qualitative Tests <1.09> (2) for chloride.

Assay Dilute an accurately measured volume of Procainamide Hydrochloride Injection, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$), with 5 mL of hydrochloric acid and water to 50 mL, cool to 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration method or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

Containers and storage Containers—Hermetic containers.

Procainamide Hydrochloride Tablets

プロカインアミド塩酸塩錠

Procainamide Hydrochloride Tablets contain not less than 95% and not more than 105% of the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$; 271.79).

Method of preparation Prepare as directed under Tablets, with Procainamide Hydrochloride.

Identification (1) Shake a quantity of powdered Procainamide Hydrochloride Tablets, equivalent to 1.5 g of Procainamide Hydrochloride according to the labeled amount, with 30 mL of water, filter, and use the filtrate as the

sample solution. To 20 mL of the sample solution add 10 mL of sodium hydroxide TS, and proceed as directed in the Identification (1) under Procainamide Hydrochloride.

(2) To 0.2 mL of the sample solution obtained in (1) add 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Procainamide Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the test solution. Take 30 mL or more of the dissolved solution 30 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.8 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 7 μg of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 0.125 g of procainamide hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Procainamide Hydrochloride Tablets in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 4.5$

W_S : Amount (mg) of procainamide hydrochloride for assay.

C : Labeled amount (mg) of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$) in 1 tablet.

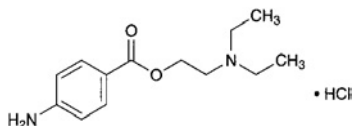
Assay Weigh accurately and powder not less than 20 Procainamide Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.5 g of procainamide hydrochloride ($C_{13}H_{21}N_3O.HCl$), stir well with 25 mL of 1 mol/L hydrochloric acid TS, centrifuge, and separate the supernatant liquid. Wash the residue with four 10-mL portions of 1 mol/L hydrochloric acid VS in the same manner. Add 10 mL of a solution of potassium bromide (3 in 10) to the combined supernatant liquid, cool to below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.18 mg of $C_{13}H_{21}N_3O.HCl$

Containers and storage Containers—Tight containers.

Procaine Hydrochloride

プロカイン塩酸塩



$C_{13}H_{20}N_2O_2 \cdot HCl$: 272.77

2-(Diethylamino)ethyl 4-aminobenzoate monohydrochloride
[51-05-8]

Procaine Hydrochloride, when dried, contains not less than 99.0% of $C_{13}H_{20}N_2O_2 \cdot HCl$.

Description Procaine Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the absorption spectrum of a solution of Procaine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procaine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Procaine Hydrochloride in 20 mL of water is between 5.0 and 6.0.

Melting point <2.60> 155 – 158°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Procaine Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Procaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—To 1.0 g of Procaine Hydrochloride add 5 mL of ethanol (95), dissolve by mixing well, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 4-aminobenzoic acid in ethanol (95) to make exactly 20 mL, then pipet 1 mL of this solution, add 4 mL of ethanol (95) and water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of dibutyl ether, *n*-hexane and acetic acid (100) (20:4:1) to a distance of about 10 cm, and air-dry the plate. After drying the plate more at 105°C

for 10 minutes, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. The principal spot from the sample solution stays at the origin.

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Procaine Hydrochloride, previously dried, dissolve in 5 mL of hydrochloric acid and 60 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool to below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

Each mL of 0.1 mol/L sodium nitrite VS
= 27.28 mg of $C_{13}H_{20}N_2O_2 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Procaine Hydrochloride Injection

プロカイン塩酸塩注射液

Procaine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$: 272.77).

Method of preparation Prepare as directed under Injections, with Procaine Hydrochloride.

Description Procaine Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To a volume of Procaine Hydrochloride Injection, equivalent to 0.01 g of Procaine Hydrochloride according to the labeled amount, add water to make 1000 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 219 nm and 223 nm, and between 289 nm and 293 nm.

(2) Procaine Hydrochloride Injection responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> 3.3 – 6.0

Extractable volume <6.05> It meets the requirement.

Assay To an exactly measured volume of Procaine Hydrochloride Injection, equivalent to about 20 mg of procaine hydrochloride ($C_{13}H_{20}N_2O_2 \cdot HCl$), add the mobile phase to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of procaine hydrochloride for assay, previously dried in a desiccator (silica gel) for 4 hours, dissolve in the mobile phase to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and the mobile phase to make 20 mL, and use this solution as the standard solu-

tion. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of procaine hydrochloride to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of procaine hydrochloride} \\ & (\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_2 \cdot \text{HCl}) \\ & = W_S \times (Q_T/Q_S) \times (2/5) \end{aligned}$$

W_S : Amount (mg) of procaine hydrochloride for assay

Internal standard solution—A solution of caffeine in the mobile phase (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Adjust the pH of 0.05 mol/L potassium dihydrogen phosphate TS to 3.0 with phosphoric acid, and add an amount of sodium 1-pentane sulfonate to make a solution so that containing 0.1%. To 800 mL of this solution add 200 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of procaine is about 10 minutes.

System suitability—

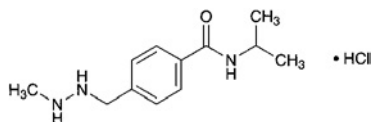
System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, procaine and the internal standard are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of procaine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Procarbazine Hydrochloride

プロカルバジン塩酸塩



$\text{C}_{12}\text{H}_{19}\text{N}_3\text{O} \cdot \text{HCl}$: 257.76

N-(1-Methylethyl)-4-[(2-methylhydrazino)methyl]benzamide monohydrochloride [366-70-1]

Procarbazine Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $\text{C}_{12}\text{H}_{19}\text{N}_3\text{O} \cdot \text{HCl}$.

Description Procarbazine Hydrochloride occurs as white to light yellowish white crystals or crystalline powder.

It is freely soluble in water, and slightly soluble in ethanol

(99.5).

It dissolves in dilute hydrochloric acid.

Melting point: about 223°C (with decomposition).

Identification (1) Dissolve 0.01 g of Procarbazine Hydrochloride in 1 mL of diluted copper (II) sulfate TS (1 in 10), and add 4 drops of sodium hydroxide TS: a green precipitate is formed immediately, and the color changes from green through yellow to orange.

(2) Determine the absorption spectrum of a solution of Procarbazine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Procarbazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Procarbazine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 0.10 g of Procarbazine Hydrochloride in 10 mL of water: the pH of this solution is between 3.0 and 5.0.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Procarbazine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 50 mg of Procarbazine Hydrochloride in 5.0 mL of a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Immerse slowly, by inclining, a plate of silica gel with fluorescent indicator for thin-layer chromatography in a solution of L-cysteine hydrochloride in diluted methanol (7 in 10) (1 in 200), allow to stand for 1 minute, lift the plate from the solution, dry it in cold wind for 10 minutes, then dry in warm wind for 5 minutes, and then dry at 60°C for 5 minutes. After cooling, spot 5 μ L each of the sample solution and standard solution on the plate. Develop the plate with a mixture of methanol and ethyl acetate (1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): not more than 1 spot other than the principal spot and the spot of the starting point from the sample solution appears, and is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.15 g of Procarbazine Hydrochloride, previously dried, place in a glass-stoppered flask, dissolve in 25 mL of water, add 25 mL of hydrochloric acid, and cool to room temperature. To this solution add 5 mL of chloroform, and titrate <2.50>, while shaking, with 0.05 mol/L potassium iodate VS until the purple color of the

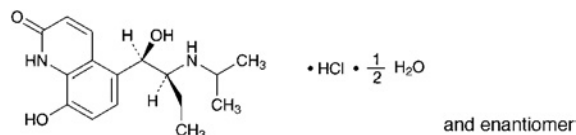
chloroform layer disappears. The end point is reached when the red-purple color of the chloroform layer no more reappears within 5 minutes after the purple color disappeared.

Each mL of 0.05 mol/L potassium iodate VS
= 8.592 mg of $C_{12}H_{19}N_3O \cdot HCl$

Containers and storage Containers—Tight containers.

Procaterol Hydrochloride Hydrate

プロカテロール塩酸塩水和物



$C_{16}H_{22}N_2O_3 \cdot HCl \cdot \frac{1}{2}H_2O$: 335.83
8-Hydroxy-5-[(1*RS*,2*SR*)-1-hydroxy-2-[(1-methylethyl)amino]butyl]} quinolin-2(1*H*)-one monohydrochloride hemihydrate [62929-91-3, anhydride]

Procaterol Hydrochloride Hydrate contains not less than 98.5% of procaterol hydrochloride ($C_{16}H_{22}N_2O_3 \cdot HCl$: 326.82), calculated on the anhydrous basis.

Description Procaterol Hydrochloride Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is soluble in water, in formic acid and in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Procaterol Hydrochloride Hydrate (1 in 100) is between 4.0 and 5.0.

It is gradually colored by light.

Melting point: about 195°C (with decomposition).

The solution of Procaterol Hydrochloride Hydrate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Procaterol Hydrochloride Hydrate (7 in 1,000,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Procaterol Hydrochloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Procaterol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Procaterol Hydrochloride Hydrate in 30 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 3.0 mL of Ferric Chloride Stock CS add water to make 50 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Procaterol Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0

mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Procaterol Hydrochloride Hydrate in 100 mL of diluted methanol (1 in 2), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than procaterol from the sample solution is not larger than the peak area of procaterol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 0.87 g of sodium 1-pentanesulfonate in 1000 mL of water. To 760 mL of this solution add 230 mL of methanol and 10 mL of glacial acetic acid.

Flow rate: Adjust the flow rate so that the retention time of procaterol is about 15 minutes.

Selection of column: Dissolve 20 mg each of Procaterol Hydrochloride Hydrate and threoprocaterol hydrochloride in 100 mL of diluted methanol (1 in 2). To 15 mL of this solution add diluted methanol (1 in 2) to make 100 mL. Proceed with 2 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of procaterol and threoprocaterol in this order with the resolution of these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of procaterol obtained from 2 μ L of the standard solution is not less than 10 mm.

Time span of measurement: 2.5 times as long as the retention time of procaterol beginning after the solvent peak.

Water <2.48> 2.5 – 3.3% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Procaterol Hydrochloride Hydrate, add 2 mL of formic acid, dissolve by warming, and add exactly 15 mL of 0.1 mol/L perchloric acid VS. Add 1 mL of acetic anhydride, heat on a water bath for 30 minutes, cool, add 60 mL of acetic anhydride, and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination.

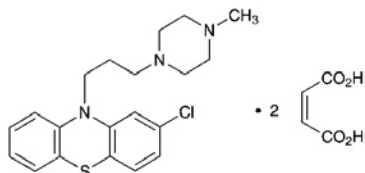
Each mL of 0.1 mol/L perchloric acid VS
= 32.68 mg of $C_{16}H_{22}N_2O_3 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Prochlorperazine Maleate

プロクロルペラジンマレイン酸塩



$C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$: 606.09

2-Chloro-10-[3-(4-methylpiperazin-1-yl)propyl]-10H-phenothiazine dimaleate [84-02-6]

Prochlorperazine Maleate, when dried, contains not less than 98.0% of $C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$.

Description Prochlorperazine Maleate occurs as a white to light yellow powder. It is odorless, and has a slightly bitter taste.

It is slightly soluble in acetic acid (100), very slightly soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It gradually acquires a red tint by light.

Melting point: 195 – 203°C (with decomposition).

Identification (1) Dissolve 5 mg of Prochlorperazine Maleate in 5 mL of sulfuric acid: a red color develops, which darkens slowly on standing. Warm a half of the solution: the color changes to red-purple. To the remainder add 1 drop of potassium dichromate TS: a green-brown color develops, which changes to brown on standing.

(2) Boil 0.5 g of Prochlorperazine Maleate with 10 mL of hydrobromic acid under a reflux condenser for 10 minutes. After cooling, add 100 mL of water, and filter through glass filter (G4). Wash the residue with three 10-mL portions of water, and dry at 105°C for 1 hour: it melts <2.60> between 195°C and 198°C (with decomposition).

(3) Dissolve 0.2 g of Prochlorperazine Maleate in 5 mL of a solution of sodium hydroxide (1 in 10), and extract with three 3-mL portions of diethyl ether [reserve the aqueous layer, and use for test (4)]. Evaporate the combined diethyl ether extracts on a water bath to dryness, dissolve the residue in 10 mL of methanol by warming, and pour into 30 mL of a solution of 2,4,6-trinitrophenol in methanol (1 in 75), previously warmed to 50°C. Allow to stand for 1 hour, collect the crystals, wash with a small amount of methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 252°C and 258°C (with decomposition).

(4) To the aqueous layer reserved in (3) add boiling chips, and heat on a water bath for 10 minutes. Cool, add 2 mL of bromine TS, heat on a water bath for 10 minutes, and heat the solution to boil. After cooling, add 2 drops of this solution to 3 mL of a solution of resorcinol in sulfuric acid (1 in 300), and heat on a water bath for 15 minutes: a red-purple color is produced.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Prochlorperazine Maleate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prochlorperazine Maleate, previously dried, dissolve in 60 mL of acetic acid (100) while stirring and warming. Cool, and titrate <2.50> with 0.05 mol/L perchloric acid VS until the color of the solution changes from orange to green (indicator: 0.5 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 15.15 mg of $C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Prochlorperazine Maleate Tablets

プロクロルペラジンマレイン酸塩錠

Prochlorperazine Maleate Tablets contain not less than 95% and not more than 105% of the labeled amount of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$: 606.09).

Method of preparation Prepare as directed under Tablets, with Prochlorperazine Maleate.

Identification (1) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 5 mg of Prochlorperazine Maleate according to the labeled amount, add 15 mL of acetic acid (100), shake, and filter. To 5 mL of the filtrate add 3 mL of sulfuric acid, and shake: a light red color develops. To this solution add 1 drop of potassium dichromate TS: a green-brown color is produced and changes to brown on standing.

(2) Weigh a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.08 g of Prochlorperazine Maleate according to the labeled amount, add 15 mL of methanol and 1 mL of dimethylamine, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 0.08 g of Prochlorperazine Maleate Reference Standard in 15 mL of a mixture of methanol and dimethylamine (15:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia TS (15:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly palladium (II) chloride TS on the plate: the spots obtained from the sample solution and standard solution show a red-purple color, and has the same *R_f* value.

(3) To a quantity of powdered Prochlorperazine Maleate Tablets, equivalent to 0.04 g of Prochlorperazine Maleate according to the labeled amount, add 10 mL of 1 mol/L hydrochloric acid TS and 20 mL of diethyl ether, shake, and centrifuge. Transfer the diethyl ether layer to a separator, wash with 5 mL of 0.05 mol/L sulfuric acid TS, and evaporate on a water bath to dryness. Dissolve the residue in

5 mL of sulfuric acid TS, filter, if necessary, and add 1 to 2 drops of potassium permanganate TS: the red color of the test solution is discharged immediately.

Assay Weigh accurately and powder not less than 20 Prochlorperazine Maleate Tablets using an agate mortar. Weigh accurately a portion of the powder, equivalent to about 16 mg of prochlorperazine maleate ($C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4$), transfer to a glass-stoppered centrifuge tube, add exactly 25 mL of a mixture of *N,N*-dimethylformamide and dimethylamine (100:1), stopper tightly, shake vigorously for 15 minutes, and centrifuge. Use the supernatant liquid as the sample solution. Separately, weigh accurately about 64 mg of Prochlorperazine Maleate Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, dissolve in a mixture of *N,N*-dimethylformamide and dimethylamine (100:1) to make exactly 100 mL, and use this solution as the standard solution. Pipet 4 mL each of the sample solution and the standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0, and 20 mL of cyclohexane, stopper tightly, and centrifuge after shaking vigorously for 5 minutes. Pipet 10 mL each of the cyclohexane layer of these solutions into glass-stoppered centrifuge tubes, add exactly 20 mL of palladium (II) chloride TS and 5 mL of *N,N*-dimethylformamide, stopper tightly, and centrifuge after shaking vigorously for 15 minutes. Determine the absorbances, A_T and A_S , of the water layers obtained from the sample solution and the standard solution at 495 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using palladium (II) chloride TS as the blank.

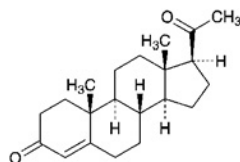
$$\begin{aligned} &\text{Amount (mg) of prochlorperazine maleate} \\ & (C_{20}H_{24}ClN_3S \cdot 2C_4H_4O_4) \\ & = W_S \times (A_T/A_S) \times (1/4) \end{aligned}$$

W_S : Amount (mg) of Prochlorperazine Maleate Reference Standard

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Progesterone

プロゲステロン



$C_{21}H_{30}O_2$: 314.46
Pregn-4-ene-3,20-dione [57-83-0]

Progesterone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{21}H_{30}O_2$.

Description Progesterone occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, in ethanol (95), in ethanol (99.5) and in 1,4-dioxane, sparingly soluble in diethyl ether, and practically insoluble in water.

Identification (1) To 0.05 g of progesterone add a solution of 0.05 g of hydroxylammonium chloride and 0.05 g of anhydrous sodium acetate in 5 mL of ethanol (95). Boil for 2 hours under a reflux condenser, evaporate the ethanol to 3 mL, and add 10 mL of water. Filter by suction, and wash the precipitate on the filter with a small amount of water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the dried crystals melt <2.60> between 235°C and 240°C.

(2) Determine the infrared absorption spectrum of Progesterone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Progesterone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Progesterone and Progesterone Reference Standard in ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: +174 – +182° (after drying, 0.2 g, 1,4-dioxane, 10 mL, 100 mm).

Melting point <2.60> 128 – 133°C or 120 – 122°C

Purity Related substances—Dissolve 80 mg of Progesterone in 2 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 10 mg of Progesterone, previously dried, and dissolve in ethanol (99.5) to make exactly 100 mL. To 5 mL of this solution, exactly measured, add ethanol (99.5) to make exactly 50 mL, and determine the absorbance A at the wavelength of maximum absorption at about 241 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of } C_{21}H_{30}O_2 = (A/540) \times 10,000$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Progesterone Injection

プロゲステロン注射液

Progesterone Injection is an oily solution for injec-

tion.

It contains not less than 90% and not more than 110% of the labeled amount of progesterone ($C_{21}H_{30}O_2$: 314.46).

Method of preparation Prepare as directed under Injections, with Progesterone.

Description Progesterone Injection is a clear, colorless to pale yellow, oily liquid.

Identification Transfer a volume of Progesterone Injection, equivalent to 0.02 g of Progesterone according to the labeled amount, to a separator. Add 40 mL of hexane, and mix thoroughly, then extract with three 20-mL portions of diluted ethanol (99.5) (9 in 10). Evaporate the combined extracts on a water bath to dryness. Add 75 mg of 2,4-dinitrophenylhydrazine and 30 mL of ethanol (95) to the residue, and boil for 15 minutes under a reflux condenser. Add 1 mL of hydrochloric acid, and heat for 15 minutes. Cool, and collect the precipitate on a glass filter (G4). Wash the precipitate with five 10-mL portions of hexane and three 5-mL portions of ethanol (95). Then wash with diluted hydrochloric acid (1 in 20) until the washings become colorless, and dry at 105°C for 3 hours: the residue melts <2.60> between 269°C and 275°C.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Progesterone Injection, equivalent to about 50 mg of progesterone ($C_{21}H_{30}O_2$), and dissolve in chloroform to make exactly 100 mL. To exactly measured 3 mL of this solution add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Progesterone Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and prepare the standard solution in the same manner as directed for the preparation of the sample solution. Pipet 5 mL each of the sample solution and standard solution, add exactly measured 10 mL of isoniazid TS and methanol to make exactly 20 mL, respectively. Allow to stand for 45 minutes, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with 5 mL of chloroform in the same manner, as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 380 nm.

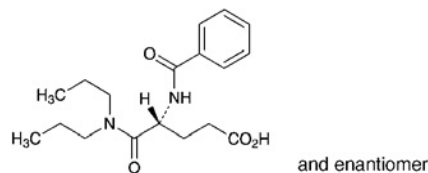
$$\begin{aligned} &\text{Amount (mg) of progesterone } (C_{21}H_{30}O_2) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Progesterone Reference Standard

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Proglumide

プログルミド



$C_{18}H_{26}N_2O_4$: 334.41
(4*RS*)-4-Benzoylamino-*N,N*-dipropylglutaramic acid
[6620-60-6]

Proglumide, when dried, contains not less than 98.5% of $C_{18}H_{26}N_2O_4$.

Description Proglumide occurs as white crystals or crystalline powder.

It is freely soluble in methanol, soluble in ethanol (95), sparingly soluble in diethyl ether, and very slightly soluble in water.

A solution of Proglumide in methanol (1 in 10) shows no optical rotation.

Identification (1) Put 0.5 g of Proglumide in a round bottom tube, add 5 mL of hydrochloric acid, seal the tube, and heat the tube carefully at 120°C for 3 hours. After cooling, open the tube, filter the content to collect crystals separated out, wash the crystals with 50 mL of water, and dry at 100°C for 1 hour: the melting point <2.60> of the crystals is between 121°C and 124°C.

(2) Determine the infrared absorption spectrum of Proglumide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (225 nm): 384 – 414 (after drying, 4 mg, methanol, 250 mL)

Melting point <2.60> 148 – 150°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Proglumide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—To 1.0 g of Proglumide add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10) and 1.5 mL of hydrogen peroxide (30), burn the ethanol, and prepare the test solution according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Proglumide in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of cyclohexane, ethyl

acetate, acetic acid (100) and methanol (50:18:5:4) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.10% (1 g, reduced pressure, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

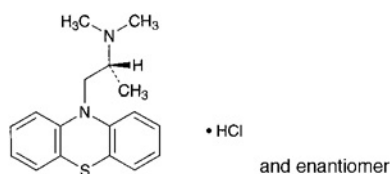
Assay Weigh accurately about 0.16 g of Proglumide, previously dried, dissolve in 40 mL of methanol, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 33.44 \text{ mg of } C_{18}H_{26}N_2O_4 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Promethazine Hydrochloride

プロメタジン塩酸塩



$C_{17}H_{20}N_2S \cdot HCl$: 320.88
(2*RS*)-*N,N*-Dimethyl-1-(10*H*-phenothiazin-10-yl)propan-2-ylamine monohydrochloride [58-33-3]

Promethazine Hydrochloride, when dried, contains not less than 98.0% of $C_{17}H_{20}N_2S \cdot HCl$.

Description Promethazine Hydrochloride occurs as a white to light yellow powder.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

It is gradually colored by light.

A solution of Promethazine Hydrochloride (1 in 25) shows on optical rotation.

Melting point: about 223°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Promethazine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Promethazine Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavenumbers.

(3) Dissolve 0.5 g of Promethazine Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. To 5 mL of the filtrate add dilute nitric acid to make acidic: the solu-

tion responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> The pH of a solution of Promethazine Hydrochloride (1 in 10) is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Promethazine Hydrochloride in 10 mL of water, protecting from direct sunlight: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Promethazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Perform the test under the protection from sunlight. Dissolve 0.10 g of Promethazine Hydrochloride in exactly 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of isopromethazine hydrochloride for thin-layer chromatography in ethanol (95) to make exactly 100 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol and diethylamine (19:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution corresponding to the spots from the standard solution (2) are not more intense than the spot from the standard solution (2), and any spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Promethazine Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

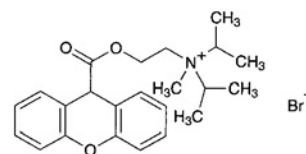
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 32.09 \text{ mg of } C_{17}H_{20}N_2S \cdot HCl \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Proprantheline Bromide

プロパンテリン臭化物



$C_{23}H_{30}BrNO_3$: 448.39
N-Methyl-*N,N*-bis(1-methylethyl)-2-[(9*H*-xanthen-9-ylcarbonyl)oxy]ethylammonium bromide [50-34-0]

Propranolol Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $C_{23}H_{30}BrNO_3$.

Description Propranolol Hydrochloride occurs as a white to yellowish white, crystalline powder. It is odorless and has a very bitter taste.

It is very soluble in water, in ethanol (95), in acetic acid (100) and in chloroform, soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Propranolol Hydrochloride (1 in 50) is between 5.0 and 6.0.

Melting point: about 161°C (with decomposition, after drying).

Identification (1) To 5 mL of a solution of Propranolol Hydrochloride (1 in 20) add 10 mL of sodium hydroxide TS, heat to boil for 2 minutes. Cool to 60°C, and add 5 mL of dilute hydrochloric acid. After cooling, collect the precipitates, and wash with water. Recrystallize from dilute ethanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 217°C and 222°C.

(2) Dissolve 0.01 g of the crystals obtained in (1) in 5 mL of sulfuric acid: a vivid yellow to yellow-red color develops.

(3) To 5 mL of a solution of Propranolol Hydrochloride (1 in 10) add 2 mL of dilute nitric acid: this solution responds to the Qualitative Tests <1.09> (1) for bromide.

Purity Xanthene-9-carboxylic acid and xanthone—Dissolve 10 mg of Propranolol Hydrochloride in exactly 2 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 1.0 mg of xanthene-9-carboxylic acid and 1.0 mg of xanthone in exactly 40 mL of chloroform, and use this solution as the standard solution. Perform the test immediately with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 25 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, and air-dry the plate for 10 minutes. Develop the plate with a mixture of 1,2-dichloroethane, methanol, water and formic acid (56:24:1:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light: the spots from the sample solution corresponding to the spots from the standard solution are not more intense than those from the standard solution.

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

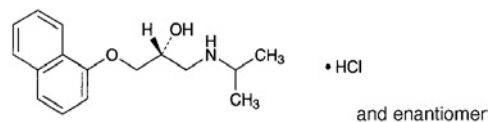
Assay Weigh accurately about 1 g of Propranolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 44.84 \text{ g of } C_{23}H_{30}BrNO_3 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Propranolol Hydrochloride

プロプラノロール塩酸塩



$C_{16}H_{21}NO_2 \cdot HCl$: 295.80

(2*RS*)-1-(1-Methylethyl)amino-3-(naphthalen-1-yloxy)propan-2-ol monohydrochloride [318-98-9]

Propranolol Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of $C_{16}H_{21}NO_2 \cdot HCl$.

Description Propranolol Hydrochloride occurs as a white, crystalline powder.

It is freely soluble in methanol, soluble in water and in acetic acid (100), and sparingly soluble in ethanol (99.5).

A solution of Propranolol Hydrochloride in methanol (1 in 40) shows no optical rotation.

It is gradually colored to yellowish white to light brown by light.

Identification (1) Determine the absorption spectrum of a solution of Propranolol Hydrochloride in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Propranolol Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Propranolol Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> The pH of a solution prepared by dissolving 0.5 g of Propranolol Hydrochloride in 50 mL of water is 5.0–6.0.

Melting point <2.60> 163–166°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Propranolol Hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Propranolol Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Propranolol Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions,

and determine each peak area by the automatic integration method: the area of the peak other than propranolol is not larger than 1/2 times the peak area of propranolol from the standard solution, and the total area of the peaks other than the peak of propranolol is not larger than 2 times the peak area of propranolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.6 g of sodium lauryl sulfate and 0.31 g of tetrabutylammonium phosphate in 450 mL of water, add 1 mL of sulfuric acid and 550 mL of acetonitrile for liquid chromatography, and adjust to pH 3.3 with 2 mol/L sodium hydroxide TS.

Flow rate: Adjust the flow rate so that the retention time of propranolol is about 4 minutes.

Time span of measurement: About 5 times as long as the retention time of propranolol.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add the mobile phase to make exactly 20 mL. Confirm that the peak area of propranolol obtained with 20 μL of this solution is equivalent to 17 to 33% of that with 20 μL of the standard solution.

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of propranolol is not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of propranolol is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Propranolol Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 29.58 mg of C₁₆H₂₁NO₂.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propranolol Hydrochloride Tablets

プロプラノロール塩酸塩錠

Propranolol Hydrochloride Tablets contain not

less than 95.0% and not more than 105.0% of the labeled amount of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl: 295.80).

Method of preparation Prepare as directed under Tablets, with Propranolol Hydrochloride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 288 nm and 292 nm, and between 317 nm and 321 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Propranolol Hydrochloride Tablets add 20 mL of water, and shake until the tablet is completely disintegrated. Add 50 mL of methanol, shake vigorously for 10 minutes, then add methanol to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add methanol to make exactly *V'* mL so that each mL contains about 20 μg of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl), and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, *A_T* and *A_S*, of the sample solution and the standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} & \text{Amount (mg) of propranolol hydrochloride} \\ & \text{(C}_{16}\text{H}_{21}\text{NO}_2\text{.HCl)} \\ & = W_S \times (A_T/A_S) \times (V'/V) \times (1/25) \end{aligned}$$

W_S: Amount (mg) of propranolol hydrochloride for assay

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Propranolol Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μm. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add water to make exactly *V'* mL so that each mL contains about 10 μg of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, *A_T* and *A_S*, of the sample solution and standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of propranolol hydrochloride (C₁₆H₂₁NO₂.HCl)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$

W_S: Amount (mg) of propranolol hydrochloride for assay

C: Labeled amount (mg) of propranolol hydrochloride

(C₁₆H₂₁NO₂·HCl) in 1 tablet

Assay Weigh accurately the mass of not less than 20 Propranolol Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg of propranolol hydrochloride (C₁₆H₂₁NO₂·HCl), add 60 mL of methanol, shake for 10 minutes, and add methanol to make exactly 100 mL. Filter, discard the first 20 mL of the filtrate, pipet 10 mL of the subsequent filtrate, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propranolol hydrochloride for assay, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, *A*_T and *A*_S, of the sample solution and the standard solution at 290 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} & \text{Amount (mg) of propranolol hydrochloride} \\ & \text{(C}_{16}\text{H}_{21}\text{NO}_2\cdot\text{HCl)} \\ & = W_S \times (A_T/A_S) \times (2/5) \end{aligned}$$

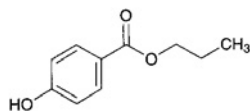
*W*_S: Amount (mg) of propranolol hydrochloride for assay

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propyl Parahydroxybenzoate

パラオキシ安息香酸プロピル



C₁₀H₁₂O₃: 180.20

Propyl 4-hydroxybenzoate [94-13-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Propyl Parahydroxybenzoate contains not less than 98.0% and not more than 102.0% of C₁₀H₁₂O₃.

♦**Description** Propyl Parahydroxybenzoate occurs as colorless crystals or a white, crystalline powder.

It is freely soluble in ethanol (95) and in acetone, and very slightly soluble in water.◆

Identification (1) The melting point <2.60> of Propyl Parahydroxybenzoate is between 96°C and 99°C.

◆(2) Determine the infrared absorption spectrum of Propyl Parahydroxybenzoate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 10 mL of ethanol (95); the solution is clear and not more intensely colored than the following control solution.

Control solution: To 5.0 mL of cobalt (II) chloride colorimetric stock solution, 12.0 mL of iron (III) chloride colorimetric stock solution and 2.0 mL of copper (II) sulfate colorimetric stock solution add water to make 1000 mL.

(2) Acidity—Dissolve 0.20 g of Propyl Parahydroxybenzoate in 5 mL of ethanol (95), add 5 mL of freshly boiled and cooled water and 0.1 mL of bromocresol green-sodium hydroxide-ethanol TS, then add 0.1 mL of 0.1 mol/L sodium hydroxide VS: the solution shows a blue color.

◆(3) Heavy metals <1.07>—Dissolve 1.0 g of Propyl Parahydroxybenzoate in 25 mL of acetone, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid, and water to make 50 mL (not more than 20 ppm).◆

(4) Related substances—Dissolve 0.10 g of Propyl Parahydroxybenzoate in 10 mL of acetone, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, water and acetic acid (100) (70:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot is not more intense than the spot obtained with the standard solution.

Residue on ignition <2.44> Not more than 0.1% (1 g).

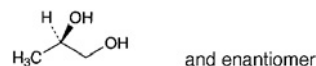
Assay Weigh accurately about 1.0 g of Propyl Parahydroxybenzoate add exactly 20 mL of 1 mol/L VS, heat at about 70°C for 1 hour, and immediately cool in ice. Titrate <2.50> the excess sodium hydroxide with 0.5 mol/L sulfuric acid VS up to the second equivalent point (potentiometric titration). Perform a blank determination.

$$\begin{aligned} & \text{Each mL of 1 mol/L sodium hydroxide VS} \\ & = 180.2 \text{ mg of C}_{10}\text{H}_{12}\text{O}_3 \end{aligned}$$

◆**Containers and storage** Containers—Well-closed containers.◆

Propylene Glycol

プロピレングリコール



C₃H₈O₂: 76.09

(2*RS*)-Propane-1,2-diol [57-55-6]

Description Propylene Glycol is a clear, colorless, viscous liquid. It is odorless, and has a slightly bitter taste.

It is miscible with water, with methanol, with ethanol (95) and with pyridine.

It is freely soluble in diethyl ether.

It is hygroscopic.

Identification (1) Mix 2 to 3 drops of Propylene Glycol with 0.7 g of triphenylchloromethane, add 1 mL of pyridine,

and heat under a reflux condenser on a water bath for 1 hour. After cooling, dissolve the mixture in 20 mL of acetone by warming, shake with 0.02 g of activated charcoal, and filter. Concentrate the filtrate to about 10 mL, and cool. Collect the separated crystals, and dry in a desiccator (silica gel) for 4 hours: the crystals melt <2.60> between 174°C and 178°C.

(2) Heat gently 1 mL of Propylene Glycol with 0.5 g of potassium hydrogen sulfate: a characteristic odor is evolved.

Specific gravity <2.56> d_{20}^{20} : 1.035 – 1.040

Purity (1) **Acidity**—Mix 10.0 mL of Propylene Glycol with 50 mL of freshly boiled and cooled water, and add 5 drops of phenolphthalein TS and 0.30 mL of 0.1 mol/L sodium hydroxide VS: the solution has a red color.

(2) **Chloride** <1.03>—Perform the test with 2.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(3) **Sulfate** <1.14>—Perform the test with 10.0 g of Propylene Glycol. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.002%).

(4) **Heavy metals** <1.07>—Perform the test with 5.0 g of Propylene Glycol according to Method 1. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) **Arsenic** <1.11>—Prepare the test solution with 1.0 g of Propylene Glycol according to Method 1, and perform the test (not more than 2 ppm).

(6) **Glycerin**—Heat 1.0 g of Propylene Glycol with 0.5 g of potassium hydrogen sulfate and evaporate to dryness: no odor of acrolein is perceptible.

Water <2.48> Not more than 0.5% (2 g, direct titration).

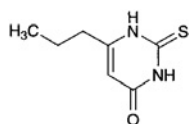
Residue on ignition <2.44> Weigh accurately about 20 g of Propylene Glycol in a tared crucible, and heat to boiling. Stop heating, and immediately ignite to burn. Cool, moisten the residue with 0.2 mL of sulfuric acid, and heat strongly with care to constant mass: the mass of the residue is not more than 0.005%.

Distilling range <2.57> 184 – 189°C, not less than 95 vol%.

Containers and storage Containers—Tight containers.

Propylthiouracil

プロピルチオウラシル



$C_7H_{10}N_2OS$: 170.23

6-Propyl-2-thiouracil [51-52-5]

Propylthiouracil, when dried, contains not less than 98.0% of $C_7H_{10}N_2OS$.

Description Propylthiouracil occurs as a white powder. It is odorless, and has a bitter taste.

It is sparingly soluble in ethanol (95), and very slightly soluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS and in ammonia TS.

Identification (1) Shake well 0.02 g of Propylthiouracil with 7 mL of bromine TS for 1 minute, and heat until the color of bromine TS disappears. Cool, filter, and add 10 mL of barium hydroxide TS to the filtrate: a white precipitate is produced. The color of the precipitate does not turn purple within 1 minute.

(2) To 5 mL of a hot saturated solution of Propylthiouracil add 2 mL of a solution of sodium pentacyanoammine ferrous (II) *n*-hydrate (1 in 100): a green color develops.

Melting point <2.60> 218 – 221°C

Purity (1) **Sulfate** <1.14>—Triturate Propylthiouracil finely in a mortar. To 0.75 g of the powder add 25 mL of water, heat for 10 minutes on a water bath, cool, filter, and wash the residue with water until the volume of the filtrate becomes 30 mL. To 10 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.077%).

(2) **Thiourea**—Dissolve 0.30 g of Propylthiouracil in 50 mL of water by heating under a reflux condenser for 5 minutes, cool, and filter. To 10 mL of the filtrate add 3 mL of ammonia TS, shake well, and add 2 mL of silver nitrate TS: the solution has no more color than the following control solution.

Control solution: Weigh exactly 60 mg of thiourea, and dissolve in water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and proceed with 10 mL of this solution in the same manner.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Propylthiouracil, previously dried, and add 30 mL of water. Add 30 mL of 0.1 mol/L sodium hydroxide VS from a burette, heat to boil, and dissolve by stirring. Wash down the solid adhering to the wall of the flask with a small amount of water, and add 50 mL of 0.1 mol/L silver nitrate VS with stirring. Boil gently for 5 minutes, add 1 to 2 mL of bromothymol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a persistent blue-green color develops. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 8.512 mg of $C_7H_{10}N_2OS$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Propylthiouracil Tablets

プロピルチオウラシル錠

Propylthiouracil Tablets contain not less than 93% and not more than 107% of the labeled amount of propylthiouracil ($C_7H_{10}N_2OS$: 170.23).

Method of preparation Prepare as directed under Tablets,

with Propylthiouracil.

Identification To a quantity of powdered Propylthiouracil Tablets, equivalent to 0.3 g of Propylthiouracil according to the labeled amount, add 5 mL of ammonia TS, allow to stand for 5 minutes with occasional shaking, add 10 mL of water, and centrifuge. To the supernatant liquid add acetic acid (31), collect the precipitate produced, recrystallize from water, and dry at 105°C for 1 hour: it melts <2.60> between 218°C and 221°C. Proceed with the residue as directed in the Identification under Propylthiouracil.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Propylthiouracil Tablets at 75 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the test solution. Take 20 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.8 μm. Discard the first 10 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of propylthiouracil for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 1000 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 274 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Propylthiouracil Tablets in 30 minutes should be not less than 80%.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ with respect to the} \\ & \text{labeled amount of propylthiouracil (C}_7\text{H}_{10}\text{N}_2\text{OS)} \\ & = W_S \times (A_T/A_S) \times (1/C) \times 90 \end{aligned}$$

W_S : Amount (mg) of propylthiouracil for assay.

C : Labeled amount (mg) of propylthiouracil (C₇H₁₀N₂OS) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Propylthiouracil Tablets. Weigh accurately a portion of the powder, equivalent to about 0.3 g of propylthiouracil (C₇H₁₀N₂OS), transfer to a Soxhlet extractor, and extract with 100 mL of acetone for 4 hours. Evaporate the acetone extract by warming on a water bath to dryness. To the residue add 30 mL of water, and proceed as directed in the Assay under Propylthiouracil.

$$\begin{aligned} & \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ & = 8.512 \text{ mg of C}_7\text{H}_{10}\text{N}_2\text{OS} \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protamine Sulfate

プロタミン硫酸塩

Protamine Sulfate is the sulfate of protamine prepared from the mature spermary of fish belonging to

the family *Salmonidae* and others.

Description Protamine Sulfate occurs as a white to light grayish yellow powder.

It is sparingly soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Protamine Sulfate (1 in 100) is between 4.0 and 7.0.

Identification (1) Dissolve 1 mg of Protamine Sulfate in 2 mL of water, add 5 drops of a solution prepared by dissolving 0.1 g of 1-naphthol in 100 mL of diluted ethanol (7 in 10) and 5 drops of sodium hypochlorite TS, then add sodium hydroxide TS until the solution becomes alkaline: a vivid red color develops.

(2) Dissolve 5 mg of Protamine Sulfate in 1 mL of water by warming, add 1 drop of a solution of sodium hydroxide (1 in 10) and 2 drops of copper (II) sulfate TS: a red-purple color develops.

(3) An aqueous solution of Protamine Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Protamine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Nitrogen—Weigh accurately about 10 mg of Protamine Sulfate, previously dried at 105°C to constant mass, and perform the test as directed under Nitrogen Determination <1.08>: not more than 0.255 mg of nitrogen (N: 14.01) is found for each mg of Protamine Sulfate.

Potency as antiheparin (i) Sample solution—Dissolve 20.0 mg of Protamine Sulfate in isotonic sodium chloride solution to make exactly 20 mL.

(ii) Heparin sodium standard solution—Dissolve 10.0 mg of Heparin Sodium Reference Standard in isotonic sodium chloride solution to make a standard solution containing exactly 0.7 mg per mL.

(iii) Sulfated whole blood—Place 250 mL of fresh bovine blood in a wide-mouthed stoppered polyethylene bottle containing 50 mL of a solution of sodium sulfate decahydrate (9 in 50), and store between 1°C and 4°C. Remove any clotted substance before use.

(iv) Thrombokinase extract—To 1.5 g of acetone-dried cattle brain add 60 mL of water, extract at 50°C for 10 to 15 minutes, and centrifuge for 2 minutes at 1500 revolutions per minute. To the supernatant add cresol to make 0.3% as a preservative, and store between 1°C and 4°C. The potency of this solution will be maintained for several days.

(v) Procedure—To one of 10 clean, glass-stoppered test tubes, 13 mm in inside diameter and 150 mm in length, transfer 1.30 mL of isotonic sodium chloride solution and 0.20 mL of thrombokinase extract, then add exactly 1 mL of sulfated whole blood, stopper the tube, mix the contents by inverting once, and note the time on a stop watch. When the solid clot which is formed at the bottom of the tube does not fall on inverting the tube, designate this time as the control clotting time. Adjust appropriately the volume of thrombokinase extract so that the control clotting time is between 2 and 3 minutes. To the nine remaining tubes add 0.50 mL of the sample solution and the same volume of thrombokinase extract as was used in the previous measurement of the control clotting time, pipet into the tubes 0.43 mL, 0.45 mL, 0.47 mL, 0.49 mL, 0.50 mL, 0.51 mL, 0.53 mL, 0.55 mL and 0.57 mL of the heparin sodium standard solution, respectively,

and make the volume in each tube up to 1.50 mL by adding isotonic sodium chloride solution. Add finally 1.0 mL of sulfated whole blood, stopper, mix the contents by inverting once, and determine the clotting times with a stop watch. The estimated ratio, v/V , is between 0.85 and 1.15, where v is the volume of the heparin sodium standard solution and V is the volume of the sample solution in that tube in which the clotting time is most nearly the same as the control clotting time.

Containers and storage Containers—Tight containers.

Protamine Sulfate Injection

プロタミン硫酸塩注射液

Protamine Sulfate Injection is an aqueous solution for injection.

The amount of Protamine Sulfate should be labeled.

Method of preparation Prepare as directed under Injections, with Protamine Sulfate.

Description Protamine Sulfate Injection is a colorless liquid. It is odorless or has the odor of preservatives.

Identification (1) Dilute a volume of Protamine Sulfate Injection, equivalent to 1 mg of Protamine Sulfate according to the labeled amount, with water to make 2 mL, and proceed as directed in the Identification (1) under Protamine Sulfate.

(2) Dilute a volume of Protamine Sulfate Injection, equivalent to 5 mg of Protamine Sulfate according to the labeled amount, with water to make 1 mL, and proceed as directed in the Identification (2) under Protamine Sulfate.

(3) Protamine Sulfate Injection responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> 5.0 – 7.0

Purity Nitrogen—Transfer an exactly measured volume of Protamine Sulfate Injection, equivalent to about 10 mg of Protamine Sulfate according to the labeled amount, to a Kjeldahl flask, and evaporate on a water bath with the aid of a current of air to dryness. Perform the test as directed under Nitrogen Determination <1.08>: 0.225 to 0.255 mg of nitrogen (N: 14.01) is found for each mg of the labeled amount of Protamine Sulfate.

Extractable volume <6.05> It meets the requirement.

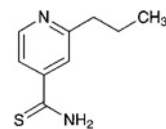
Potency as antiheparin Proceed as directed in the Potency as antiheparin under Protamine Sulfate, but use the following sample solution.

Sample solution: Dilute an exactly measured volume of Protamine Sulfate Injection, equivalent to 20.0 mg of Protamine Sulfate according to the labeled amount, with isotonic sodium chloride solution to make exactly 20 mL.

Containers and storage Containers—Hermetic containers.

Prothionamide

プロチオナミド



$C_9H_{12}N_2S$: 180.27

2-Propylpyridine-4-carbothioamide [14222-60-7]

Prothionamide, when dried, contains not less than 98.0% of $C_9H_{12}N_2S$.

Description Prothionamide occurs as yellow crystals or crystalline powder. It has a slight, characteristic odor.

It is freely soluble in methanol and in acetic acid (100), soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in dilute sulfuric acid.

Identification (1) Mix 0.05 g of Prothionamide with 0.1 g of 1-chloro-2,4-dinitrobenzene, transfer about 10 mg of this mixture to a test tube, and heat for several seconds over a small flame until the mixture is fused. Cool, and add 3 mL of potassium hydroxide-ethanol TS: a red to orange-red color develops.

(2) Place 0.5 g of Prothionamide in a 100-mL beaker, and dissolve in 20 mL of sodium hydroxide TS by heating while shaking occasionally: the gas evolved turns a moistened red litmus paper to blue. Boil gently, and evaporate the solution to 3 to 5 mL. After cooling, add gradually 20 mL of acetic acid (100), and heat on a water bath: the gas evolved darkens moistened lead (II) acetate paper. Evaporate the solution on a water bath to 3 to 5 mL with the aid of a current of air, cool, add 10 mL of water, and mix well. Filter the crystals by suction, recrystallize from water immediately, and dry in a desiccator (in vacuum, silica gel) for 6 hours: the crystals melt <2.60> between 198°C and 203°C (with decomposition).

Melting point <2.60> 142 – 145°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Prothionamide in 20 mL of ethanol (95): the solution is clear, and shows a yellow color.

(2) Acidity—Dissolve 3.0 g of Prothionamide in 20 mL of methanol with warming. Add 100 mL of water to the solution, cool in an ice water bath with agitation, and remove any precipitate by filtration. Allow 80 mL of the filtrate to cool to room temperature, and add 0.8 mL of cresol red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Prothionamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.6 g of Prothionamide according to Method 3, and perform the test. To the test solution add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and ignite to burn (not more

than 3.3 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Prothionamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from orange-red to dark orange-brown (indicator: 2 mL of *p*-naphtholbenzein TS). Perform a blank determination.

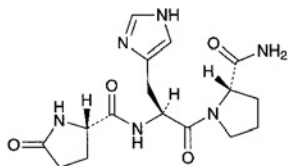
Each mL of 0.1 mol/L perchloric acid VS
= 18.03 mg of C₉H₁₂N₂S

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Protirelin

プロチレリン



C₁₆H₂₂N₆O₄: 362.38

5-Oxo-L-prolyl-L-histidyl-L-prolinamide [24305-27-9]

Protirelin contains not less than 98.5% of C₁₆H₂₂N₆O₄, calculated on the dehydrated basis.

Description Protirelin occurs as a white powder.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100).

It is hygroscopic.

Identification (1) Take 0.01 g of Protirelin in a test tube made of hard glass, add 0.5 mL of 6 mol/L hydrochloric acid TS, seal the upper part of the tube, and heat carefully at 110°C for 5 hours. After cooling, open the seal, transfer the contents into a beaker, and evaporate on a water bath to dryness. Dissolve the residue in 1 mL of water, and use this solution as the sample solution. Separately, dissolve 0.08 g of L-glutamic acid, 0.12 g of L-histidine hydrochloride monohydrate and 0.06 g of L-proline in 20 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry the plate at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the three spots obtained from the sample solution show the same color and the same *R_f* value as each corresponding spots obtained from the standard solution.

(2) Determine the infrared absorption spectrum of Protirelin, previously dried, as directed in the potassium bromide

disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: -66.0 - -69.0° (0.1 g calculated on the dehydrated basis, water, 20 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of Protirelin in 10 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Protirelin in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Protirelin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.20 g of Protirelin in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography, and spot 5 μL of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of 1-butanol, water, pyridine and acetic acid (100) (4:2:1:1) to a distance of about 12 cm, and dry the plates at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plates. Successively spray evenly a solution of sodium carbonate decahydrate (1 in 10) on it: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and heat at 80°C for 5 minutes: no colored spot appears.

Water <2.48> Not more than 5.0% (0.1 g, direct titration).

Residue on ignition <2.44> Not more than 0.3% (0.2 g).

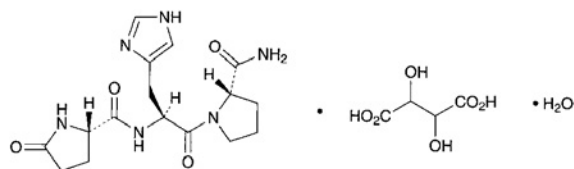
Assay Weigh accurately about 70 mg of Protirelin dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.02 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.02 mol/L perchloric acid VS
= 7.248 mg of C₁₆H₂₂N₆O₄

Containers and storage Containers—Tight containers.

Protirelin Tartrate Hydrate

プロチレリン酒石酸塩水和物



$C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6 \cdot H_2O$: 530.49

5-Oxo-L-prolyl-L-histidyl-L-prolinamide monotartrate monohydrate [24305-27-9, Protirelin]

Protirelin Tartrate Hydrate, calculated on the anhydrous basis, contains not less than 98.5% of protirelin tartrate ($C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6$: 512.48).

Description Protirelin Tartrate Hydrate occurs as white to pale yellowish white crystals or crystalline powder.

It is freely soluble in water, sparingly soluble in acetic acid (100), and practically insoluble in ethanol (95) and in diethyl ether.

Melting point: about 187°C (with decomposition).

Identification (1) To 1 mL of a solution of Protirelin Tartrate Hydrate (1 in 1000) add 2 mL of a solution of 4-nitrobenzene diazonium fluoroborate (1 in 2000) and 2 mL of boric acid-potassium chloride-sodium hydroxide buffer solution, pH 9.0: a red color develops.

(2) Dissolve 0.03 g of Protirelin Tartrate Hydrate in 5 mL of sodium hydroxide TS, add 1 drop of copper (II) sulfate TS: a purple color develops.

(3) To 0.20 g of Protirelin Tartrate Hydrate add 5.0 mL of 6 mol/L hydrochloric acid TS, and boil for 7 hours under a reflux condenser. After cooling, evaporate 2.0 mL of this solution on a water bath to dryness, dissolve the residue in 2.0 mL of water and use this solution as the sample solution. Separately, dissolve 22 mg of L-glutamic acid, 32 mg of L-histidine hydrochloride (monohydrate) and 17 mg of L-proline in 2.0 mL of 0.1 mol/L hydrochloric acid TS by heating, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, acetic acid (100) and pyridine (4:1:1:1) to a distance of about 12 cm, and dry at 100°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and dry at 80°C for 5 minutes: the three spots obtained from the sample solution show, respectively, the same color and the same R_f value as the corresponding spot from the standard solution.

(4) A solution of Protirelin Tartrate Hydrate (1 in 40) responds to the Qualitative Tests <1.09> for tartrate.

Optical rotation <2.49> $[\alpha]_D^{20}$: -50.0 - -53.0° (0.5 g calculated on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Protirelin Tartrate Hydrate in 100 mL of water: the pH of this solution is between 3.0 and 4.0.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Protirelin Tartrate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Protirelin Tartrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Take 1.0 g of Protirelin Tartrate Hydrate in a porcelain crucible. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), ignite the ethanol, and heat gradually to incinerate. If a carbonized material still remains in this method, moisten with a small quantity of nitric acid, and ignite to incinerate. After cooling, add 10 mL of dilute hydrochloric acid, heat on a water bath to dissolve the residue, use this solution as the test solution, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.60 g of Protirelin Tartrate Hydrate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate (1) of silica gel for thin-layer chromatography. Spot 5 μ L of the sample solution on a plate (2) of silica gel for thin-layer chromatography. Develop the plates with a mixture of chloroform, methanol and ammonia solution (28) (6:4:1) to a distance of about 10 cm, and dry at 100°C for 30 minutes. Spray evenly a mixture of a solution of sulfanilic acid in 1 mol/L hydrochloric acid TS (1 in 200) and a solution of sodium nitrite (1 in 20) (1:1) on the plate (1), and air-dry the plate. Then, spray evenly a solution of sodium carbonate decahydrate (1 in 10) on the plate: the spots other than the principal spot from the sample solution are not more intense than those from the standard solution in color. On the other hand, spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate (2), and dry at 80°C for 5 minutes: no colored spot is obtained.

Water <2.48> Not more than 4.5% (0.2 g, direct titration).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

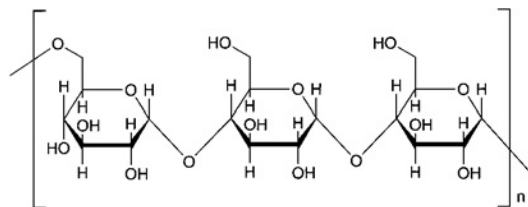
Assay Weigh accurately about 0.5 g of Protirelin Tartrate Hydrate, dissolve in 80 mL of acetic acid (100) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 51.25 mg of $C_{16}H_{22}N_6O_4 \cdot C_4H_6O_6$

Containers and storage Containers—Well-closed containers.

Pullulan

プルラン



$(C_{18}H_{30}O_{15})_n$

Poly[6- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow 4)- α -D-glucopyranosyl-(1 \rightarrow)]
[9057-02-7]

Pullulan is a neutral simple polysaccharide produced by the growth of *Aureobasidium pullulans*. It has a chain structure of repeated α -1,6 binding of maltotriose composed of three glucoses in α -1,4 binding.

Description Pullulan occurs as a white powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification (1) Dissolve 10 g of Pullulan in 100 mL of water with stirring by adding in small portions: a viscous solution is produced.

(2) Mix 10 mL of the viscous solution obtained in (1) with 0.1 mL of pullulanase TS, and allow to stand: the solution loses its viscosity.

(3) To 10 mL of a solution of Pullulan (1 in 50) add 2 mL of macrogol 600: a white precipitate is formed immediately.

Viscosity <2.53> Take exactly 10.0 g of Pullulan, previously dried, dissolve in water to make exactly 100 g, and perform the test at $30 \pm 0.1^\circ\text{C}$ as directed in Method 1: the kinematic viscosity is between 100 and 180 mm^2/s .

pH <2.54> Dissolve 1.0 g of Pullulan in 10 mL of freshly boiled and cooled water: the pH is between 4.5 and 6.5.

Purity (1) Heavy metals <1.07>—Proceed with 4.0 g of Pullulan according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(2) Nitrogen—Weigh accurately about 3 g of Pullulan, previously dried, and perform the test as directed under Nitrogen Determination <1.08>: the amount of nitrogen (N: 14.01) is not more than 0.05%. Use 12 mL of sulfuric acid for the decomposition, and add 40 mL of a solution of sodium hydroxide (2 in 5).

(3) Monosaccharide and oligosaccharides—Dissolve 0.8 g of Pullulan, previously dried, in 100 mL of water, and designate this solution as the sample stock solution. To 1 mL of the sample stock solution add 0.1 mL of potassium chloride saturated solution, and shake vigorously with 3 mL of methanol. Centrifuge, and use the supernatant liquid as the sample solution. Separately, pipet 1 mL of the sample stock solution, add water to make exactly 50 mL, and use this solution as the standard solution. Pipet 0.2 mL each of the sample solution, the standard solution and water, transfer them

gently to each test tube containing 5 mL of a solution of anthrone in diluted sulfuric acid (3 in 4) (1 in 500) and cooling in ice water, stir immediately, then heat at 90°C for 10 minutes, and cool immediately. Perform the test with these solutions so obtained as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as a blank, and determine the absorbances at 620 nm, A_T , A_S and A_B : the amount of monosaccharide and oligosaccharides is not more than 10.0%.

Amount (%) of monosaccharide and oligosaccharides
 $= \{(A_T - A_B)/(A_S - A_B)\} \times 8.2$

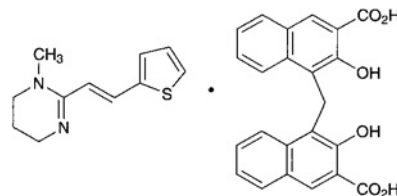
Loss on drying <2.41> Not more than 6.0% (1 g, in vacuum, 90°C , 6 hours).

Residue on ignition <2.44> Not more than 0.3% (2 g).

Containers and storage Containers—Well-closed containers.

Pyrantel Pamoate

ピランテルパモ酸塩



$C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$: 594.68

1-Methyl-2-[(1E)-2-(thien-2-yl)vinyl]-1,4,5,6-tetrahydropyrimidine mono[4,4'-methylenebis(3-hydroxy-2-naphthoate)] (1/1) [22204-24-6]

Pyrantel Pamoate, when dried, contains not less than 98.0% of $C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$.

Description Pyrantel Pamoate occurs as a light yellow to yellow, crystalline powder. It is odorless and tasteless.

It is sparingly soluble in *N,N*-dimethylformamide, very slightly soluble in methanol and in ethanol (95), and practically insoluble in water, in ethyl acetate and in diethyl ether.

Melting point: $256 - 264^\circ\text{C}$ (with decomposition).

Identification (1) To 0.05 g of Pyrantel Pamoate add 10 mL of methanol and 1 mL of a mixture of hydrochloric acid and methanol (1:1), and shake vigorously: a yellow precipitate is produced. Filter the solution, and use the filtrate as the sample solution. Use the precipitate for the test (2). To 0.5 mL of the sample solution add 1 mL of a solution of 2,3-indolinedione in sulfuric acid (1 in 1000): a red color develops.

(2) Collect the precipitate obtained in the test (1), wash with methanol, and dry at 105°C for 1 hour. To 0.01 g of the dried precipitate add 10 mL of methanol, shake well, and filter. To 5 mL of the filtrate add 1 drop of iron (III) chloride TS: a green color develops.

(3) Dissolve 0.1 g of Pyrantel Pamoate in 50 mL of *N,N*-dimethylformamide, and add methanol to make 200 mL. To 2 mL of the solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-

visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Pyrantel Pamoate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Chloride <1.03>—To 1.0 g of Pyrantel Pamoate add 10 mL of dilute nitric acid and 40 mL of water, and heat on a water bath with shaking for 5 minutes. After cooling, add water to make 50 mL, and filter. To 20 mL of the filtrate add 2 mL of dilute nitric acid and water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.036%).

(2) Sulfate <1.14>—To 0.75 g of Pyrantel Pamoate add 5 mL of dilute hydrochloric acid and water to make 100 mL, and heat on a water bath for 5 minutes with shaking. After cooling, add water to make 100 mL, and filter. To 20 mL of the filtrate add water to make 50 mL. Proceed the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.144%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Pyrantel Pamoate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pyrantel Pamoate according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—The procedure should be performed under protection from direct sunlight in light-resistant vessels. Dissolve 0.10 g of Pyrantel Pamoate in 10 mL of *N,N*-dimethylformamide, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the spot of pyrantel and the spot of pamoic acid from the sample solution are not more intense than the spot of pyrantel (*R_f* value: about 0.3) from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.3% (1 g).

Assay Weigh accurately about 0.5 g of Pyrantel Pamoate, previously dried, add 25 mL of chloroform and 25 mL of sodium hydroxide TS, shake for 15 minutes, and extract. Extract further with two 25-mL portions of chloroform. Filter each extract through 5 g of anhydrous sodium sulfate on a pledget of absorbent cotton. Combine the chloroform extracts, add 30 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any

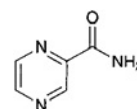
necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 59.47 mg of $C_{11}H_{14}N_2S \cdot C_{23}H_{16}O_6$

Containers and storage Containers—Tight containers.

Pyrazinamide

ピラジナミド



$C_5H_5N_3O$: 123.11

Pyrazine-2-carboxamide [98-96-4]

Pyrazinamide, when dried, contains not less than 99.0% and not more than 101.0% of $C_5H_5N_3O$.

Description Pyrazinamide occurs as white crystals or crystalline powder.

It is sparingly soluble in water and in methanol, and slightly soluble in ethanol (99.5) and in acetic anhydride.

Identification (1) Determine the absorption spectrum of a solution of Pyrazinamide in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrazinamide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 188 – 193°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Pyrazinamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Pyrazinamide in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.1 g of Pyrazinamide,

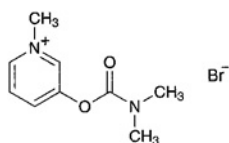
previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.31 mg of C₅H₅N₃O

Containers and storage Containers—Well-closed containers.

Pyridostigmine Bromide

ピロドスチグミン臭化物



C₉H₁₃BrN₂O₂: 261.12

3-Dimethylcarbamoyloxy-1-methylpyridinium bromide
[101-26-8]

Pyridostigmine Bromide, when dried, contains not less than 98.5% of C₉H₁₃BrN₂O₂.

Description Pyridostigmine Bromide occurs as a white, crystalline powder. It is odorless or has a slightly characteristic odor.

It is very soluble in water, freely soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

The pH of a solution of Pyridostigmine Bromide (1 in 10) is between 4.0 and 6.0.

It is deliquescent.

Identification (1) Dissolve 0.02 g of Pyridostigmine Bromide in 10 mL of water, add 5 mL of Reinecke salt TS: a light red precipitate is produced.

(2) To 0.1 g of Pyridostigmine Bromide add 0.6 mL of sodium hydroxide TS: the unpleasant odor of dimethylamine is perceptible.

(3) Determine the absorption spectrum of a solution of Pyridostigmine Bromide in 0.1 mol/L hydrochloric acid TS (1 in 30,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Pyridostigmine Bromide (1 in 50) responds to the Qualitative Tests <1.09> for Bromide.

Melting point <2.60> 153 – 157°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridostigmine Bromide in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pyridostigmine Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Pyridostigmine Bromide according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Pyridostig-

mine Bromide in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add ethanol (95) to make exactly 10 mL. Pipet 1 mL of this solution, add ethanol (95) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and ammonium chloride TS (5:4:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 100°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Pyridostigmine Bromide, previously dried, dissolve in 10 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

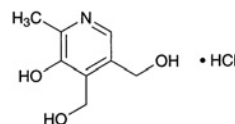
Each mL of 0.1 mol/L perchloric acid VS
= 26.11 mg of C₉H₁₃BrN₂O₂

Containers and storage Containers—Hermetic containers.

Pyridoxine Hydrochloride

Vitamin B₆

ピリドキシン塩酸塩



C₈H₁₁NO₃.HCl: 205.64

4,5-Bis(hydroxymethyl)-2-methylpyridin-3-ol
monohydrochloride [58-56-0]

Pyridoxine Hydrochloride, when dried, contains not less than 98.0% and not more than 101.0% of C₈H₁₁NO₃.HCl.

Description Pyridoxine Hydrochloride occurs as a white to pale yellow, crystalline powder.

It is freely soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

It is gradually affected by light.

Melting point: about 206°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Pyridoxine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a

solution of Pyridoxine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyridoxine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyridoxine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Pyridoxine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Pyridoxine Hydrochloride in 50 mL of water is between 2.5 and 3.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Pyridoxine hydrochloride in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Pyridoxine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 1.0 g of Pyridoxine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 2.5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate, and air-dry: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Pyridoxine Hydrochloride, previously dried, add 5 mL of acetic acid (100) and 5 mL of acetic anhydride, dissolve by gentle boiling, cool, add 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.56 mg of $C_8H_{11}NO_3.HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Pyridoxine Hydrochloride Injection

Vitamin B₆ Injection

ピリドキシン塩酸塩注射液

Pyridoxine Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of pyridoxine hydrochloride ($C_8H_{11}NO_3.HCl$: 205.64).

Method of preparation Prepare as directed under Injections, with Pyridoxine Hydrochloride.

Description Pyridoxine Hydrochloride Injection is a colorless or pale yellow, clear liquid.

It is gradually affected by light.

pH: 3.0 – 6.0

Identification (1) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.05 g of Pyridoxine Hydrochloride according to the labeled amount, add 0.1 mol/L hydrochloric acid TS to make 100 mL. To 2 mL of this solution add 0.1 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible spectrophotometry <2.24>: it exhibits a maximum between 288 nm and 292 nm.

(2) To a volume of Pyridoxine Hydrochloride Injection, equivalent to 0.01 g of Pyridoxine Hydrochloride according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.01 g of Pyridoxine Hydrochloride Reference Standard in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, and air-dry the plate. Develop the plate with a mixture of acetone, tetrahydrofuran, hexane and ammonia solution (28) (65:13:13:9) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of sodium carbonate in diluted ethanol (99.5) (3 in 10) (1 in 20) on the plate. After air-drying, spray evenly a solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (99.5) (1 in 1000) on the plate: the spots obtained from the sample solution and the standard solution are blue in color and have the same *R_f* value.

Bacterial endotoxins <4.01> Less than 3.0 EU/mg.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Pyridoxine Hydrochloride Injection, equivalent to about 20 mg of pyridoxine hydrochloride ($C_8H_{11}NO_3.HCl$), dilute with water, if necessary, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add water to make exactly 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Pyridoxine Hydrochloride Reference Standard, previously dried in a desiccator (in vacuum, silica gel) for 4 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 1

mL each of the sample solution and standard solution, add 2.0 mL of barbital buffer solution, 9.0 mL of 2-propanol and 2.0 mL of a freshly prepared solution of 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000), shake well, add 2-propanol to make exactly 25 mL, and allow to stand for 90 minutes. Determine the absorbances, A_T and A_S , of the subsequent sample solution and subsequent standard solution, respectively, at 650 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared in the same manner with 1 mL of water, as the blank.

$$\begin{aligned} &\text{Amount (mg) of pyridoxine hydrochloride} \\ &(\text{C}_8\text{N}_{11}\text{NO}_3\cdot\text{HCl}) \\ &= W_S \times (A_T/A_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Pyridoxine Hydrochloride Reference Standard

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Pyroxylin

ピロキシリン

Pyroxylin is a nitric acid ester of cellulose. It is usually moistened with 2-propanol or some other solvent.

Description Pyroxylin occurs as a white cotton-like substance or white flakes.

It is freely soluble in acetone, and very slightly soluble in diethyl ether.

Upon heating or exposure to light, it is decomposed with the evolution of nitrous acid vapors.

Identification Ignite Pyroxylin: it burns very rapidly with a luminous flame.

Purity (1) Clarity of solution—Dissolve 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, in 25 mL of a mixture of diethyl ether and ethanol (95) (3:1): the solution is clear.

(2) Acidity—Shake 1.0 g of Pyroxylin, previously dried at 80°C for 2 hours, with 20 mL of water for 10 minutes: the filtrate is neutral.

(3) Water-soluble substances—Evaporate 10 mL of the filtrate obtained in (2) on a water bath to dryness, and dry at 105°C for 1 hour: the mass of the residue is not more than 1.5 mg.

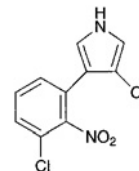
(4) Residue on ignition—Weigh accurately about 2 g of Pyroxylin, previously dried at 80°C for 2 hours, and moisten with 10 mL of a solution of castor oil in acetone (1 in 20) to gelatinize the sample. Ignite the contents to carbonize the sample, heat strongly at about 500°C for 2 hours, and allow to cool over silica gel: the amount of the residue is not more than 0.30%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, packed loosely, remote from fire, and preferably in a cold place.

Pyrrolnitrin

ピロールニトリン



$\text{C}_{10}\text{H}_6\text{Cl}_2\text{N}_2\text{O}_2$: 257.07
3-Chloro-4-(3-chloro-2-nitrophenyl)pyrrole [1018-71-9]

Pyrrolnitrin contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Pyrrolnitrin is expressed as mass (potency) of pyrrolnitrin ($\text{C}_{10}\text{H}_6\text{Cl}_2\text{N}_2\text{O}_2$).

Description Pyrrolnitrin occurs as yellow to yellow-brown, crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Pyrrolnitrin in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Pyrrolnitrin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Pyrrolnitrin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Pyrrolnitrin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 124 – 128°C

Purity Related substances—Dissolve 0.10 g of Pyrrolnitrin in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 3 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of xylene, ethyl acetate and formic acid (18:2:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly diluted sulfuric acid (1 in 3) on the plate, and heat at 100°C for 30 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure using light-resistant vessels.

Weigh accurately an amount of Pyrrolnitrin and Pyrrolnitrin Reference Standard, equivalent to about 50 mg (potency), dissolve in diluted acetonitrile (3 in 5) to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL of the internal standard solution, add diluted acetonitrile (3 in 5) to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of pyrrolnitrin to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{10}\text{H}_6\text{Cl}_2\text{N}_2\text{O}_2 \\ &= W_S \times (Q_T / Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Pyrrolnitrin Reference Standard

Internal standard solution—A solution of benzyl benzoate in diluted acetonitrile (3 in 5) (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of pyrrolnitrin is about 9 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, pyrrolnitrin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

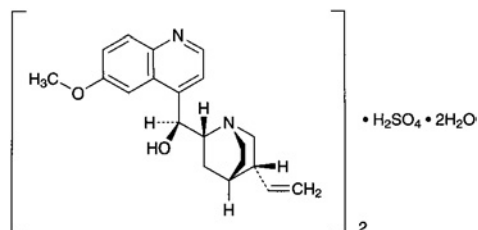
System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of pyrrolnitrin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Quinidine Sulfate Hydrate

キニジン硫酸塩水和物



$(\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2)_2 \cdot \text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$: 782.94
(9*S*)-6'-Methoxycinchonan-9-ol hemisulfate monohydrate [6591-63-5]

Quinidine Sulfate Hydrate, when dried, contains not

less than 98.5% of quinidine sulfate $[(\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2)_2 \cdot \text{H}_2\text{SO}_4 \cdot 746.91]$.

Description Quinidine Sulfate Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is freely soluble in ethanol (95) and in boiling water, sparingly soluble in water, and practically insoluble in diethyl ether. Quinidine Sulfate Hydrate, previously dried, is freely soluble in chloroform.

It darkens gradually by light.

Optical rotation $[\alpha]_D^{20}$: +275 – +287° (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

Identification (1) Dissolve 0.01 g of Quinidine Sulfate Hydrate in 10 mL of water and 2 to 3 drops of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 1000) add 1 to 2 drops of bromine TS, then add 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of an aqueous solution of Quinidine Sulfate Hydrate (1 in 100) add 1 mL of silver nitrate TS, stir with a glass rod, and allow to stand for a short interval: a white precipitate is produced, and it dissolves on addition of nitric acid.

(4) Dissolve 0.4 g of Quinidine Sulfate Hydrate in 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Quinidine Sulfate Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity (1) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinidine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at about 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

(2) Related substances—Dissolve 20 mg of Quinidine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate their amount by the area percentage method: the amount of dihydroquinidine sulfate is not more than 15.0%, and those of quinidine sulfate and dihydroquinine sulfate are not more than 1.0%. The total area of the peaks other than the principal peak and the above peaks is not larger than the peak area of cinchonine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilylated silica gel (10 μ m in particle diameter).

Temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methane-

sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinidine is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Quinidine Sulfate Hydrate and quinine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with a resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonine obtained from 50 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinidine beginning after the solvent peak.

(3) Readily carbonizable substances <1.15>—Take 0.20 g of Quinidine Sulfate Hydrate and perform the test: the solution has no more color than Matching fluid M.

Loss on drying <2.41> Not more than 5.0% (1 g, 130 °C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Quinidine Sulfate Hydrate, previously dried, dissolve in 20 mL of acetic acid (100), and add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

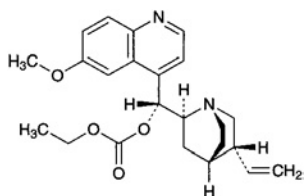
Each mL of 0.1 mol/L perchloric acid VS
= 24.90 mg of (C₂₀H₂₄N₂O₂)₂·H₂SO₄

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Quinine Ethyl Carbonate

キニーネエチル炭酸エステル



C₂₃H₂₈N₂O₄: 396.48

Ethyl (8*S*,9*R*)-6'-methoxycinchonan-9-yl carbonate
[83-75-0]

Quinine Ethyl Carbonate contains not less than 98.5% of C₂₃H₂₈N₂O₄, calculated on the dehydrated basis.

Description Quinine Ethyl Carbonate occurs as white crystals. It is odorless, and tasteless at first but slowly develops a bitter taste.

It is very soluble in methanol, freely soluble in ethanol (95) and in ethanol (99.5), soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid.

Identification (1) Determine the absorption spectrum of a solution of Quinine Ethyl Carbonate in methanol (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Ethyl Carbonate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -42.2 - -44.0° (0.5 g, calculated on the dehydrated basis, methanol, 50 mL, 100 mm).

Melting point <2.60> 91 - 95°C

Purity (1) Chloride <1.03>—Dissolve 0.30 g of Quinine Ethyl Carbonate in 10 mL of dilute nitric acid and 20 mL of water. To 5 mL of the solution add 2 to 3 drops of silver nitrate TS: no color develops.

(2) Sulfate <1.14>—Dissolve 1.0 g of Quinine Ethyl Carbonate in 5 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS, 5 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Ethyl Carbonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Related substances—Dissolve 20 mg of Quinine Ethyl Carbonate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of quinine sulfate in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method, and calculate the amount of a main impurity in the sample solution which appears at about 1.2 times of the retention time of quinine ethyl carbonate by the area percentage method: it is not more than 10.0%. The total peak area other than the principal peak and above mentioned peak from the sample solution is not larger than the peak area of Quinine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.2 g of sodium 1-octanesulfonate in 1000 mL of a mixture of water and methanol (1:1), and ad-

just to pH 3.5 with diluted phosphoric acid (1 in 20).

Flow rate: Adjust the flow rate so that the retention time of the peak of quinine ethyl carbonate is about 20 minutes.

Selection of column: Dissolve 5 mg each of Quinine Ethyl Carbonate and quinine sulfate in the mobile phase to make 50 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quinine, dihydroquinine, quinine ethyl carbonate and the main impurity of quinine ethyl carbonate in this order with the resolution between the peaks of quinine and dihydroquinine being not less than 2.7, and between the peaks of quinine and quinine ethyl carbonate being not less than 5.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of quinine obtained from 10 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About 2 times as long as the retention time of quinine ethyl carbonate.

Water <2.48> Not more than 3.0% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

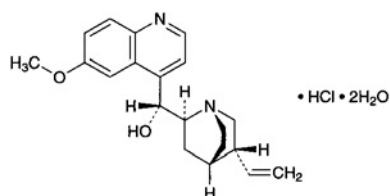
Assay Weigh accurately about 0.3 g of Quinine Ethyl Carbonate, dissolve in 60 mL of acetic acid (100), add 2 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 19.82 \text{ mg of } C_{23}H_{28}N_2O_4 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Quinine Hydrochloride Hydrate

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$C_{20}H_{24}N_2O_2 \cdot HCl \cdot 2H_2O$: 396.91
(8*S*,9*R*)-6'-Methoxycinchonan-9-ol monohydrochloride dihydrate [6119-47-7]

Quinine Hydrochloride Hydrate, when dried, contains not less than 98.5% of quinine hydrochloride ($C_{20}H_{24}N_2O_2 \cdot HCl$: 360.88).

Description Quinine Hydrochloride Hydrate occurs as white crystals. It is odorless, and has a very bitter taste.

It is very soluble in ethanol (99.5), freely soluble in acetic acid (100), in acetic anhydride and in ethanol (95), soluble in water, and practically insoluble in diethyl ether. Quinine Hydrochloride Hydrate, previously dried, is freely soluble in chloroform.

It gradually changes to brown by light.

Identification (1) A solution of Quinine Hydrochloride Hydrate (1 in 50) shows no fluorescence. To 1 mL of the solu-

tion add 100 mL of water and 1 drop of dilute sulfuric acid: a blue fluorescence is produced.

(2) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 1000) add 1 to 2 drops of bromine TS and 1 mL of ammonia TS: a green color develops.

(3) To 5 mL of a solution of Quinine Hydrochloride Hydrate (1 in 50) add 1 mL of dilute nitric acid and 1 mL of silver nitrate TS: a white precipitate is produced. Collect the precipitate, and add an excess of ammonia TS: it dissolves.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-245 - -255^\circ$ (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Quinine Hydrochloride Hydrate in 100 mL of freshly boiled and cooled water: the pH of this solution is between 6.0 and 7.0.

Purity (1) Sulfate <1.14>—Perform the test with 1.0 g of Quinine Hydrochloride Hydrate. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Barium—Dissolve 0.5 g of Quinine hydrochloride Hydrate in 10 mL of water by warming, and add 1 mL of dilute sulfuric acid: no turbidity is produced.

(3) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinine Hydrochloride Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue so obtained is not more than 2.0 mg.

(4) Related substances—Dissolve 20 mg of Quinine Hydrochloride Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonidine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of the sample solution by the automatic integration method, and calculate the amount of dihydroquinine hydrochloride by the area percentage method: it is not more than 10.0%. The total area of the peaks other than the main peak and the above peaks is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of water, acetonitrile, methanesulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

Selection of column: Dissolve 10 mg each of Quinine Hydrochloride and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μ L of this solution under the above operating conditions. Use

a column giving elution of quinidine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine, and that between quinine and dihydroquinidine being not less than 1.2, respectively.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine from 50 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinine beginning after the solvent peak.

(5) Readily carbonizable substances <1.15>—Perform the test with 0.25 g of Quinine Hydrochloride Hydrate. The solution has no more color than Matching Fluid M.

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 5 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Quinine Hydrochloride Hydrate, previously dried, dissolve in 100 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

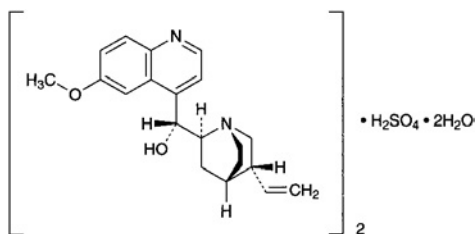
$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 18.04 \text{ mg of } C_{20}H_{24}N_2O_2 \cdot HCl \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Quinine Sulfate Hydrate

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$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4 \cdot 2H_2O$: 782.94
(8*S*,9*R*)-6'-Methoxycinchonon-9-ol hemisulfate monohydrate [6119-70-6]

Quinine Sulfate Hydrate contains not less than 98.5% of quinine sulfate [$(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$: 746.91], calculated on the dried basis.

Description Quinine Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless, and has a very bitter taste.

It is freely soluble in acetic acid (100), slightly soluble in water, in ethanol (95), in ethanol (99.5) and in chloroform, and practically insoluble in diethyl ether.

It gradually changes to brown by light.

Identification (1) Determine the absorption spectrum of a solution of Quinine Sulfate Hydrate (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both

spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Quinine Sulfate Hydrate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 0.4 g of Quinine Sulfate Hydrate add 20 mL of water and 1 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-235 - -245^\circ$ (after drying, 0.5 g, 0.1 mol/L hydrochloric acid VS, 25 mL, 100 mm).

pH <2.54> Shake 2.0 g of Quinine Sulfate Hydrate in 20 mL of freshly boiled and cooled water, and filter: the pH of this filtrate is between 5.5 and 7.0.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Quinine Sulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Chloroform-ethanol-insoluble substances—Warm 2.0 g of Quinine Sulfate Hydrate with 15 mL of a mixture of chloroform and ethanol (99.5) (2:1) at 50°C for 10 minutes. After cooling, filter through a tared glass filter (G4) by gentle suction. Wash the residue with five 10-mL portions of a mixture of chloroform and ethanol (99.5) (2:1), dry at 105°C for 1 hour, and weigh: the mass of the residue is not more than 2.0 mg.

(3) Related substances—Dissolve 20 mg of Quinine Sulfate Hydrate in the mobile phase to make exactly 100 mL, and use this solution as the sample solution. Separately, dissolve 25 mg of cinchonidine in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from the sample solution by the automatic integration method, and calculate the amount of dihydroquinine sulfate by the area percentage method: it is not more than 5%. The total area of the peaks other than the main peak and the above peak is not larger than the peak area of cinchonidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 235 nm).

Column: A column about 4 mm in inside diameter and about 25 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Temperature: Room temperature

Mobile phase: A mixture of water, acetonitrile, methane sulfonic acid TS and a solution of diethylamine (1 in 10) (43:5:1:1).

Flow rate: Adjust the flow rate so that the retention time of quinine is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Quinine Sulfate Hydrate and quinidine sulfate in 5 mL of methanol, and add the mobile phase to make 50 mL. Proceed with 50 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of quini-

dine, quinine, dihydroquinidine and dihydroquinine in this order with the resolution between quinidine and quinine and that between quinine and dihydroquinidine being not less than 1.2.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of cinchonidine obtained from 50 μ L of the standard solution is between 5 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of quinine beginning after the solvent peak.

Loss on drying <2.41> 3.0% – 5.0% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Quinine Sulfate Hydrate, dissolve in 20 mL of acetic acid (100), add 80 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.90 mg of $(C_{20}H_{24}N_2O_2)_2 \cdot H_2SO_4$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Freeze-dried Inactivated Tissue Culture Rabies Vaccine

乾燥組織培養不活化狂犬病ワクチン

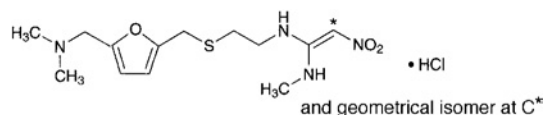
Freeze-dried Inactivated Tissue Culture Rabies Vaccine is a dried preparation containing inactivated rabies virus.

It conforms to the requirements of Freeze-dried Inactivated Tissue Culture Rabies Vaccine in the Minimum Requirements of Biologic Products.

Description Freeze-dried Inactivated Tissue Culture Rabies Vaccine becomes a colorless or light yellow-red clear liquid on addition of solvent.

Ranitidine Hydrochloride

ラニチジン塩酸塩



$C_{13}H_{22}N_4O_3S \cdot HCl$: 350.86

(1*EZ*)-*N*-{2-[(5-[(Dimethylamino)methyl]furan-2-yl)methyl]sulfanyl]ethyl}-*N*'-methyl-2-nitroethene-1,1-diamine monohydrochloride [66357-59-3]

Ranitidine Hydrochloride, when dried, contains not less than 97.5% and not more than 102.0% of $C_{13}H_{22}N_4O_3S \cdot HCl$.

Description Ranitidine Hydrochloride occurs as a white to pale yellow, crystalline or fine granular powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

It is gradually colored by light.

Melting point: about 140°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Ranitidine Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ranitidine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ranitidine Hydrochloride as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Ranitidine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ranitidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Ranitidine Hydrochloride in 100 mL of water is between 4.5 and 6.0.

Purity (1) Clarity and color of solution—A solution of Ranitidine Hydrochloride (1 in 10) is clear and pale yellow to light yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ranitidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ranitidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(4) Related substances—Conduct this procedure without exposure to light, using light-resistant vessels. Dissolve 0.22 g of Ranitidine Hydrochloride in methanol to make exactly 10 mL, and use this solution as the sample solution. Pipet 0.5 mL of the sample solution, add methanol to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 6 mL, 4 mL, 2 mL and 1 mL of the standard solution (1), add to each methanol to make exactly 10 mL, and use these solutions as the standard solution (2), the standard solution (3), the standard solution (4) and the standard solution (5), respectively. Separately, dissolve 12.7 mg of ranitidinediamine in methanol to make exactly 10 mL, and use this solution as the standard solution (6). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2), (3), (4) and (5) on a plate of silica gel for thin-layer chromatography. Separately, spot 10 μ L of the sample solution on the plate, then spot 10 μ L of the standard solution (6) on the spotted position of the sample solution. Immediately develop the plate with a mixture of ethyl acetate, 2-propanol, ammonia solution (28) and water (25:15:5:1) to a distance of about 15 cm, and air-dry the plate. Allow the plate to stand in iodine vapor until the spot from the standard solution (5) appears: the spot obtained from the standard so-

lution (6) is completely separated from the principal spot from the sample solution. The spot having *Rf* value of about 0.7 from the sample solution is not more intense than the spot from the standard solution (1), the spots other than the principal spot and the spot of *Rf* 0.7 from the sample solution are not more intense than the spot from the standard solution (2), and the total amount of these related substances, calculated by comparison with the spots from the standard solutions (1), (2), (3), (4) and (5), is not more than 1.0%.

Loss on drying <2.41> Not more than 0.75% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 20 mg of Ranitidine Hydrochloride and Ranitidine Hydrochloride Reference Standard, previously dried, dissolve each in the mobile phase to make exactly 200 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 10 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of ranitidine.

$$\text{Amount (mg) of } C_{13}H_{22}N_4O_3S \cdot HCl = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Ranitidine Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 322 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 20 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and diluted 0.5 mol/L ammonium acetate TS (1 in 5) (17:3).

Flow rate: Adjust the flow rate so that the retention time of ranitidine is about 5 minutes.

System suitability—

System performance: Dissolve 20 mg of Ranitidine Hydrochloride and 5 mg of benzaldehyde in 200 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, benzaldehyde and ranitidine are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ranitidine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Rape Seed Oil

Oleum Rapae

ナタネ油

Rape Seed Oil is the fixed oil obtained from the seed

of *Brassica campestris* Linné subsp. *napus* Hooker fil. et Anderson var. *nippo-oleifera* Makino (*Cruciferae*).

Description Rape Seed Oil is a clear, pale yellow, slightly viscous oil. It is odorless or has a slight odor and a mild taste.

It is miscible with diethyl ether and with petroleum diethyl ether. It is slightly soluble in ethanol (95).

Specific gravity d_{25}^{25} : 0.906 – 0.920

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 169 – 195

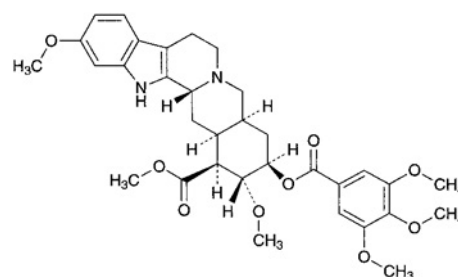
Unsaponifiable matters <1.13> Not more than 1.5%.

Iodine value <1.13> 95 – 127

Containers and storage Containers—Tight containers.

Reserpine

レセルピン



$C_{33}H_{40}N_2O_9$: 608.68

Methyl (3*S*,16*S*,17*R*,18*R*,20*R*)-11,17-dimethoxy-18-(3,4,5-trimethoxybenzoyloxy)yohimban-16-carboxylate [50-55-5]

Reserpine, when dried, contains not less than 96.0% of $C_{33}H_{40}N_2O_9$.

Description Reserpine occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in acetic acid (100) and in chloroform, slightly soluble in acetonitrile, very slightly soluble in ethanol (95), and practically insoluble in water and in diethyl ether.

It is affected by light.

Identification (1) To 1 mg of Reserpine add 1 mL of vanillin-hydrochloric acid TS, and warm: a vivid red-purple color develops.

(2) Determine the absorption spectrum of a solution of Reserpine in acetonitrile (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Reserpine Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Reserpine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Reserpine Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-114 - -127^\circ$ (after drying, 0.25 g, chloroform, 25 mL, 100 mm).

Purity Related substances—Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve 50 mg of Reserpine in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area from these solutions by the automatic integration method: the total area of all peaks other than reserpine peak from the sample solution is not larger than the peak area of reserpine from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (13:7).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 20 minutes.

Time span of measurement: About twice as long as the retention time of reserpine.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add acetonitrile to make exactly 50 mL. Confirm that the peak area of reserpine obtained from 10 μ L of this solution is equivalent to 3 to 5% of that of reserpine obtained from 10 μ L of the standard solution.

System performance: Dissolve 0.01 g of Reserpine and 4 mg of butyl parahydroxybenzoate in 100 mL of acetonitrile. To 5 mL of this solution add acetonitrile to make 50 mL. When the procedure is run with 20 μ L of this solution according to the operating conditions in the Assay, reserpine and butyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of reserpine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (0.2 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.2 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 10 mg each of Reserpine and Reserpine Reference Standard, previously dried, and dissolve each in acetonitrile to make exactly 100 mL. Pipet 5 mL each of these solutions, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of reserpine to that of the internal standard.

$$\text{Amount (mg) of } C_{33}H_{40}N_2O_9 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Reserpine Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate, pH 3.0 and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of reserpine is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, reserpine and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of reserpine to that of the internal standard is not more than 2.0%.

Containers and Storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Injection

レセルピン注射液

Reserpine Injection is an aqueous solution for injection. It contains not less than 90% and not more than 110% of the labeled amount of reserpine ($C_{33}H_{40}N_2O_9$: 608.68).

Method of preparation Prepare as directed under Injections, with Reserpine.

Description Reserpine Injection is a clear, colorless or pale yellow liquid.

pH: 2.5 – 4.0

Identification Measure a volume of Reserpine Injection, equivalent to 1.5 mg of Reserpine according to the labeled amount, add 10 mL of diethyl ether, shake for 10 minutes, and take the aqueous layer. If necessary, add 10 mL of diethyl ether to the aqueous layer, and shake for 10 minutes to repeat the process. To the aqueous layer add water to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 265 nm and 269 nm.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Reserpine Injection, equivalent to about 4 mg of reserpine ($C_{33}H_{40}N_2O_9$). Separately, weigh accurately about 4 mg of Reserpine Reference Standard, previously dried in vacuum at 60°C for 3

hours. Transfer them to separate separator, add 10 mL each of water and 5 mL each of ammonia TS, and extract with one 20-mL portion of chloroform, then with three 10-mL portions of chloroform with shaking vigorously. Combine the chloroform extracts, wash with two 50-mL portions of diluted hydrochloric acid (1 in 1000), and combine the washings. Then wash the chloroform extract with two 50-mL portions of a solution of sodium hydrogen carbonate (1 in 100), and combine the all washings. Extract the combined washing with two 10-mL portions of chloroform, and combine the washings with the former chloroform extract. Transfer the chloroform solution to a 100-mL volumetric flask through a pledget of absorbent cotton previously wetted with chloroform, wash with a small amount of chloroform, dilute with chloroform to make 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution, respectively, at 295 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of reserpine (C}_{33}\text{H}_{40}\text{N}_2\text{O}_9) \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Reserpine Reference Standard

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

0.1% Reserpine Powder

Reserpine Powder

レセルピン散 0.1%

0.1% Reserpine Powder contains not less than 0.09% and not more than 0.11% of reserpine ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$; 608.68).

Method of preparation

Reserpine	1 g
Lactose Hydrate	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Identification To 0.4 g of 0.1% Reserpine Powder add 20 mL of acetonitrile, shake for 30 minutes, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately a quantity of 0.1% Reserpine Powder, equivalent to about 0.5 mg of reserpine ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$), disperse in 12 mL of water, add exactly 10 mL of the internal standard solution and 10 mL of acetonitrile, and dissolve by warming at 50°C for 15 minutes, then add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Reserpine Reference Standard, previously dried at 60°C in

vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and standard solution as directed in the Assay under Reserpine.

$$\begin{aligned} \text{Amount (mg) of reserpine (C}_{33}\text{H}_{40}\text{N}_2\text{O}_9) \\ = W_S \times (Q_T/Q_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Reserpine Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Reserpine Tablets

レセルピン錠

Reserpine Tablets contain not less than 90% and not more than 110% of the labeled amount of reserpine ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$; 608.68).

Method of preparation Prepare as directed under Tablets, with Reserpine.

Identification Take a portion of powdered Reserpine Tablets, equivalent to 0.4 mg of Reserpine according to the labeled amount, add 20 mL of acetonitrile, shake for 30 minute, and centrifuge. Determine the absorption spectrum of the supernatant liquid as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 265 nm and 269 nm, and between 294 nm and 298 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Conduct this procedure without exposure to daylight, using light-resistant vessels. To one tablet of Reserpine Tablets add 2 mL of water, disintegrate by warming at 50°C for 15 minutes while shaking. After cooling, add exactly 2 mL of the internal standard solution per 0.1 mg of reserpine ($\text{C}_{33}\text{H}_{40}\text{N}_2\text{O}_9$) according to the labeled amount, add 2 mL of acetonitrile, warm at 50°C for 15 minutes while shaking, and after cooling, add water to make 10 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine Reference Standard, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and standard solution as directed in the Assay under Reserpine.

$$\begin{aligned} \text{Amount (mg) of reserpine (C}_{33}\text{H}_{40}\text{N}_2\text{O}_9) \\ = W_S \times (Q_T/Q_S) \times (C/10) \end{aligned}$$

W_S : Amount (mg) of Reserpine Reference Standard

C : Labeled amount (mg) of reserpine in each tablet.

Internal standard solution—A solution of butyl parahydroxy-

ybenzoate in acetonitrile (1 in 50,000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Take 1 tablet of Reserpine Tablets, and perform the test at 100 revolutions per minute with 500 mL of a solution of polysorbate 80 (1 in 20,000) in diluted dilute acetic acid (1 in 200) as the test solution according to the Paddle method. Take 20 mL or more of the dissolved solution 30 minutes after starting the dissolution test, filter through a filter laminated with polyester fibers, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Reserpine Reference Standard at 60°C in vacuum for 3 hours, weigh accurately an amount 100 times the labeled amount, dissolve in 1 mL of chloroform and 80 mL of ethanol (95), and add a solution of polysorbate 80 in diluted dilute acetic acid (1 in 200) (1 in 20,000) to make exactly 200 mL. Pipet 1 mL of this solution, add a solution of polysorbate 80 in diluted dilute acetic acid (1 in 200) (1 in 20,000) to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, transfer to glass-stoppered brown test tubes T and S, respectively, add exactly 5 mL each of ethanol (99.5), shake well, add exactly 1 mL each of diluted vanadium (V) oxide (1 in 2), shake vigorously, and allow to stand for 30 minutes. Perform the test with these solutions as directed under Fluorometry <2.22>, and determine the intensity of fluorescence, F_T and F_S , at the wavelength of excitation at 400 nm and at the wavelength of fluorescence at 500 nm. Dissolution rate of Reserpine Tablets after 30 minutes should be not less than 70%.

$$\begin{aligned} & \text{Dissolution rate (\%)} \text{ to the labeled} \\ & \text{amount of reserpine (C}_{33}\text{H}_{40}\text{N}_2\text{O}_9) \\ & = W_S \times (F_T/F_S) \times (1/C) \end{aligned}$$

W_S : Amount (mg) of Reserpine Reference Standard.

C: Labeled amount (mg) of reserpine (C₃₃H₄₀N₂O₉) in each tablet.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately and powder not less than 20 Reserpine Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.5 mg of reserpine (C₃₃H₄₀N₂O₉), add 3 mL of water, and warm at 50°C for 15 minutes while shaking. After cooling, add exactly 10 mL of the internal standard solution, 10 mL of acetonitrile and warm at 50°C for 15 minutes while shaking. After cooling, add water to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of Reserpine Reference Standard, previously dried at 60°C in vacuum for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and standard solution as directed in Assay under Reserpine.

$$\begin{aligned} & \text{Amount (mg) of reserpine (C}_{33}\text{H}_{40}\text{NO}_9) \\ & = W_S \times (Q_T/Q_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Reserpine Reference Standard

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

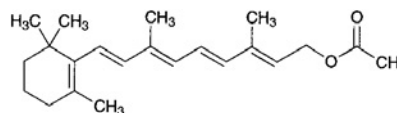
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Retinol Acetate

Vitamin A Acetate

レチノール酢酸エステル



C₂₂H₃₂O₂: 328.49

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl acetate [I27-47-9]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil.

It contains not less than 2,500,000 Vitamin A Units per gram.

A suitable antioxidant may be added.

Retinol Acetate contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

It is freely soluble in petroleum ether, soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Acetate and Retinol Acetate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and R_f value with the blue spot from the standard solution.

Purity (1) Acid value <1.13>—Take exactly 5.0 g of Retinol Acetate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Acetate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate <2.50> this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

Amount (meq/kg) of peroxide = $(V/W) \times 10$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS consumed

W: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <2.55>.

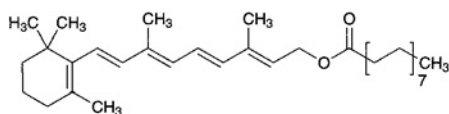
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Retinol Palmitate

Vitamin A Palmitate

レチノールパルミチン酸エステル



$C_{36}H_{60}O_2$: 524.86

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl palmitate [79-81-2]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil, and contains not less than 1,500,000 Vitamin A Units in each gram.

It may contain a suitable antioxidant.

Retinol Palmitate contains not less than 95.0% and not more than 105.0% of the labeled Units.

Description Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

It is very soluble in petroleum ether, slightly soluble in ethanol (95), and practically insoluble in water.

It is decomposed by air and by light.

Identification Dissolve Retinol Palmitate and Retinol Palmitate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution is the same in color tone and *R_f* value with the blue spot from the standard solution.

Purity (1) Acid value <1.13>—Take exactly 5.0 g of Retinol Palmitate, and perform the test: not more than 2.0.

(2) Peroxide—Weigh accurately about 5 g of Retinol Palmitate, transfer in a 250-mL glass-stoppered conical flask, add 50 mL of a mixture of acetic acid (100) and isooctane (3:2), and gently mix to dissolve completely. Replace the air of the inside gradually with about 600 mL of Nitrogen, then add 0.1 mL of saturated potassium iodide TS under a current

of Nitrogen. Immediately stopper tightly, and mix with a swirling motion for 1 minute. Add 30 mL of water, stopper tightly, and shake vigorously for 5 to 10 seconds. Titrate <2.50> this solution with 0.01 mol/L sodium thiosulfate VS until the blue color of the solution disappears after addition of 0.5 mL of starch TS near the end point where the solution is a pale yellow color. Calculate the amount of peroxide by the following formula: not more than 10 meq/kg.

Amount (meq/kg) of peroxide = $(V/W) \times 10$

V: Volume (mL) of 0.01 mol/L sodium thiosulfate VS

W: Amount (g) of the sample

Assay Proceed as directed in Method 1-1 under the Vitamin A Assay <2.55>.

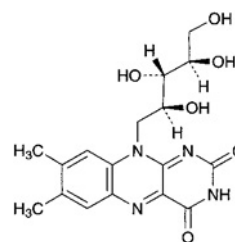
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere, and in a cold place.

Riboflavin

Vitamin B₂

リポフラビン



$C_{17}H_{20}N_4O_6$: 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione [83-88-5]

Riboflavin, when dried, contains not less than 98.0% of $C_{17}H_{20}N_4O_6$.

Description Riboflavin occurs as yellow to orange-yellow crystals. It has a slight odor.

It is very slightly soluble in water, practically insoluble in ethanol (95), in acetic acid (100), and in diethyl ether.

It dissolves in sodium hydroxide TS.

A saturated solution of Riboflavin is neutral.

It is decomposed by light.

Melting point: about 290°C (with decomposition).

Identification (1) A solution of Riboflavin (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31),

and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin in phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Riboflavin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-128 - -142^\circ$ Weigh accurately about 0.1 g of dried Riboflavin, dissolve in exactly 4 mL of dilute sodium hydroxide TS, add 10 mL of freshly boiled and cooled water, add exactly 4 mL of aldehyde-free alcohol while shaking, add freshly boiled and cooled water to make exactly 20 mL, and determine the rotation in a 100-mm cell within 30 minutes after preparing the solution.

Purity Lumiflavin—Shake 25 mg of Riboflavin with 10 mL of ethanol-free chloroform for 5 minutes, and filter: the filtrate has no more color than the following control solution.

Control solution: To 2.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

Loss on drying <2.41> Not more than 1.5% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 15 mg of Riboflavin, previously dried, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the sample solution. Dry Riboflavin Reference Standard at 105°C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_S , at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, $A_{T'}$ and $A_{S'}$, of the solutions.

$$\begin{aligned} & \text{Amount (mg) of } C_{17}H_{20}N_4O_6 \\ & = W_S \times \{(A_T - A_{T'}) / (A_S - A_{S'})\} \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Powder

Vitamin B₂ Powder

リボフラビン散

Riboflavin Powder contains not less than 95% and not more than 115% of the labeled amount of riboflavin ($C_{17}H_{20}N_4O_6$; 376.36).

Method of preparation Prepare as directed under Powders,

with Riboflavin.

Identification Shake a portion of Riboflavin Powder, equivalent to 1 mg of Riboflavin according to the labeled amount, with 100 mL of water, filter, and proceed with the filtrate as directed in the Identification (1) and (2) under Riboflavin.

Purity Rancidity—Riboflavin Powder is free from any unpleasant or rancid odor or taste.

Assay The procedure should be performed under protection from direct sunlight and in light-resistant vessels. Weigh accurately Riboflavin Powder equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add 800 mL of diluted acetic acid (100) (1 in 400), and extract by warming for 30 minutes with occasional shaking. Cool, dilute with water to make exactly 1000 mL, and filter through a glass filter (G4). Use this filtrate as the sample solution, and proceed as directed in the Assay under Riboflavin.

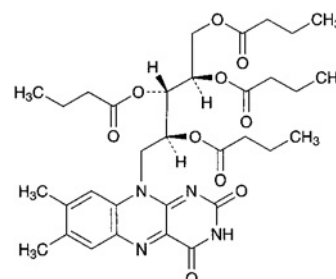
$$\begin{aligned} & \text{Amount (mg) of riboflavin } (C_{17}H_{20}N_4O_6) \\ & = W_S \times \{(A_T - A_{T'}) / (A_S - A_{S'})\} \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Butyrate

リボフラビン酪酸エステル



$C_{33}H_{44}N_4O_{10}$: 656.72
(2*R*,3*S*,4*S*)-5-(7,8-Dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)pentan-1,2,3,4-tetraol tetrabutanoate [752-56-7]

Riboflavin Butyrate, when dried, contains not less than 98.5% of $C_{33}H_{44}N_4O_{10}$.

Description Riboflavin Butyrate occurs as orange-yellow crystals or crystalline powder. It has a slight, characteristic odor and a slightly bitter taste.

It is freely soluble in methanol, in ethanol (95) and in chloroform, slightly soluble in diethyl ether, and practically insoluble in water.

It is decomposed by light.

Identification (1) A solution of Riboflavin Butyrate in ethanol (95) (1 in 100,000) shows a light yellow-green color with a strong yellowish green fluorescence. To the solution add dilute hydrochloric acid or sodium hydroxide TS: the fluorescence disappears.

(2) Dissolve 0.01 g of Riboflavin Butyrate in 5 mL of

ethanol (95), add 2 mL of a mixture of a solution of hydroxylammonium chloride (3 in 20) and a solution of sodium hydroxide (3 in 20) (1:1), and shake well. To this solution add 0.8 mL of hydrochloric acid and 0.5 mL of iron (III) chloride TS, and add 8 mL of ethanol (95): a deep red-brown color develops.

(3) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 146 – 150°C

Purity (1) Chloride—Dissolve 2.0 g of Riboflavin Butyrate in 10 mL of methanol, and add 24 mL of dilute nitric acid and water to make 100 mL. After shaking well, allow to stand for 10 minutes, filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 25 mL of the sample solution add water to make 50 mL, then add 1 mL of silver nitrate TS, and allow to stand for 5 minutes: the turbidity of the solution is not thicker than that of the following control solution.

Control solution: To 25 mL of the sample solution add 1 mL of silver nitrate TS, allow to stand for 10 minutes, and filter. Wash the precipitate with four 5-mL portions of water, and combine the washings with the filtrate. To this solution add 0.30 mL of 0.01 mol/L hydrochloric acid VS and water to make 50 mL, add 1 mL of water, and mix (not more than 0.021%).

(2) Heavy metals <1.07>—Proceed with 2.0 g of Riboflavin Butyrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Free acid—To 1.0 g of Riboflavin Butyrate add 50 mL of freshly boiled and cooled water, shake, and filter. To 25 mL of the filtrate add 0.50 mL of 0.01 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution shows a red color.

(4) Related substances—Dissolve 0.10 g of Riboflavin Butyrate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add chloroform to make exactly 50 mL. Pipet 5 mL of this solution, add chloroform to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and 2-propanol (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 40 mg of Riboflavin Butyrate, previously dried, dissolve in ethanol (95) to make exactly 500 mL, and pipet 10 mL of this solution, add ethanol (95) to make exactly 50 mL, and use

this solution as the sample solution. Separately, weigh accurately about 50 mg of Riboflavin Reference Standard, previously dried at 105°C for 2 hours, dissolve in 150 mL of diluted acetic acid (100) (2 in 75) by warming, and after cooling, add water to make exactly 500 mL. Pipet 5 mL of this solution, add ethanol (95) to make exactly 50 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 445 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{33}H_{44}N_4O_{10} \\ = W_S \times (A_T/A_S) \times 1.7449 \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

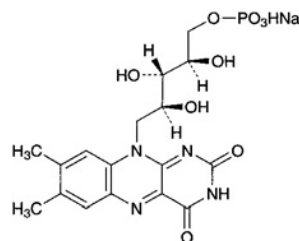
Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate

Riboflavin Phosphate

Vitamin B₂ Phosphate Ester

リボフラビンリン酸エステルナトリウム



$C_{17}H_{20}N_4NaO_9P$: 478.33

Monosodium (2*R*,3*S*,4*S*)-5-(7,8-dimethyl-2,4-dioxo-3,4-dihydrobenzo[*g*]pteridin-10(2*H*)-yl)-2,3,4-trihydroxypentyl monohydrogenphosphate [130-40-5]

Riboflavin Sodium Phosphate contains not less than 92% of $C_{17}H_{20}N_4NaO_9P$, calculated on the anhydrous basis.

Description Riboflavin Sodium Phosphate is a yellow to orange-yellow, crystalline powder. It is odorless, and has a slightly bitter taste.

It is soluble in water, and practically insoluble in ethanol (95), in chloroform and in diethyl ether.

It is decomposed on exposure to light.

It is very hygroscopic.

Identification (1) A solution of Riboflavin Sodium Phosphate (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin Sodium Phosphate (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30

minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin Sodium Phosphate in phosphate buffer solution, pH 7.0, (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) To 0.05 g of Riboflavin Sodium Phosphate add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite. Boil the residue with 10 mL of nitric acid (1 in 50) for 5 minutes, after cooling, neutralize this solution with ammonia TS, and filter, if necessary: the solution responds to the Qualitative Tests <1.09> for sodium salt and phosphate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +38 – +43° (0.3 g, calculated on the anhydrous basis, 5 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of Riboflavin Sodium Phosphate in 20 mL of water: the pH of the solution is between 5.0 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.20 g of Riboflavin Sodium Phosphate in 10 mL of water: the solution is clear and yellow to orange-yellow in color.

(2) Lumiflavin—To 35 mg of Riboflavin Sodium Phosphate add 10 mL of ethanol-free chloroform, and shake for 5 minutes, then filter: the filtrate has no more color than the control solution.

Control solution: To 3.0 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(3) Free phosphoric acid—Weigh accurately about 0.4 g of Riboflavin Sodium Phosphate, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Measure exactly 5 mL each of the sample solution and Phosphoric Acid Standard Solution, transfer to separate 25-mL volumetric flasks, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS to each of these flasks, mix, and add water to make 25 mL. Allow to stand for 30 minutes at 20 ± 1°C, and perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 5 mL of water in the same manner as a blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard phosphoric acid solution at 740 nm: the free phosphoric acid content is not more than 1.5%.

$$\begin{aligned} &\text{Content (\% of free phosphoric acid (H}_3\text{PO}_4\text{))} \\ &= (A_T/A_S) \times (1/W) \times 257.8 \end{aligned}$$

W : Amount (mg) of Riboflavin Sodium Phosphate calculated on the anhydrous basis.

Water <2.48> Place 25 mL of a mixture of methanol for Karl Fischer method and ethylene glycol for Karl Fischer method (1:1) in a dry flask for titration, and titrate with water determination TS to the end point. Weigh accurately about 0.1 g of Riboflavin Sodium Phosphate, place quickly into the flask, add a known excess volume of Karl Fischer TS, mix for 10 minutes, and perform the test: the water content is not more than 10.0%.

Assay Conduct this procedure without exposure to day-

light, using light-resistant vessels. To about 0.1 g of Riboflavin Sodium Phosphate, accurately weighed, dissolve in diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, then pipet 10 mL of this solution, and add diluted acetic acid (100) (1 in 500) to make exactly 50 mL. Use this solution as the sample solution. Separately, dry Riboflavin Reference Standard at 105°C for 2 hours, weigh accurately about 15 mg, dissolve in 800 mL of diluted acetic acid (100) (1 in 400) by warming, cool, add water to make exactly 1000 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and determine the absorbances, A_T and A_S , at 445 nm. Add 0.02 g of sodium hydrosulfite to 5 mL of each solution, shake until decolorized, and immediately measure the absorbances, A_T' and A_S' , of the solutions.

$$\begin{aligned} &\text{Amount (mg) of C}_{17}\text{H}_{20}\text{N}_4\text{NaO}_9\text{P} \\ &= W_S \times \{(A_T - A_T') / (A_S - A_S')\} \times 1.2709 \times 5 \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Riboflavin Sodium Phosphate Injection

Riboflavin Phosphate Injection

Vitamin B₂ Phosphate Ester Injection

リボフラビンリン酸エステルナトリウム注射液

Riboflavin Sodium Phosphate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 120% of the labeled amount of riboflavin (C₁₇H₂₀N₄O₆: 376.36).

The concentration of Riboflavin Sodium Phosphate Injection should be stated as the amount of riboflavin (C₁₇H₂₀N₄O₆).

Method of preparation Prepare as directed under Injections, with Riboflavin Sodium Phosphate.

Description Riboflavin Sodium Phosphate Injection is a clear, yellow to orange-yellow liquid.

pH: 5.0 – 7.0

Identification (1) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 1 mg of Riboflavin according to the labeled amount, add water to make 100 mL, and proceed with this solution as directed in the Identification (1) and (2) under Riboflavin Sodium Phosphate.

(2) To a measured volume of Riboflavin Sodium Phosphate Injection, equivalent to 0.05 g of Riboflavin according to the labeled amount, and evaporate on a water bath to dryness. Proceed with this residue as directed in the Identification (4) under Riboflavin Sodium Phosphate.

Extractable volume <6.05> It meets the requirement.

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. To an accurately measured

volume of Riboflavin Sodium Phosphate Injection, equivalent to about 15 mg of riboflavin ($C_{17}H_{20}N_4O_6$), add diluted acetic acid (100) (1 in 500) to make exactly 1000 mL, and use this solution as the sample solution. Proceed as directed in the Assay under Riboflavin Sodium Phosphate.

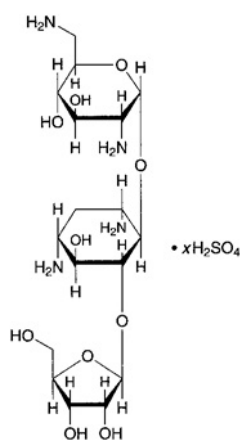
$$\begin{aligned} &\text{Amount (mg) of Riboflavin (C}_{17}\text{H}_{20}\text{N}_4\text{O}_6\text{)} \\ &= W_S \times \{(A_T - A_T') / (A_S - A_S')\} \end{aligned}$$

W_S : Amount (mg) of Riboflavin Reference Standard

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Ribostamycin Sulfate

リボスタマイシン硫酸塩



$C_{17}H_{34}N_4O_{10} \cdot xH_2SO_4$

2,6-Diamino-2,6-dideoxy- α -D-glucopyranosyl-(1 \rightarrow 4)-
[β -D-ribofuranosyl-(1 \rightarrow 5)]-2-deoxy-D-streptamine sulfate
[53797-35-6]

Ribostamycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces ribosidificus*.

It contains not less than 680 μ g (potency) and not more than 780 μ g (potency) per mg, calculated on the dried basis. The potency of Ribostamycin Sulfate is expressed as mass (potency) of ribostamycin ($C_{17}H_{34}N_4O_{10}$: 454.47).

Description Ribostamycin Sulfate occurs as a white to yellowish white powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) Dissolve 20 mg of Ribostamycin Sulfate in 2 mL of phosphate buffer solution, pH 6.0, add 1 mL of ninhydrin TS, and boil: a blue-purple color develops.

(2) Dissolve 0.12 g each of Ribostamycin Sulfate and Ribostamycin Sulfate Reference Standard in 20 mL of water, and use these solutions as the sample solution and standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly

0.2% ninhydrin-water saturated 1-butanol TS, and heat at 100°C for 10 minutes: the principal spots obtained from the sample solution and standard solution show a purple-brown color and the same R_f value.

(3) To 2 mL of a solution of Ribostamycin Sulfate (1 in 5) add 1 drop of barium chloride TS: a white turbidity is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +42 – +49° (after drying, 0.25 g, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Ribostamycin Sulfate in 20 mL of water is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Ribostamycin Sulfate in 5 mL of water: the solution is clear, and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ribostamycin Sulfate according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.12 g of Ribostamycin Sulfate in water to make exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (3 in 40) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 10 minutes: the spot other than the principal spot obtained from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under <4.02> Microbial Assay for Antibiotics according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Ribostamycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5 to 15°C and use within 20 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μ g (potency) and 5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

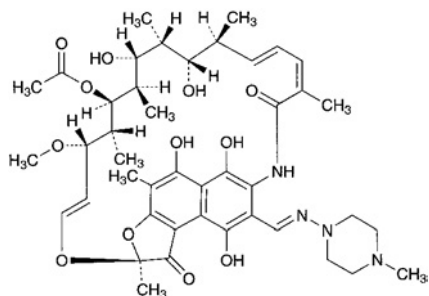
(iv) Sample solutions—Weigh accurately an amount of Ribostamycin Sulfate, equivalent to about 20 mg (potency),

and dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 20 μg (potency) and 5 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Rifampicin

リファンピシン



$\text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12}$: 822.94
(2*S*,12*Z*,14*E*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*,24*E*)-5,6,9,17,19-Pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1-yliminomethyl)-1,11-dioxo-1,2-dihydro-2,7-(epoxypentadeca[1,11,13]trienimino)naphtho[2,1-*b*]furan-21-yl acetate [13292-46-1]

Rifampicin is a derivative of a substance having antibacterial activity produced by the growth of *Streptomyces mediterranei*.

It contains not less than 970 μg (potency) and not more than 1020 μg (potency) per mg, calculated on the dried basis. The potency of Rifampicin is expressed as mass (potency) of rifampicin ($\text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12}$).

Description Rifampicin occurs as orange-red to red-brown, crystals or crystalline powder.

It is slightly soluble in water, in acetonitrile, in methanol and in ethanol (95).

Identification (1) To 5 mL of a solution of Rifampicin in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rifampicin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rifampicin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Rifampicin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Rifampicin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead

Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Rifampicin according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Perform the test immediately after preparing of the sample and standard solutions. Dissolve 0.10 g of Rifampicin in 50 mL of acetonitrile, and use this solution as the sample stock solution. Pipet 5 mL of the sample stock solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the sample solution. Separately, pipet 1 mL of the sample stock solution, and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add citric acid-phosphate-acetonitrile TS to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak appeared at the relative retention time of about 0.7 with respect to rifampicin from the sample solution is not more than 1.5 times the peak area of rifampicin from the standard solution, the area of the peak other than rifampicin and the peak mentioned above from the sample solution is not more than the peak area of rifampicin from the standard solution, and the total area of the peaks other than rifampicin and the peak mentioned above from the sample solution is not more than 3.5 times the peak area of rifampicin from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of rifampicin beginning after the peak of the solvent.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add citric acid-phosphate-acetonitrile TS to make exactly 20 mL. Confirm that the peak area of rifampicin obtained from 50 μL of this solution is equivalent to 7 to 13% of that from 50 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Rifampicin and Rifampicin Reference Standard, equivalent to about 40 mg (potency), and dissolve each in acetonitrile to make exactly 200 mL. Pipet 10 mL each of these solutions, add citric acid-phosphate-acetonitrile TS to make exactly 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of rifampicin.

Amount [μg (potency)] of $\text{C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12}$

$$= W_S \times (A_T/A_S) \times 1000$$

W_S : Amount [mg (potency)] of Rifampicin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 10 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 4.2 g of citric acid monohydrate and 1.4 g of sodium perchlorate in 1000 mL of a mixture of water, acetonitrile and phosphate buffer solution, pH 3.1 (11:7:2).

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 8 minutes.

System suitability—

System performance: To 5 mL of a solution of Rifampicin in acetonitrile (1 in 5000) add 1 mL of a solution of butyl parahydroxybenzoate in acetonitrile (1 in 5000) and citric acid-phosphate-acetonitrile TS to make 50 mL. When the procedure is run with 50 μ L of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Rifampicin Capsules

リファンピシカプセル

Rifampicin Capsules contain not less than 93.0% and not more than 105.0% of rifampicin ($C_{43}H_{58}N_4O_{12}$; 822.94).

Method of preparation Prepare as directed under Capsules, with Rifampicin.

Identification Dissolve an amount of the content of Rifampicin Capsules, equivalent to 20 mg (potency) of Rifampicin according to the labeled amount, in methanol to make 100 mL, and filter. To 5 mL of the filtrate add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 234 nm and 238 nm, between 252 nm and 256 nm, between 331 nm and 335 nm, and between 472 nm and 476 nm.

Purity Related substances—Perform the test quickly after the sample solution and the standard solution are prepared. Open the capsules of not less than 20 Rifampicin Capsules, carefully take out the content, weigh accurately, and powder. Weigh accurately a portion of the powder, equivalent to about 20 mg (potency) of Rifampicin according to the labeled amount, and dissolve in acetonitrile to make exactly 10 mL.

Pipet 2 mL of this solution, add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Rifampicin Reference Standard, and dissolve in acetonitrile to make exactly 10 mL. Pipet 2 mL of this solution, and add the mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Pipet 1 mL of this solution, add the mixture of acetonitrile and methanol (1:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the amount of the peaks of quinone substance and *N*-oxide substance, having the relative retention time of about 0.5 and about 1.2 with respect to rifampicin, respectively, are not more than 4.0% and not more than 1.5%, respectively. The amount of the peak other than the peaks mentioned above is not more than 1.0%, and the total amount of these related substances is not more than 2.0%. For these calculations, use the areas of the peaks of the quinone substance and *N*-oxide substance after multiplying by their relative response factors, 1.24 and 1.16, respectively.

$$\begin{aligned} &\text{Amount (mg) of quinone substance} \\ &= (W_S/W_T) \times (A_{Tq}/A_S) \times 2.48 \end{aligned}$$

$$\begin{aligned} &\text{Amount (mg) of } N\text{-oxide substance} \\ &= (W_S/W_T) \times (A_{To}/A_S) \times 2.32 \end{aligned}$$

Each amount (mg) of related substances other than quinone and *N*-oxide substances = $(W_S/W_T) \times (A_{Ti}/A_S) \times 2$

W_S : Amount [mg (potency)] of Rifampicin Reference Standard

W_T : Amount [mg (potency)] of sample

A_S : Peak area of the standard solution

A_{Tq} : Peak area of quinone substance

A_{To} : Peak area of *N*-oxide substance

A_{Ti} : Each peak area of related substances other than quinone and *N*-oxide substances

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 2.1 g of sodium perchlorate, 6.5 g of citric acid monohydrate and 2.3 g of potassium dihydrogen phosphate in 1100 mL of water, and add 900 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rifampicin is about 12 minutes.

Time span of measurement: About 2.5 times as long as the retention time of rifampicin.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add a mixture of acetonitrile and methanol (1:1) to make exactly 20 mL. Confirm that the peak area of rifampicin obtained with 20 μ L of this solution is equivalent to 3.5 to 6.5% of that with 20 μ L of the standard solution.

System performance: When the procedure is run with 20

μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of rifampicin is not less than 2500 and not more than 4.0, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rifampicin is not more than 2.0%.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Assay Open the capsules of not less than 20 Rifampicin Capsules, take out the content, weigh accurately the mass of the content, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg (potency) of Rifampicin, dissolve in a mixture of acetonitrile and methanol (1:1) to make exactly 50 mL. Pipet 10 mL of this solution, and add acetonitrile to make exactly 50 mL. Pipet 5 mL of this solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg (potency) of Rifampicin Reference Standard, dissolve in 20 mL of a mixture of acetonitrile and methanol (1:1), and add acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add the solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of the mixture of water and acetonitrile (3:1) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of rifampicin.

$$\begin{aligned} &\text{Amount [mg (potency)] of rifampicin (C}_{43}\text{H}_{58}\text{N}_4\text{O}_{12}) \\ &= W_S \times (A_T/A_S) \times (5/2) \end{aligned}$$

W_S : Amount [mg (potency)] of Rifampicin Reference Standard

Operating conditions—

Proceed as directed in the operating conditions in the Assay under Rifampicin.

System suitability—

System performance: Dissolve 30 mg (potency) of Rifampicin Reference Standard in 20 mL of the mixture of acetonitrile and methanol (1:1), and add acetonitrile to make 100 mL. To 5 mL of this solution add 2 mL of a solution of butyl parahydroxybenzoate in the mixture of acetonitrile and methanol (1:1) (1 in 5000), then add the solution prepared by dissolving 2.1 g of citric acid monohydrate, 27.6 g of disodium hydrogen phosphate dodecahydrate and 3.1 g of potassium dihydrogen phosphate in 1000 mL of a mixture of water and acetonitrile (3:1) to make exactly 50 mL. When the procedure is run with 50 μL of this solution under the above operating conditions, butyl parahydroxybenzoate and rifampicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 5 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of

rifampicin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Ringer's Solution

リンゲル液

Ringer's Solution is an aqueous solution for injection.

It contains not less than 0.53 w/v% and not more than 0.58 w/v% of chlorine [as (Cl: 35.45)], and not less than 0.030 w/v% and not more than 0.036 w/v% of calcium chloride hydrate (CaCl₂·2H₂O: 147.01).

Method of preparation

Sodium Chloride	8.6 g
Potassium Chloride	0.3 g
Calcium Chloride Hydrate	0.33 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Ringer's Solution is a clear and colorless liquid. It has a slightly saline taste.

Identification (1) Evaporate 10 mL of Ringer's Solution to 5 mL: the solution responds to the Qualitative Tests <1.09> for potassium salt and calcium salt.

(2) Ringer's Solution responds to the Qualitative Tests <1.09> for sodium salt and chloride.

pH <2.54> 5.0 – 7.5

Purity (1) Heavy metals <1.07>—Evaporate 100 mL of Ringer's Solution to about 40 mL on a water bath. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Control solution: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic <1.11>—Perform the test with 20 mL of Ringer's Solution as the test solution (not more than 0.1 ppm).

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay (1) Chlorine—To 20 mL of Ringer's Solution, accurately measured, add 30 mL of water. Titrate <2.50> with 0.1 mol/L silver nitrate VS while shaking vigorously (indicator: 3 drops of sodium fluorescein TS).

$$\begin{aligned} &\text{Each mL of 0.1 mol/L silver nitrate VS} \\ &= 3.545 \text{ mg of Cl} \end{aligned}$$

(2) Calcium chloride Hydrate—To 50 mL of Ringer's Solution, exactly measured, add 2 mL of 8 mol/L potassium hydroxide TS and 0.05 g of NN indicator, and titrate <2.50> immediately with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS, until the color of the solution changes from red-purple to blue.

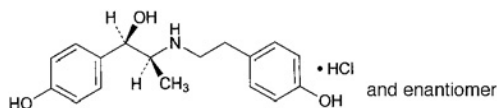
Each mL of 0.01 mol/L disodium dihydrogen

ethylenediamine tetraacetate VS
= 1.470 mg of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous infusions may be used.

Ritodrine Hydrochloride

リトドリン塩酸塩



$\text{C}_{17}\text{H}_{21}\text{NO}_3 \cdot \text{HCl}$: 323.81
(1*RS*,2*SR*)-1-(4-Hydroxyphenyl)-2-
{[2-(4-hydroxyphenyl)ethyl]amino} propan-1-ol
monohydrochloride
[23239-51-2]

Ritodrine Hydrochloride, when dried, contains not less than 98.0% and not more than 102.0% of $\text{C}_{17}\text{H}_{21}\text{NO}_3 \cdot \text{HCl}$.

Description Ritodrine Hydrochloride occurs as a white crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of Ritodrine Hydrochloride (1 in 10) shows no optical rotation.

Melting point: about 196°C (with decomposition).

It is gradually colored to a light yellow by light.

Identification (1) Determine the absorption spectrum of a solution of Ritodrine Hydrochloride (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Ritodrine Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Ritodrine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ritodrine Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Ritodrine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 50 mL of water is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Ritodrine Hydrochloride in 10 mL of water is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Ritodrine Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 20 mg of Ritodrine

Hydrochloride in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak of ritodrine threo-isomer, having the relative retention time of about 1.2 with respect to ritodrine, is not larger than 4/5 times the peak area of ritodrine from the standard solution, the area of the peak other than ritodrine and ritodrine threo-isomer is not larger than 3/10 times the peak area of ritodrine from the standard solution, and the total area of the peaks other than ritodrine and ritodrine threo-isomer is not larger than 4 times the peak area of ritodrine from the standard solution.

Operating conditions—

Column, column temperature, and mobile phase: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 10 minutes.

Time span of measurement: About 3 times as long as the retention time of ritodrine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add exactly 50 mL of the mobile phase. Confirm that the peak area of ritodrine obtained with 10 μL of this solution is equivalent to 7 to 13% of that with 10 μL of the standard solution.

System performance: To about 20 mg of ritodrine hydrochloride add 50 mL of the mobile phase and 5.6 mL of sulfuric acid, and add the mobile phase to make 100 mL. Heat a portion of this solution at about 85°C for about 2 hours, and allow to cool. Pipet 10 mL of this solution, and add exactly 10 mL of 2 mol/L sodium hydroxide TS. When the procedure is run with 10 μL of this solution under the above operating conditions, ritodrine and the threo-isomer are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Ritodrine Hydrochloride and Ritodrine Hydrochloride Reference Standard, previously dried, and dissolve in methanol to make exactly 50 mL. Pipet 25 mL of these solutions, add exactly 5 mL of the internal standard solution, then add water to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ritodrine to that of the internal standard.

Amount (mg) of $\text{C}_{17}\text{H}_{21}\text{NO}_3 \cdot \text{HCl}$

$$= W_S \times (Q_T / Q_S)$$

W_S : Amount (mg) of Ritodrine Hydrochloride Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ritodrine Hydrochloride Tablets

リトドリン塩酸塩錠

Ritodrine Hydrochloride Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of ritodrine hydrochloride ($C_{17}H_{21}NO_3 \cdot HCl$; 323.81).

Method of preparation Prepare as directed under Tablets, with Ritodrine Hydrochloride.

Identification To 10 mL of the filtrate obtained in the Assay add 0.01 mol/L hydrochloric acid TS to make 100 mL. Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 272 nm and 276 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Ritodrine Hydrochloride Tablets add 9 mL of 0.01 mol/L hydrochloric acid TS, shake until the tablet is completely disintegrated, add 0.01 mol/L hydrochloric acid TS to make exactly 10 mL, and filter through a membrane filter having pore size of 0.45 μ m. Pipet 3 mL of the filtrate, add exactly 1 mL of the internal standard solution, and use

this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 3 mL of this solution, add exactly 1 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3 \cdot HCl$)
 $= W_S \times (Q_T / Q_S) \times (1/5)$

W_S : Amount (mg) of Ritodrine Hydrochloride Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 10,000)

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Ritodrine Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 5.6 μ g of ritodrine hydrochloride ($C_{17}H_{21}NO_3 \cdot HCl$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Ritodrine Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, and dissolve in water to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 80 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of ritodrine. The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of ritodrine hydrochloride ($C_{17}H_{21}NO_3 \cdot HCl$)

$$= W_S \times (A_T / A_S) \times (V' / V) \times (1/C) \times 18$$

W_S : Amount (mg) of Ritodrine Hydrochloride Reference Standard

C : Labeled amount (mg) of ritodrine hydrochloride ($C_{17}H_{21}NO_3 \cdot HCl$) in 1 tablet

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 80 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ritodrine are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 80 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ritodrine is not more than 1.5%.

Assay To 20 Ritodrine Hydrochloride Tablets add 150 mL of 0.01 mol/L hydrochloric acid TS, shake for 20 minutes, and add 0.01 mol/L hydrochloric acid TS to make exactly 200 mL. Filter through a glass filter (G4), and discard the first 20 mL of the filtrate. Pipet 30 mL of the subsequent filtrate, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Ritodrine Hydrochloride Reference Standard, previously dried at 105°C for 2 hours, and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 30 mL of this solution, add exactly 5 mL of the internal standard solution and 0.01 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of ritodrine to that of the internal standard.

Amount (mg) of ritodrine hydrochloride ($\text{C}_{17}\text{H}_{21}\text{NO}_3 \cdot \text{HCl}$)
 $= W_S \times (Q_T/Q_S) \times 4$

W_S : Amount (mg) of Ritodrine Hydrochloride Reference Standard

Internal standard solution—A solution of methyl parahydroxybenzoate in methanol (3 in 5000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 6.6 g of diammonium hydrogen phosphate and 1.1 g of sodium 1-heptanesulfonate in 700 mL of water, and add 300 mL of methanol. Adjust to pH 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of ritodrine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, ritodrine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating

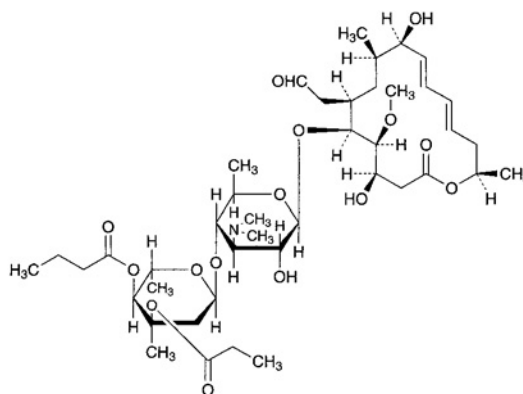
conditions, the relative standard deviation of the ratio of the peak area of ritodrine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Rokitamycin

ロキタマイシン



$\text{C}_{42}\text{H}_{69}\text{NO}_{15}$: 827.99

(3*R*,4*S*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-[4-*O*-Butanoyl-2,6-dideoxy-3-*C*-methyl-3-*O*-propanoyl- α -*L*-ribo-hexopyranosyl-(1 \rightarrow 4)-3,6-dideoxy-3-dimethylamino- β -*D*-glucopyranosyloxy]-6-formylmethyl-3,9-dihydroxy-4-methoxy-8-methylhexadeca-10,12-dien-15-olide [74014-51-0]

Rokitamycin is a derivative of leucomycin A₅, which is a macrolide antibiotic produced by the growth of the mutants of *Streptomyces kitasatoensis*.

Rokitamycin contains not less than 900 μg (potency) and not more than 1050 μg (potency) per mg, calculated on the anhydrous basis. The potency of Rokitamycin is expressed as mass (potency) of rokitamycin ($\text{C}_{42}\text{H}_{69}\text{NO}_{15}$).

Description Rokitamycin occurs as a white to yellowish white powder.

It is very soluble in methanol and in chloroform, freely soluble in ethanol (99.5) and in acetonitrile, and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Rokitamycin in methanol (1 in 50,000), as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rokitamycin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Rokitamycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Rokitamycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Determine the spectrum of a solution of Rokitamycin in deuterated chloroform for nuclear magnetic resonance spectroscopy (1 in 20), using tetramethylsilane for nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits single signals A, B, C and D at around δ 1.4 ppm, at around δ 2.5 ppm, at around δ 3.5 ppm and at around δ 9.8 ppm, respectively. The ratio of integrated intensity of these signals, A:B:C:D, is about 3:6:3:1.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Rokitamycin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Rokitamycin in 50 mL of acetonitrile, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add acetonitrile to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the peak areas of 3''-O-propionylleucomycin A₇ having the relative retention time of about 0.72, 3''-O-propionylisoleucomycin A₅ having the relative retention time of about 0.86 and 3''-O-propionylleucomycin A₁ having the relative retention time of about 1.36 with respect to rokitamycin obtained with the sample solution are not larger than the peak area of rokitamycin with the standard solution, the area of the peak other than rokitamycin, 3''-O-propionylleucomycin A₇, 3''-O-propionylisoleucomycin A₅ and 3''-O-propionylleucomycin A₁ obtained with the sample solution is not larger than 23/100 times the peak area of rokitamycin with the standard solution, and the total area of the peaks other than rokitamycin obtained with the sample solution is not larger than 3 times the peak area of rokitamycin with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 55°C.

Mobile phase: A mixture of methanol, diluted 0.5 mol/L ammonium acetate TS (2 in 5) and acetonitrile (124:63:13).

Flow rate: Adjust the flow rate so that the retention time of rokitamycin is about 11 minutes.

Time span of measurement: About 2.5 times as long as the retention time of rokitamycin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add acetonitrile to make exactly 10 mL. Confirm that the peak area of rokitamycin obtained with 5 μ L of this solution is equivalent to 7 to 13% of that with 5 μ L of the standard solution.

System performance: When the procedure is run with 5 μ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of

the peak of rokitamycin are not less than 3000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of rokitamycin is not more than 2.0%.

Water <2.48> Not more than 3.0% (0.2 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH <2.54> of the medium so that it will be 7.8 to 8.0 after sterilization.

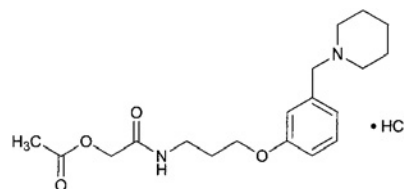
(iii) Standard solutions—Weigh accurately an amount of Rokitamycin Reference Standard equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μ g (potency) and 0.5 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Rokitamycin equivalent to about 40 mg (potency), dissolve in 50 mL of methanol, and add 0.1 mol/L phosphate buffer solution, pH 4.5 to make exactly 100 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 containing 0.01% of polysorbate 80 to make solutions so that each mL contains 2 μ g (potency) and 0.5 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride

ロキサチジン酢酸エステル塩酸塩



C₁₉H₂₈N₂O₄·HCl: 384.90

(3-{3-[(Piperidin-1-yl)methyl]phenoxy}propylcarbamoyl)methyl acetate monohydrochloride [93793-83-0]

Roxatidine Acetate Hydrochloride, when dried, con-

tains not less than 99.0% and not more than 101.0% of $C_{19}H_{28}N_2O_4 \cdot HCl$.

Description Roxatidine Acetate Hydrochloride occurs as white, crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and sparingly soluble in ethanol (99.5).

Identification (1) Determine the absorption spectrum of a solution of Roxatidine Acetate Hydrochloride in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Roxatidine Acetate Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Roxatidine Acetate Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Roxatidine Acetate Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Roxatidine Acetate Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 20 mL of water: the pH of this solution is between 4.0 and 6.0.

Melting point <2.60> 147 – 151°C (after drying).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Roxatidine Acetate Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Roxatidine Acetate Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Roxatidine Acetate Hydrochloride in 10 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than roxatidine acetate is not larger than 1/5 times the peak area of roxatidine acetate obtained from the standard solution, and the total area of the peaks other than roxatidine acetate is not larger than 1/2 times the peak area of roxatidine acetate from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 10 minutes.

Time span of measurement: About 1.5 times as long as the retention time of roxatidine acetate beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add ethanol (99.5) to make exactly 10 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of roxatidine acetate obtained with 10 μ L of this solution is equivalent to 7 to 13% of that with 10 μ L of the solution for system suitability test.

System performance: Dissolve 50 mg of Roxatidine Acetate Hydrochloride and 10 mg of benzoic acid in 25 mL of ethanol (99.5). When the procedure is run with 10 μ L of this solution under the above operating conditions, benzoic acid and roxatidine acetate are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Loss on drying <2.41> Not more than 0.3% (1 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.3 g of Roxatidine Acetate Hydrochloride, previously dried, dissolve in 5 mL of acetic acid (100), add 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.49 mg of $C_{19}H_{28}N_2O_4 \cdot HCl$

Containers and storage Containers—Tight containers.

Roxatidine Acetate Hydrochloride Extended-release Capsules

ロキサチジン酢酸エステル塩酸塩徐放カプセル

Roxatidine Acetate Hydrochloride Extended-release Capsules contains not less than 93.0% and not more than 107.0% of the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$: 384.90).

Method of preparation Prepare as directed under Capsules, with Roxatidine Acetate Hydrochloride.

Identification To 1 mL of the filtrate obtained in the Assay add ethanol (99.5) to make 20 mL, and determine the absorption spectrum as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 275 nm and 278 nm, and between 282 nm and 285 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Take out the contents of 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules, add exactly V mL of ethanol (99.5) so that each mL contains about 2.5 mg of

roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) according to the labeled amount, disperse the particles with the aid of ultrasonic wave, and filter through a membrane filter with pore size of not more than $1.0 \mu m$. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Proceed as directed in the Assay.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) = $W_S \times (Q_T/Q_S) \times (V/20)$

W_S : Amount (mg) of Roxatidine Acetate Hydrochloride Reference Standard

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 capsule of Roxatidine Acetate Hydrochloride Extended-release Capsules at 50 revolutions per minute according to the Paddle method, using the sinker, using 900 mL of water as the dissolution medium. Withdraw exactly 20 mL each of the dissolution medium 45 minutes, 90 minutes and 8 hours after starting the test for a 37.5-mg capsule and 60 minutes, 90 minutes and 8 hours after starting the test for a 75-mg capsule. Supply exactly 20 mL of water, warmed at $37 \pm 0.5^\circ C$, immediately after withdrawing of the dissolution medium every time. Filter the dissolution media withdrawn through a membrane filter with pore size of not more than $0.45 \mu m$. Discard the first 10 mL of the filtrate, pipet V' mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about $42 \mu g$ of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) according to the labeled amount, and use these solutions as the sample solutions. Separately, weigh accurately about 21 mg of Roxatidine Acetate Hydrochloride Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in water to make exactly 50 mL. Pipet 2 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly $100 \mu L$ each of the sample solutions and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, $A_{T(n)}$ and A_S , of roxatidine acetate of each solution. The dissolution rates for a 37.5-mg capsule in 45 minutes, in 90 minutes and in 8 hours are 10 – 40%, 35 – 65 %, and not less than 70%, respectively, and for a 75-mg capsule in 60 minutes, in 90 minutes and in 8 hours are 20 – 50%, 35 – 65%, and not less than 70%, respectively.

Dissolution rate (%) with respect to the labeled amount of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) on the n th dissolution medium withdrawing ($n = 1, 2, 3$)

$$= W_S \times \left[\frac{A_{T(n)}}{A_S} + \sum_{i=1}^{n-1} \left(\frac{A_{T(i)}}{A_S} \times \frac{1}{45} \right) \right] \times \frac{V'}{V} \times \frac{1}{C} \times 180$$

W_S : Amount (mg) of Roxatidine Acetate Hydrochloride Reference Standard

C : Labeled amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5 \mu m$ in particle diameter).

Column temperature: A constant temperature of about $40^\circ C$.

Mobile phase: A mixture of water, acetonitrile, triethylamine and acetic acid (100) (340:60:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 5 minutes.

System suitability—

System performance: When the procedure is run with $100 \mu L$ of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of roxatidine acetate are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with $100 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of roxatidine acetate is not more than 1.0%.

Assay Take out the contents of not less than 20 Roxatidine Acetate Hydrochloride Extended-release Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 75 mg of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$), add exactly 30 mL of ethanol (99.5), shake, and filter through a membrane filter with pore size of not more than $1.0 \mu m$. To exactly 8 mL of the filtrate add exactly 2 mL of the internal standard solution, mix, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Roxatidine Acetate Hydrochloride Reference Standard, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours, and dissolve in ethanol (99.5) to make exactly 20 mL. To exactly 8 mL of this solution add exactly 2 mL of the internal standard solution, mix, and use this solution as the standard solution. Perform the test with $10 \mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of roxatidine acetate to that of the internal standard.

Amount (mg) of roxatidine acetate hydrochloride ($C_{19}H_{28}N_2O_4 \cdot HCl$) = $W_S \times (Q_T/Q_S) \times (3/2)$

W_S : Amount (mg) of Roxatidine Acetate Hydrochloride Reference Standard

Internal standard solution—A solution of benzoic acid in ethanol (99.5) (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cyanopropylsilanized silica gel for liquid chromatography ($5 \mu m$ in particle diameter).

Column temperature: A constant temperature of about $35^\circ C$.

Mobile phase: A mixture of hexane, ethanol (99.5), triethylamine and acetic acid (100) (384:16:2:1).

Flow rate: Adjust the flow rate so that the retention time of roxatidine acetate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10

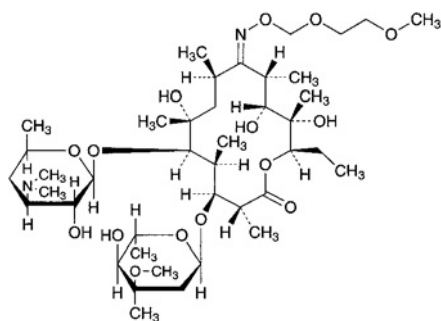
μL of the standard solution under the above operating conditions, the internal standard and roxatidine acetate are elute in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with $10\ \mu\text{L}$ of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of roxatidine acetate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Roxithromycin

ロキシシロマイシン



$\text{C}_{41}\text{H}_{76}\text{N}_2\text{O}_{15}$: 837.05
(2*R*,3*S*,4*S*,5*R*,6*R*,8*R*,10*R*,11*R*,12*S*,13*R*)-
5-(3,4,6-Trideoxy-3-dimethylamino- β -D-xylo-
hexopyranosyloxy)-3-(2,6-dideoxy-3-C-methyl-3-O-methyl- α -L-ribo-hexopyranosyloxy)-6,11,12-trihydroxy-9-
(2-methoxyethoxy)methoxyimino-2,4,6,8,10,12-
hexamethylpentadecan-13-olide [80214-83-1]

Roxithromycin is a derivative of erythromycin.

It contains not less than $970\ \mu\text{g}$ (potency) per mg, calculated on the anhydrous basis. The potency of Roxithromycin is expressed as mass (potency) of roxithromycin ($\text{C}_{41}\text{H}_{76}\text{N}_2\text{O}_{15}$).

Description Roxithromycin occurs as a white crystalline powder.

It is freely soluble in ethanol (95) and in acetone, soluble in methanol, sparingly soluble in acetonitrile, and practically insoluble in water.

Identification Determine the infrared absorption spectrum of Roxithromycin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Roxithromycin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: -93 – -96° (0.5 g calculated on the anhydrous basis, acetone, 50 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Roxithromycin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 40 mg of Roxithromy-

cin in the mobile phase A to make 10 mL, and use this solution as the sample solution. Separately, dissolve 20 mg of Roxithromycin Reference Standard in the mobile phase A to make exactly 10 mL. Pipet 1 mL of this solution, add the mobile phase A to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly $20\ \mu\text{L}$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas by the automatic integration method: the area of a peak having the relative retention time of about 1.05 to the retention time of roxithromycin from the sample solution is not larger than 2 times of the peak area of roxithromycin from the standard solution. The areas of other than the peak of roxithromycin and the peak having the relative retention time of about 1.05 to the retention time of roxithromycin are not larger than the peak area of roxithromycin from the standard solution, and the total area of the peaks other than roxithromycin from the sample solution is not larger than 6 times of the peak area of roxithromycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography ($5\ \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 25°C .

Mobile phase A: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Mobile phase B: A mixture of acetonitrile and water (7:3).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 38	100	0
38 – 39	100→90	0→10
39 – 80	90	10

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 21 minutes.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase A to make exactly 10 mL. Confirm that the peak area of roxithromycin obtained from $20\ \mu\text{L}$ of this solution is equivalent to 15 to 25% of that of roxithromycin obtained from $20\ \mu\text{L}$ of the standard solution.

System performance: Dissolve 5 mg each of Roxithromycin Reference Standard and *N*-demethylroxithromycin in the mobile phase A to make 100 mL. When the procedure is run with $20\ \mu\text{L}$ of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 5 times with $20\ \mu\text{L}$ of the standard solution under the above operating

conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 2.0%.

Water <2.48> Not more than 3.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Roxithromycin and Roxithromycin Reference Standard, equivalent to about 20 mg (potency), and dissolve separately in the mobile phase to make exactly 10 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak areas, A_T and A_S , of roxithromycin.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{41}\text{H}_{76}\text{N}_2\text{O}_{15} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Roxithromycin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 205 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 200 mL of a solution of ammonium dihydrogenphosphate (17 in 100) add 510 mL of water, and adjust to pH 5.3 with 2 mol/L sodium hydroxide TS. To this solution add 315 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of roxithromycin is about 11 minutes.

System suitability—

System performance: Dissolve 5 mg each of Roxithromycin Reference Standard and *N*-demethylroxithromycin in the mobile phase to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, *N*-demethylroxithromycin and roxithromycin are eluted in this order with the resolution between these peaks being not less than 6 and the symmetry factor of the peak of roxithromycin is not more than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of roxithromycin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Freeze-dried Live Attenuated Rubella Vaccine

乾燥弱毒生風しんワクチン

Freeze-dried Live Attenuated Rubella Vaccine is a preparation for injection which is dissolved before use. It contains live attenuated rubella virus.

It conforms to the requirements of Freeze-dried Live Attenuated Rubella Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Live Attenuated Rubella Vaccine becomes a colorless, yellowish or reddish clear liquid on addition of solvent.

Saccharated Pepsin

含糖ペプシン

Saccharated Pepsin is a mixture of pepsin obtained from the gastric mucosa of hog or cattle and Lactose Hydrate, and it is an enzyme drug having a proteolytic activity.

Saccharated Pepsin contains not less than 3800 units and not more than 6000 units per g.

Description Saccharated Pepsin occurs as a white powder. It has a characteristic odor, and has a slightly sweet taste.

It dissolves in water to give a slightly turbid liquid, and does not dissolve in ethanol (95) and in diethyl ether.

It is slightly hygroscopic.

Purity (1) Rancidity—Saccharated Pepsin has no unpleasant or rancid odor.

(2) Acidity—Dissolve 0.5 g of Saccharated Pepsin in 50 mL of water, and add 0.50 mL of 0.1 mol/L sodium hydroxide VS and 2 drops of phenolphthalein TS: the solution is red in color.

Loss on drying <2.41> Not more than 1.0% (1 g, 80°C, 4 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay (i) Substrate solution—Use the substrate solution 1 described in (2) Assay for protein digestive activity under the Digestion Test <4.03> after adjusting the pH to 2.0.

(ii) Sample solution—Weigh accurately an amount of Saccharated Pepsin equivalent to about 1250 units, dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make exactly 50 mL.

(iii) Standard solution—Weigh accurately a suitable amount of Saccharated Pepsin Reference Standard, and dissolve in ice-cold 0.01 mol/L hydrochloric acid TS to make a solution containing about 25 units per mL.

(iv) Procedure—Proceed as directed in (2) Assay for protein digestive activity under Digestion Test <4.03>, and determine the absorbances, A_T and A_{TB} , of the sample solution, using trichloroacetic acid TS A as the precipitation reagent. Separately, determine the absorbances, A_S and A_{SB} , of the standard solution in the same manner as the sample solution.

$$\begin{aligned} \text{Units in 1 g of Saccharated Pepsin} \\ = U_S \times \{(A_T - A_{TB}) / (A_S - A_{SB})\} \times (1/W) \end{aligned}$$

U_S : Units per mL of the standard solution

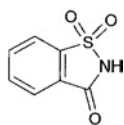
W : Amount (g) of Saccharated Pepsin per mL of the sample solution

Containers and storage Containers—Tight containers.

Storage—Not exceeding 30°C.

Saccharin

サッカリン



$C_7H_5NO_3S$: 183.18

1,2-Benzo[*d*]isothiazol-3(2*H*)-one 1,1-dioxide
[81-07-2]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Saccharin contains not less than 99.0% and not more than 101.0% of $C_7H_5NO_3S$, calculated on the dried basis.

♦**Description** Saccharin occurs as colorless or white crystals or a white crystalline powder. It has a very sweet taste.

It is sparingly soluble in ethanol (95), and slightly soluble in water.

It dissolves in sodium hydroxide TS.◆

♦**Identification** Determine the infrared absorption spectrum of Saccharin as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

Melting point <2.60> 226 – 230°C

Purity ♦(1) Clarity and color of solution—Dissolve 1.0 g of Saccharin in 30 mL of hot water or in 50 mL of ethanol (95): the solution is clear and colorless in each case.◆

♦(2) Heavy metals <1.07>—Proceed with 2.0 g of Saccharin according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).◆

(3) Benzoate and salicylate—To 10 mL of a saturated solution of Saccharin in hot water add 3 drops of iron (III) chloride TS: no precipitate is formed, and no red-purple to purple color develops.

♦(4) *o*-Toluene sulfonamide—Dissolve 10 g of Saccharin in 70 mL of sodium hydroxide TS, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, then evaporate the solvent. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of *o*-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate to dryness on a water bath, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of *o*-toluene sulfonamide to that of the internal standard: Q_T is

not larger than Q_S .

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography coated 3% with diethylene glycol succinate polyester for gas chromatography (180 – 250 μ m in particle diameter).

Column temperature: A constant temperature of about 200°C.

Temperature of injection port: A constant temperature of about 225°C.

Temperature of detector: A constant temperature of about 250°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 μ L of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 1 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.◆

(5) Readily carbonizable substances <1.15>—Perform the test with 0.20 g of Saccharin, by warming at 48 to 50°C for 10 minutes: the color of the solution is not more intense than the matching fluid A.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.5 g of Saccharin, dissolve in 40 mL of ethanol (95), add 40 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination in the same manner, and make any necessary correction.

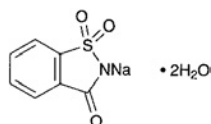
Each mL of 0.1 mol/L sodium hydroxide VS
= 18.32 mg of $C_7H_5NO_3S$

Containers and storage Containers—Well-closed containers.

Saccharin Sodium Hydrate

Saccharin Sodium

サッカリンナトリウム水和物



$C_7H_4NNaO_3S \cdot 2H_2O$: 241.20

2-Sodio-1,2-benzo[d]isothiazol-3(2H)-one 1,1-dioxide dihydrate [6155-57-3]

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

Saccharin Sodium Hydrate, contains not less than 99.0% and not more than 101.0% of saccharin sodium ($C_7H_4NNaO_3S$: 205.17), calculated on the anhydrous basis.

♦**Description** Saccharin Sodium Hydrate occurs as colorless crystals or a white, crystalline powder. It has an intensely sweet taste, even in 10,000 dilutions.

It is freely soluble in water and in methanol, and sparingly soluble in ethanol (95) and in acetic acid (100).

It effloresces slowly and loses about half the amount of water of crystallization in air.◆

Identification ♦(1) Determine the infrared absorption spectrum of Saccharin Sodium Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.◆

(2) A solution of Saccharin Sodium Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity ♦(1) Clarity and color of solution—Dissolve 1.0 g of Saccharin Sodium Hydrate in 1.5 mL of water or in 50 mL of ethanol (95): the solution is clear and colorless.◆

(2) Acidity or alkalinity—Dissolve 1.0 g of Saccharin Sodium Hydrate in 10 mL of water, and add 1 drop of phenolphthalein TS: the solution is colorless. Add 1 drop of 0.1 mol/L sodium hydroxide VS to the solution: the color changes to red.

♦(3) Heavy metals <1.07>—Dissolve 2.0 g of Saccharin Sodium Hydrate in 40 mL of water, add 0.7 mL of dilute hydrochloric acid, dilute with water to make 50 mL, and rub the inner wall of the vessel with a glass rod until crystallization begins. Allow the solution to stand for 1 hour after the beginning of crystallization, and then filter through dry filter paper. Reject the first 10 mL of the filtrate, and take 25 mL of the subsequent filtrate. Add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test, using this solution as the test solution. To 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than

10 ppm).◆

(4) Benzoate and salicylate—Dissolve 0.5 g of Saccharin Sodium Hydrate in 10 mL of water, add 5 drops of acetic acid (31) and 3 drops of iron (III) chloride TS: no turbidity is produced, and no red-purple to purple color develops.

♦(5) *o*-Toluene sulfonamide—Dissolve 10 g of Saccharin Sodium Hydrate in 50 mL of water, and extract with three 30-mL portions of ethyl acetate. Combine all the ethyl acetate extracts, wash with 30 mL of a solution of sodium chloride (1 in 4), dehydrate with 5 g of anhydrous sodium sulfate, and evaporate ethyl acetate. To the residue add exactly 5 mL of the internal standard solution to dissolve, and use this solution as the sample solution. Separately, dissolve 0.10 g of *o*-toluene sulfonamide in ethyl acetate to make exactly 100 mL. Pipet 1 mL of this solution, evaporate on a water bath to dryness, dissolve the residue in exactly 5 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of *o*-toluene sulfonamide to that of the internal standard: Q_T is not more than Q_S .

Internal standard solution—A solution of caffeine in ethyl acetate (1 in 500).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with siliceous earth for gas chromatography (180 to 250 μ m in diameter), coated with diethyleneglycol succinate polyester for gas chromatography at the ratio of 3 %.

Column temperature: A constant temperature of about 200°C.

Injection port temperature: A constant temperature of about 225°C.

Detector temperature: A constant temperature of about 250°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of caffeine is about 6 minutes.

System suitability—

System performance: When the procedure is run with 1 μ L of the standard solution under the above operating conditions, the internal standard and *o*-toluene sulfonamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 1 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak height of *o*-toluene sulfonamide to that of the internal standard is not more than 2.0%.◆

(6) Readily carbonizable substances <1.15>—Perform the test with 0.20 g of Saccharin Sodium Hydrate. Allow the solution to stand between 48°C and 50°C for 10 minutes: the solution has no more color than Matching Fluid A.

Water <2.48> Not more than 15.0% (0.1 g, volumetric titration, direct titration).

Assay Weigh accurately about 0.15 g of Saccharin Sodium Hydrate, dissolve in 50 mL of acetic acid (100), heat slightly if necessary, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determina-

tion, and make any necessary correction.

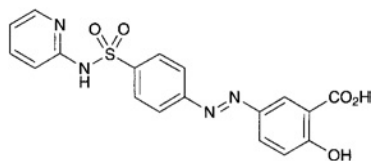
Each mL of 0.1 mol/L perchloric acid VS
= 20.52 mg of $C_7H_4NNaO_3S$

◆ **Containers and storage** Containers—Well-closed containers. ◆

Salazosulfapyridine

Sulfasalazine

サラゾスルファピリジン



$C_{18}H_{14}N_4O_5S$: 398.39

2-Hydroxy-5-[4-(pyridin-2-ylsulfamoyl)phenylazo]benzoic acid [599-79-1]

Salazosulfapyridine, when dried, contains not less than 96.0% of $C_{18}H_{14}N_4O_5S$.

Description Salazosulfapyridine occurs as a yellow to yellow-brown, fine powder. It is odorless and tasteless.

It is sparingly soluble in pyridine, slightly soluble in ethanol (95), practically insoluble in water, in chloroform and in diethyl ether.

It dissolves in sodium hydroxide TS.

Melting point: 240 – 249°C (with decomposition).

Identification (1) Dissolve 0.1 g of Salazosulfapyridine in 20 mL of dilute sodium hydroxide TS: a red-brown color develops. This color gradually fades upon gradual addition of 0.5 g of sodium hydrosulfite with shaking. Use this solution in the following tests (2) to (4).

(2) To 1 mL of the solution obtained in (1) add 40 mL of water, neutralize with 0.1 mol/L hydrochloric acid TS, and add water to make 50 mL. To 5 mL of this solution add 2 to 3 drops of dilute iron (III) chloride TS: a red color develops and changes to purple, then fades when dilute hydrochloric acid is added dropwise.

(3) The solution obtained in (1) responds to the Qualitative Tests <1.09> for primary aromatic amines.

(4) To 1 mL of the solution obtained in (1) add 1 mL of pyridine and 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(5) Determine the absorption spectrum of a solution of Salazosulfapyridine in dilute sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Chloride <1.03>—Dissolve 2.0 g of Salazosulfapyridine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of nitric acid, shake, and filter. To 25 mL of the filtrate add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test so-

lution. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(2) Sulfate <1.14>—Dissolve 2.0 g of Salazosulfapyridine in 12 mL of sodium hydroxide TS and 36 mL of water, add 2 mL of hydrochloric acid, shake, and filter. To 25 mL of the filtrate add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Salazosulfapyridine according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Take 1.0 g of Salazosulfapyridine in a decomposition flask, add 20 mL of nitric acid, and heat gently until it becomes fluid. After cooling, add 5 mL of sulfuric acid, and heat until white fumes are evolved. Add, if necessary, 5 mL of nitric acid after cooling, and heat again. Repeat this operation until the solution becomes colorless to slightly yellow. After cooling, add 15 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved again. After cooling, add water to make 25 mL. Perform the test with 5 mL of this solution as the test solution: the color of the test solution is not deeper than that of the following standard stain.

Standard stain: Proceed in the same manner without Salazosulfapyridine, transfer 5 mL of the obtained solution to a generator bottle, add exactly 2 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.20 g of Salazosulfapyridine in 20 mL of pyridine, and use this solution as the sample solution. Pipet 1 mL of this solution, add pyridine to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with diluted methanol (9 in 10) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

(6) Salicylic acid—To 0.10 g of Salazosulfapyridine add 15 mL of diethyl ether, and shake vigorously. Add 5 mL of dilute hydrochloric acid, shake vigorously for 3 minutes, collect the diethyl ether layer, and filter. To the water layer add 15 mL of diethyl ether, shake vigorously for 3 minutes, collect the diethyl ether layer, filter, and combine the filtrates. Wash the residue on the filter paper with a small quantity of diethyl ether, and combine the washings and the filtrate. Evaporate the diethyl ether with the aid of air-stream at room temperature. To the residue add dilute ammonium iron (II) sulfate TS, shake, and filter, if necessary. Wash the residue on the filter paper with a small quantity of dilute ammonium iron (III) sulfate TS, combine the washings and the filtrate, add dilute ammonium iron (III) sulfate TS to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, dissolve in dilute ammonium iron (III) sulfate TS to make exactly 400 mL, and use this solution as the standard solution. De-

termine the absorbances, A_T and A_S , at 535 nm of the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: salicylic acid content is not more than 0.5%.

$$\begin{aligned} \text{Content (\% of salicylic acid (C}_7\text{H}_6\text{O}_3)) \\ = W_S \times (A_T/A_S) \times 0.05 \end{aligned}$$

W_S : Amount (mg) of salicylic acid for assay

Loss on drying <2.41> Not more than 2.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

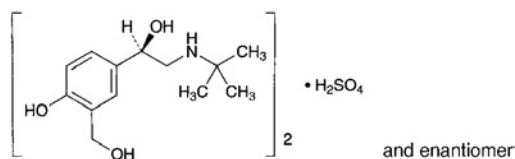
Assay Weigh accurately about 20 mg of Salazosulfapyridine, previously dried, and perform the test as directed in the procedure of determination for sulfur under the Oxygen Flask Combustion Method <1.06>, using 10 mL of diluted hydrogen peroxide (30) (1 in 40) as an absorbing liquid.

Each mL of 0.005 mol/L barium perchlorate VS
= 1.992 mg of $\text{C}_{18}\text{H}_{14}\text{N}_4\text{O}_5\text{S}$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Salbutamol Sulfate

サルブタモール硫酸塩



$(\text{C}_{13}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$: 576.70
(1*RS*)-2-(1,1-Dimethylethyl)amino-1-(4-hydroxy-3-hydroxymethylphenyl)ethanol hemisulfate [51022-70-9]

Salbutamol Sulfate, when dried, contains not less than 98.0% of $(\text{C}_{13}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$.

Description Salbutamol Sulfate occurs as a white powder. It is freely soluble in water, slightly soluble in ethanol (95), and in acetic acid (100) and practically insoluble in diethyl ether.

A solution of Salbutamol Sulfate (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Salbutamol Sulfate in 0.1 mol/L hydrochloric acid TS (1 in 12,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Salbutamol Sulfate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Salbutamol Sulfate (1 in 20) responds to the Qualitative Tests <1.09> for sulfate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Salbutamol Sulfate in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Salbutamol Sulfate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 20 mg of Salbutamol Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 2-propanol, water and ammonia solution (28) (25:15:8:2) to a distance of about 15 cm, and air-dry the plate. Leave the plate in a well-closed vessel saturated with diethylamine vapor for 5 minutes, and spray evenly 4-nitrobenzenediazonium chloride TS: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution in color.

(4) Boron—Take 50 mg of Salbutamol Sulfate and 5.0 mL of the boron standard solution, and transfer to a platinum crucible. Add 5 mL of potassium carbonate-sodium carbonate TS, evaporate on a water bath to dryness, and dry at 120°C for 1 hour. Ignite the residue immediately. After cooling, add 0.5 mL of water and 3 mL of curcumin TS to the residue, warm gently in a water bath for 5 minutes. After cooling, add 3 mL of acetic acid-sulfuric acid TS, mix, and allow to stand for 30 minutes. Add ethanol (95) to make exactly 100 mL, and filter. Discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution and standard solution. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using ethanol (95) as the blank: the absorbance of the sample solution at 555 nm is not larger than that of the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 100°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.9 g of Salbutamol Sulfate, previously dried, and dissolve in 50 mL of acetic acid (100) by warming. After cooling, titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 57.67 mg of $(\text{C}_{13}\text{H}_{21}\text{NO}_3)_2 \cdot \text{H}_2\text{SO}_4$

Containers and storage Containers—Tight containers.

Salicylated Alum Powder

サリチル・ミョウバン散

Salicylated Alum Powder contains not less than 2.7% and not more than 3.3% of salicylic acid ($\text{C}_7\text{H}_6\text{O}_3$: 138.12).

Method of preparation

Salicylic Acid, finely powdered	30 g
Dried Aluminum Potassium Sulfate, very finely powdered	640 g
Talc, very finely powdered	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Description Salicylated Alum Powder occurs as a white powder.

Identification (1) The colored solution obtained in the Assay has a red-purple color and exhibits an absorption maximum <2.24> between 520 nm and 535 nm (salicylic acid).

(2) Shake 0.3 g of Salicylated Alum Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g of salicylic acid in 5 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on the plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same *R_f* value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution and the corresponding spot from the sample solution reveal a purple color.

Assay Weigh accurately about 0.33 g of Salicylated Alum Powder, add 80 mL of ethanol (95), and shake vigorously. Dilute with ethanol (95) to make exactly 100 mL, filter, and discard the first 10 mL of the filtrate. Use the subsequent filtrate as the sample solution. Dissolve about 0.1 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in sufficient ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, dilute with ethanol (95) to make exactly 100 mL, and use the solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution into stoppered test tubes respectively, to each add exactly 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200), and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 25 mL. Determine the absorbances, *A_T* and *A_S*, of both solutions at 530 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared in the same manner with ethanol (95), instead of the sample solution, as the blank.

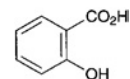
$$\begin{aligned} &\text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3\text{)} \\ &= W_S \times (A_T/A_S) \times (1/10) \end{aligned}$$

W_S: Amount (mg) of salicylic acid for assay

Containers and storage Containers—Well-closed containers.

Salicylic Acid

サリチル酸



C₇H₆O₃: 138.12
2-Hydroxybenzoic acid [69-72-7]

Salicylic Acid, when dried, contains not less than 99.5% of C₇H₆O₃.

Description Salicylic Acid occurs as white crystals or crystalline powder. It is odorless and has a slightly acid, followed by an acrid taste.

It is freely soluble in ethanol (95), in acetone and in diethyl ether, soluble in hot water, and slightly soluble in water.

Identification A solution of Salicylic Acid (1 in 500) responds to the Qualitative Tests <1.09> (1) and (3) for salicylate.

Melting point <2.60> 158 – 161°C

Purity (1) Chloride <1.03>—Dissolve 5.0 g of Salicylic Acid in 90 mL of water by heating, cool, dilute with water to 100 mL, and filter. Discard the first 20 mL of the filtrate, take subsequent 30 mL of the filtrate, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.008%).

(2) Sulfate <1.14>—To 30 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Dissolve 2.0 g of Salicylic Acid in 25 mL of acetone, add 4 mL of sodium hydroxide TS, 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 25 mL of acetone, 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(4) Readily carbonizable substances <1.15>—Perform the test with 0.5 g of Salicylic Acid: the solution has no more color than Matching Fluid C.

Loss on drying <2.41> Not more than 0.5% (2 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (1 g).

Assay Weigh accurately about 0.5 g of Salicylic Acid, previously dried, dissolve in 25 mL of neutralized ethanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

$$\begin{aligned} &\text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ &= 13.81 \text{ mg of C}_7\text{H}_6\text{O}_3 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Salicylic Acid Adhesive Plaster

サリチル酸絆創膏

Method of preparation

Adhesive Plaster consists of a mixture of the below ingredients with carefully selected rubber, resins, zinc oxide and other substances. It has adhesive properties. It spreads evenly on a fabric.

Salicylic Acid, finely powdered	500 g
Adhesive plaster base	a sufficient quantity
To make 1000 g	

Description The surface of Salicylic Acid Adhesive Plaster is whitish in color and adheres well to the skin.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Salicylic Acid Spirit

サリチル酸精

Salicylic Acid Spirit contains not less than 2.7 w/v% and not more than 3.3 w/v% of salicylic acid ($C_7H_6O_3$; 138.12).

Method of preparation

Salicylic Acid	30 g
Glycerin	50 mL
Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Salicylic Acid Spirit is a clear, colorless liquid. Specific gravity d_{20}^{20} : about 0.86

Identification The solution obtained in the Assay has a red-purple color. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 520 nm and 535 nm (salicylic acid).

Alcohol number <1.01> Not less than 8.8 (Method 2).

Assay Measure exactly 10 mL of Salicylic Acid Spirit, add 10 mL of ethanol (95) and water to make exactly 100 mL. Pipet 3 mL of this solution, and dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL. Use this solution as the sample solution. Dissolve about 0.3 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours and accurately weighed, in 10 mL of alcohol and water to make exactly 100 mL. Pipet 3 mL of this solution, dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and standard solution, to each add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in

200), dilute with hydrochloric acid-potassium chloride buffer solution, pH 2.0, to exactly 25 mL. Determine the absorbances <2.24>, A_T and A_S , of both solutions at 530 nm, using a blank solution prepared in the same manner with water instead of the sample solution.

$$\begin{aligned} &\text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3\text{)} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of salicylic acid for assay

Containers and storage Containers—Tight containers.

Compound Salicylic Acid Spirit

複方サリチル酸精

Compound Salicylic Acid Spirit contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$; 138.12), and not less than 0.43 w/v% and not more than 0.53 w/v% of phenol (C_6H_6O ; 94.11).

Method of preparation

Salicylic Acid	20 g
Liquefied Phenol	5 mL
Glycerin	40 mL
Ethanol	800 mL
Water or Purified Water	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Compound Salicylic Acid Spirit is a clear, colorless to light red liquid.

Specific gravity d_{20}^{20} : about 0.88

Identification (1) To 1 mL of Compound Salicylic Acid Spirit add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(2) To 1 mL of Compound Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, and extract with 20 mL of diethyl ether. Wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and extract with 10 mL of dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, allow to stand for 10 minutes, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(3) To 0.5 mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution (1). To 2 mL of Compound Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, wash the extract with two 5-mL portions of sodium hydrogen carbonate TS, and use the chloroform extract as the sample solution (2). Separately, dissolve 0.01 g each of salicylic acid and phenol in 5 mL each of chloroform, and use both solutions as the standard solutions (1) and (2). Perform the test with these solutions as directed under Thin-layer Chro-

matography <2.03>. Spot 5 μL each of the sample solutions and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots from the sample solution (1) and standard solution (1) show the same R_f value, and the spots from the sample solution (2) and standard solution (2) show the same R_f value. Spray evenly iron (III) chloride TS upon the plate: the spot from the standard solution (1) and the corresponding spot from the sample solution (1) reveal a purple color.

Alcohol number <1.01> Not less than 7.5 (Method 2).

Assay Measure accurately 2 mL of Compound Salicylic Acid Spirit, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 50 mg of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 20 mL of this solution, add exactly 5 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. Perform the test with 15 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

$$\begin{aligned} \text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3\text{)} \\ = W_{\text{Sa}} \times (Q_{\text{Ta}}/Q_{\text{Sa}}) \times (1/5) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of phenol (C}_6\text{H}_6\text{O)} \\ = W_{\text{Sb}} \times (Q_{\text{Ta}}/Q_{\text{Tb}}) \times (1/5) \end{aligned}$$

W_{Sa} : Amount (mg) of salicylic acid for assay

W_{Sb} : Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 1250).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography, 5 μm in particle diameter.

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

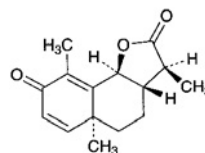
Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μL of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Santonin

サントニン



$\text{C}_{15}\text{H}_{18}\text{O}_3$: 246.30
(3*S*,3*aS*,5*aS*,9*bS*)-3,5*a*,9-Trimethyl-3*a*,5,5*a*,9*b*-tetrahydronaphtho[1,2-*b*]furan-2,8(3*H*,4*H*)-dione [481-06-1]

Santonin, when dried, contains not less than 98.5% and not more than 101.0% of $\text{C}_{15}\text{H}_{18}\text{O}_3$.

Description Santonin occurs as colorless crystals, or a white, crystalline powder.

It is freely soluble in chloroform, sparingly soluble in ethanol (95), and practically insoluble in water.

It becomes yellow by light.

Identification (1) Determine the absorption spectrum of a solution of Cortisone Acetate in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Cortisone Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_{\text{D}}^{20}$: $-170 - -175^\circ$ (0.2 g, chloroform, 10 mL, 100 mm).

Melting point <2.60> 172 – 175°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Santonin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm)

(2) Alkaloids—Boil 0.5 g of Santonin with 20 mL of diluted sulfuric acid (1 in 100), cool, and filter. Dilute 10 mL of the filtrate with water to 30 mL, add 3 drops of iodine TS, and allow to stand for 3 hours: no turbidity is produced.

(3) Artemisin—Dissolve 1.0 g of powdered Santonin in 2 mL of chloroform by slight warming: the solution is clear and colorless, or any yellow color produced is not darker than Matching Fluid A.

(4) Phenols—Boil 0.20 g of Santonin with 10 mL of water, cool, and filter. To the filtrate add bromine TS until the color of the solution becomes yellow: no turbidity is produced.

(5) Acid-coloring substances—Moisten 10 mg of Santonin with nitric acid: no color develops immediately. Moisten Santonin with sulfuric acid, previously cooled to 0°C: no color is produced immediately.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3

hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

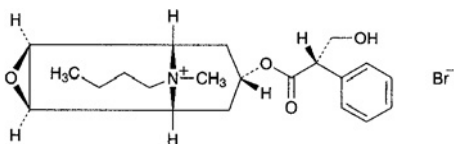
Assay Weigh accurately about 0.25 g of Santonin, previously dried, dissolve in 10 mL of ethanol (95) by warming, add exactly 20 mL of 0.1 mol/L sodium hydroxide VS, and heat on a water bath under a reflux condenser for 5 minutes. Cool quickly, and titrate <2.50> the excess sodium hydroxide with 0.05 mol/L hydrochloric acid VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 24.63 mg of $C_{15}H_{18}O_3$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Scopolamine Butylbromide

ブチルスコポラミン臭化物



$C_{21}H_{30}BrNO_4$; 440.37
(1*S*,2*S*,4*R*,5*R*,7*S*)-9-Butyl-7-[(2*S*)-3-hydroxy-2-phenylpropanoate]-9-methyl-3-oxa-9-azoniatricclo[3.3.1.0^{2,4}]nonane bromide [149-64-4]

Scopolamine Butylbromide, when dried, contains not less than 98.5% of $C_{21}H_{30}BrNO_4$.

Description Scopolamine Butylbromide occurs as white crystals or crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), sparingly soluble in methanol, slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 140°C (with decomposition).

Identification (1) To 1 mg of Scopolamine Butylbromide add 3 to 4 drops of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color develops.

(2) Determine the absorption spectrum of a solution of Scopolamine Butylbromide (1 in 1000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Scopolamine Butylbromide, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Scopolamine Butylbromide (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-18.0 - -20.0^\circ$ (after

drying, 1 g, water, 10 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Scopolamine Butylbromide in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Matching Fluid F add diluted hydrochloric acid (1 in 40) to make 20 mL.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Scopolamine Butylbromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Scopolamine Butylbromide in the mobile phase to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of scopolamine hydrobromide in the mobile phase to make exactly 100 mL. Pipet 10 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution (1). Pipet 5 mL of the standard solution (1), add the mobile phase to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with exactly 20 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the peak area of scopolamine from the sample solution is not larger than that from the standard solution (2), and each area of the peaks other than the peak appearing in the first elution and the peak of scopolamine and butylscopolamine from the sample solution are not larger than the peak area from the standard solution (1).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 2 g of sodium lauryl sulfate in 370 mL of water and 680 mL of methanol, and adjust the pH to 3.6 with diluted phosphoric acid (1 in 10).

Flow rate: Adjust the flow rate so that the retention time of butylscopolamine is about 7 minutes.

Time span of measurement: About twice as long as the retention time of butylscopolamine.

System suitability—

System performance: Dissolve 5 mg each of Scopolamine Butylbromide and scopolamine hydrobromide in 50 mL of the mobile phase. When the procedure is run with 20 μ L of this solution under the above operating conditions, scopolamine and butylscopolamine are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution (2) under the above operating conditions, the relative standard deviation of the peak area of scopolamine is not more than 2.0%.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C,

4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

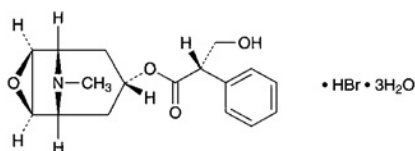
Assay Weigh accurately about 0.8 g of Scopolamine Butylbromide, previously dried, dissolve in 40 mL of acetic acid (100) and 30 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.04 mg of $C_{21}H_{30}BrNO_4$

Containers and storage Containers—Tight containers.

Scopolamine Hydrobromide Hydrate

スコポラミン臭化水素酸塩水和物



$C_{17}H_{21}NO_4 \cdot HBr \cdot 3H_2O$: 438.31

(1*S*,2*S*,4*R*,5*R*,7*S*)-9-Methyl-3-oxa-9-azatricyclo-[3.3.1.0^{2,4}]non-7-yl (2*S*)-3-hydroxy-2-phenylpropanoate monohydrobromide trihydrate [6533-68-2]

Scopolamine Hydrobromide Hydrate, when dried, contains not less than 98.5% of scopolamine hydrobromide ($C_{17}H_{21}NO_4 \cdot HBr$: 384.26).

Description Scopolamine Hydrobromide Hydrate occurs as colorless or white crystals, or white granules or powder. It is odorless.

It is freely soluble in water, sparingly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in diethyl ether.

Identification (1) To 1 mg of Scopolamine Hydrobromide Hydrate add 3 to 4 drops of fuming nitric acid, evaporate on a water bath to dryness, and cool. Dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 6 drops of tetraethylammonium hydroxide TS: a red-purple color is produced.

(2) A solution of Scopolamine Hydrobromide Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for bromide.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-24.0 - -26.0^\circ$ (after drying, 0.5 g, water, 10 mL, 100 mm).

Melting point <2.60> 195 – 199°C (after drying; previously heat the bath to 180°C).

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Scopolamine Hydrobromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Acidity—Dissolve 0.50 g of Scopolamine Hydrobromide Hydrate in 15 mL of water, and add 0.50 mL of 0.02 mol/L sodium hydroxide and 1 drop of methyl red-methylene blue TS: a green color develops.

(3) Apotatropine—Dissolve 0.20 g of Scopolamine Hydrobromide Hydrate in 20 mL of water, add 0.60 mL of 0.002 mol/L potassium permanganate VS, and allow to stand for 5 minutes: the red color in the solution does not disappear.

(4) Related substances—Dissolve 0.15 g of Scopolamine Hydrobromide Hydrate in 3 mL of water, and use this solution as the sample solution.

(i) To 1 mL of the sample solution add 2 to 3 drops of ammonia TS: no turbidity is produced.

(ii) To 1 mL of the sample solution add 2 to 3 drops of potassium hydroxide TS: a transient white turbidity might be produced, and disappears clearly in a little while.

Loss on drying <2.41> Not more than 13.0% [1.5 g; first dry in a desiccator (silica gel) for 24 hours, then dry at 105°C for 3 hours].

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Scopolamine Hydrobromide Hydrate, previously dried in 10 mL of acetic acid (100) by warming. After cooling, add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.43 mg of $C_{17}H_{21}NO_4 \cdot HBr$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Serrapeptase

セラペプターゼ

[95077-02-4]

Serrapeptase is the enzyme preparation having proteolytic activity, produced by the growth of *Serratia* species.

Usually, it is diluted with Lactose Hydrate.

It contains not less than 2000 serrapeptase Units and not more than 2600 serrapeptase Units per mg.

It is hygroscopic.

Description Serrapeptase occurs as a grayish white to light brown powder, having a slight characteristic odor.

Identification Dissolve 0.4 g of Serrapeptase in 100 mL of acetic acid-sodium acetate buffer solution, pH 5.0, transfer exactly 1 mL each of this solution into three tubes, and refer to them as A, B and C. To tube A add exactly 1 mL of water, to tubes B and C add exactly 1 mL of 0.04 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, mix gently, and allow them to stand in a water bath at $4 \pm 1^\circ\text{C}$ for about 1 hour. Then, to the tube B add exactly 2 mL of 0.04 mol/L zinc chloride TS, to the tubes A and C add exactly 2 mL of water, mix gently, and allow them to stand in a water bath at $4 \pm 1^\circ\text{C}$ for about 1 hour. Pipet 1 mL each of these solutions, add borate-hydrochloric acid buffer solution, pH 9.0 to the solutions A and B to make exactly 200 mL, to the solution C to make exactly 50 mL, and use these solutions as the sample solutions. Proceed with these sample solutions as directed in

the Assay: the activities of the solutions A and B are almost the same, and the activity of the solution C is not more than 5% of that of the solution A.

Activity of solutions A, B or C = $(A_T/A_S) \times (1/20) \times D \times 176$

A_S : Absorbance of the standard solution

A_T : Absorbance of the sample solution

20: Reaction time (minute)

D : Dilution rate (200 for solution A and B, 50 for solution C)

176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken \times amount of tyrosine in 2 mL of tyrosine standard solution)

Purity (1) Heavy metals <1.07>—Put 1.0 g of Serrapeptase in a porcelain crucible, add 2 drops each of sulfuric acid and nitric acid, and incinerate by ignition. After cooling, to the residue add 2 mL of hydrochloric acid, evaporate to dryness on a water bath, add 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. After cooling, filter if necessary, wash the filter paper with 10 mL of water, put the filtrate and washing in a Nessler tube, add water to make 50 mL, and use this solution as the test solution. Prepare the control solution as follows: Evaporate to dryness 2 drops each of sulfuric acid and nitric acid on a sand bath, add 2 mL of hydrochloric acid to the residue, evaporate to dryness on a water bath, add 2.0 mL of Standard Lead Solution, 10 mL of a solution of hydroxylamine hydrochloride (3 in 100) and 2 mL of dilute acetic acid, and heat on a water bath for 5 minutes. Proceed in the same manner as directed for the preparation of the test solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Serrapeptase according to Method 3, excepting addition of 5 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (3 in 10) instead of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), evaporating to dryness on a water bath, then incinerating with a small flame, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 1.5% (1 g).

Assay (i) Sample solution: Dissolve exactly 0.100 g of Serrapeptase in a solution of ammonium sulfate (1 in 20) to make exactly 100 mL. Pipet 1 mL of this solution, add borate-hydrochloric acid buffer solution, pH 9.0 to make exactly 200 mL, and use this solution as the sample solution.

(ii) Tyrosine standard solution: Dissolve exactly 0.160 g of Tyrosine Reference Standard, previously dried at 105°C for 3 hours, in 0.2 mol/L hydrochloric acid TS to make exactly 1000 mL. Pipet 10 mL of this solution, and add 0.2 mol/L hydrochloric acid TS to make exactly 100 mL. Prepare before use.

(iii) Substrate solution: Previously determine the loss on drying <2.41> (60°C, reduced pressure not exceeding 0.67 kPa, 3 hours) of milk casein, previously dried. To exactly 1.20 g of the milk casein, calculated based on the loss on drying, add 160 mL of a solution of sodium borate (19 in 1000), and heat in a water bath to dissolve. After cooling, adjust the pH to exactly 9.0 with 1 mol/L hydrochloric acid TS, and add borate-hydrochloric acid buffer solution, pH 9.0 to

make exactly 200 mL. Use after warming to 37 \pm 0.5°C. Prepare before use.

(iv) Precipitation reagent: Trichloroacetic acid TS for serrapeptase. Use after warming to 37 \pm 0.5°C.

(v) Procedure: Pipet 1 mL of the sample solution, put in a glass-stoppered tube (15 \times 130 mm), allow to stand at 37 \pm 0.5°C for 5 minutes, add exactly 5 mL of the substrate solution, and mix well immediately. Allow to stand at 37 \pm 0.5°C for exactly 20 minutes, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, allow to stand at 37 \pm 0.5°C for 30 minutes, and filter through a dried filter paper. Pipet 2 mL of the filtrate, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and allow to stand at 37 \pm 0.5°C for 30 minutes. Determine the absorbance of this solution at 660 nm, A_1 , as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank. Separately, pipet 1 mL of the sample solution, add exactly 5 mL of trichloroacetic acid TS for serrapeptase, mix, add exactly 5 mL of the substrate solution, allow to stand at 37 \pm 0.5°C for 30 minutes, and proceed in the same manner as directed above to determine the absorbance A_2 . Separately, pipet 2 mL of the tyrosine standard solution, add exactly 5 mL of a solution of anhydrous sodium carbonate (3 in 50), mix, add exactly 1 mL of diluted Folin's TS (1 in 3), mix well, and proceed in the same manner as directed above to determine the absorbance A_3 . Separately, pipet 2 mL of 0.2 mol/L hydrochloric acid TS, and proceed in the same manner as directed above to determine the absorbance A_4 .

$$\begin{aligned} &\text{Serrapeptase Unit per mg of Serrapeptase} \\ &= \{(A_1 - A_2)/(A_3 - A_4)\} \times (1/20) \times 200 \times 176 \end{aligned}$$

20: Reaction time (minute)

200: Dilution rate

176: Conversion factor (Total volume of enzyme reaction solution/volume of filtrate taken \times amount of tyrosine in 2 mL of tyrosine standard solution)

One serrapeptase Unit corresponds to the amount of serrapeptase which produces 5 μ g of tyrosine per minute from 5 mL of the substrate solution under the above conditions.

Containers and storage Containers—Tight containers.

Sesame Oil

Oleum Sesami

ゴマ油

Sesame Oil is the fixed oil obtained from the seeds of *Sesamum indicum* Linné (*Pedaliaceae*).

Description Sesame Oil is a clear, pale yellow oil. It is odorless or has a faint, characteristic odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether.

It is slightly soluble in ethanol (95).

It congeals between 0°C and -5°C.

Congealing point of the fatty acids: 20 - 25°C

Identification To 1 mL of Sesame Oil add 0.1 g of sucrose and 10 mL of hydrochloric acid, and shake for 30 seconds: the acid layer becomes light red and changes to red on stand-

ing.

Specific gravity <1.13> d_{20}^{20} : 0.914 – 0.921

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 187 – 194

Unsaponifiable matters <1.13> Not more than 2.0%.

Iodine value <1.13> 103 – 118

Containers and storage Containers—Tight containers.

Purified Shellac

精製セラック

Purified Shellac is a resin-like substance obtained from a purified secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description Purified Shellac occurs as light yellow-brown to brown, lustrous, hard, brittle scutella. It has no odor or has a faint, characteristic odor.

It is freely soluble in ethanol (95) and in ethanol (99.5), and practically insoluble in water and in diethyl ether.

It dissolves in sodium hydroxide TS.

Acid value 60 – 80 <1.13> Weigh accurately about 1 g of Purified Shellac, add 40 mL of neutralized ethanol, and dissolve by warming. After cooling, titrate <2.50> with 0.1 mol/L potassium hydroxide VS (potentiometric titration).

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Purified Shellac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Purified Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(3) Ethanol-insoluble substances—Dissolve about 5 g of Purified Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(4) Rosin—Dissolve 2.0 g of Purified Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(5) Wax—Dissolve 10.0 g of Purified Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with

shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 2.0%. Weigh accurately about 1 g of medium powder of Purified Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

Total ash <5.01> Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

White Shellac

白色セラック

White Shellac is a resin-like substance obtained from a bleached secretion of *Laccifer lacca* Kerr (*Coccidae*).

Description White Shellac occurs as yellowish white to light yellow, hard, brittle granules. It is odorless or has a faint, characteristic odor.

It is sparingly soluble in ethanol (95), very slightly soluble in petroleum ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Acid value <1.13> 65 – 90 Weigh accurately about 0.5 g of White Shellac, add 50 mL of neutralized ethanol as a solvent, and dissolve by warming. After cooling, perform the test as directed in the Acid value under Purified Shellac.

Purity (1) Chloride <1.03>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) while warming, add 40 mL of water, and cool. Add 12 mL of dilute nitric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.80 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.140%).

(2) Sulfate <1.14>—Shake and dissolve 0.40 g of White Shellac in 5 mL of ethanol (95) by warming, add 40 mL of water, and cool. Add 2 mL of dilute hydrochloric acid and water to make 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 0.45 mL of 0.005 mol/L sulfuric acid VS add 2.5 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.110%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of White Shellac according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm.)

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of White Shellac according to Method 3, and perform the test. Add 10 mL of a solution of magnesium nitrate hexahy-

drate in ethanol (95) (1 in 50), then add 1.5 mL of hydrogen peroxide (30), and fire to burn (not more than 5 ppm).

(5) Ethanol-insoluble substances—Dissolve about 5 g of White Shellac, accurately weighed, in 50 mL of ethanol (95) on a water bath while shaking. Pour the ethanol solution into a tared extraction thimble, previously dried at 105°C for 2 hours, in a Soxhlet extractor, and extract with ethanol (95) for 3 hours: the mass of the residue is not more than 2.0%. Use a cylindrical weighing bottle for taring the extraction thimble.

(6) Rosin—Dissolve 2.0 g of White Shellac in 10 mL of ethanol (99.5) with thorough shaking, add gradually 50 mL of petroleum ether while shaking, and filter, if necessary. Wash the solution with two 50-mL portions of water, filter the upper layer, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 2 mL of a mixture of carbon tetrachloride and phenol (2:1), transfer the solution to a depression of a spot plate, and fill the neighboring depression with a mixture of carbon tetrachloride and bromine (4:1). Immediately cover both depressions with a watch glass, and allow to stand: the solution of the residue exhibits no purple or blue color within 1 minute.

(7) Wax—Dissolve 10.0 g of White Shellac in 150 mL of a solution of sodium carbonate decahydrate (9 in 200) with shaking on a water bath, and continue the heating for 2 hours. After cooling, collect the floating wax by filtration, wash the wax and the filter paper with water, transfer to a beaker, and dry at 65°C until the water is almost evaporated. Transfer the wax together with the filter paper to an extraction thimble in a Soxhlet extractor. Dissolve the wax remaining in the beaker with a suitable quantity of chloroform by warming. Pour the solution into the thimble, and extract with chloroform for 2 hours. Evaporate the chloroform solution to dryness, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 20 mg.

Loss on drying Not more than 6.0%. Weigh accurately about 1 g of medium powder of White Shellac, and dry at 40°C for 4 hours, then for 15 hours in a desiccator (calcium chloride for drying).

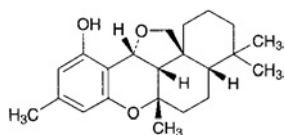
Total ash <5.01> Not more than 1.0% (1 g).

Containers and storage Containers—Well-closed containers.

Storage—In a cold place.

Siccanin

シッカニン



$C_{22}H_{30}O_3$: 342.47
(4a*S*,6a*S*,11b*R*,13a*S*,13b*S*)-4,4,6a,9-Tetramethyl-1,2,3,4,4a,5,6,6a,11b,13b-decahydro-13*H*-benzo[*a*]furo[2,3,4-*mn*]xanthen-11-ol [22733-60-4]

Siccanin is a substance having antifungal activity produced by the growth of *Helminthosporium siccanis*.

It contains not less than 980 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Siccanin is expressed as mass (potency) of siccanin ($C_{22}H_{30}O_3$).

Description Siccanin occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Siccanin in ethanol (99.5) (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Siccanin Reference Standard obtained in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Siccanin as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Siccanin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: -165 - -175° (0.1 g, ethanol (99.5), 10 mL, 100 mm).

Melting point <2.60> 138 - 142°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Siccanin according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.20 g of Siccanin in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and acetone (5:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-chlorobenzenediazonium TS on the plate: the number of the spots other than the principal spot obtained from the sample solution is not more than three, and they are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, reduced pressure not exceeding 0.67 kPa, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately an amount of Siccanin and Siccanin Reference Standard, equivalent to about 50 mg (potency), dissolve each in the internal standard solution to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of siccanin to that of the internal standard.

$$\begin{aligned} &\text{Amount } [\mu\text{g (potency)}] \text{ of } C_{22}H_{30}O_3 \\ &= W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Siccanin Reference Stand-

ard

Internal standard solution—A solution of 1,4-diphenylbenzene in methanol (1 in 30,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and phosphate buffer solution, pH 5.9 (19:6).

Flow rate: Adjust the flow rate so that the retention time of siccanin is about 17 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, siccanin and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of siccanin to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Light Anhydrous Silicic Acid

軽質無水ケイ酸

Light Anhydrous Silicic Acid, calculated on the incinerated basis, contains not less than 98.0% of silicon dioxide (SiO₂: 60.08).

Description Light Anhydrous Silicic Acid occurs as a white to bluish white, light, fine powder. It is odorless and tasteless, and smooth to the touch.

It is practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in hydrofluoric acid, in hot potassium hydroxide TS and in hot sodium hydroxide TS, and does not dissolve in dilute hydrochloric acid.

Identification (1) Dissolve 0.1 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, and add 12 mL of ammonium chloride TS: a white, gelatinous precipitate is produced. The precipitate does not dissolve in dilute hydrochloric acid.

(2) To the precipitate obtained in (1) add 10 mL of a solution of methylene blue trihydrate (1 in 10,000), and wash with water: the precipitate has a blue color.

(3) Prepare a bead by fusing ammonium sodium hydrogenphosphate tetrahydrate on a platinum loop. Bring the hot, transparent bead into contact with Light Anhydrous Silicic Acid, and fuse again: an insoluble matter is perceptible in the bead. The resulting bead, upon cooling, becomes opaque and acquires a reticulated appearance.

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Light Anhy-

drous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, filter if necessary, and wash with 10 mL of water. Combine the filtrate and washings, add 18 mL of dilute nitric acid, shake, and add water to make 50 mL. Perform the test using this solution as the test solution. To 0.15 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of sodium hydroxide TS, 18 mL of dilute nitric acid and water to make 50 mL, and use this solution as the control solution (not more than 0.011%).

(2) **Heavy metals** <1.07>—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 20 mL of sodium hydroxide TS by boiling, cool, add 15 mL of acetic acid (31), shake, filter if necessary, wash with 10 mL of water, combine the filtrate and washings, and add water to make 50 mL. Perform the test using this solution as the test solution. Add acetic acid (31) to 20 mL of sodium hydroxide TS and 1 drop of phenolphthalein TS until the color of this solution disappears, add 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL, and use this solution as the control solution (not more than 40 ppm).

(3) **Aluminum**—Dissolve 0.5 g of Light Anhydrous Silicic Acid in 40 mL of sodium hydroxide TS by boiling, cool, add sodium hydroxide TS to make 50 mL, and filter. Measure 10 mL of the filtrate, add 17 mL of acetic acid (31), shake, add 2 mL of aluminon TS and water to make 50 mL, and allow to stand for 30 minutes: the color of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.176 g of aluminum potassium sulfate 12-water in water, and add water to make 1000 mL. To 15.5 mL of this solution add 10 mL of sodium hydroxide TS, 17 mL of acetic acid (31), 2 mL of aluminon TS and water to make 50 mL.

(4) **Iron** <1.10>—To 40 mg of Light Anhydrous Silicic Acid add 10 mL of dilute hydrochloric acid, and heat for 10 minutes in a water bath while shaking. After cooling, add 0.5 g of L-tartaric acid to dissolve by shaking. Prepare the test solution with this solution according to Method 2, and perform the test according to Method B. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 500 ppm).

(5) **Calcium**—Dissolve 1.0 g of Light Anhydrous Silicic Acid in 30 mL of sodium hydroxide TS by boiling, cool, add 20 mL of water, 1 drop of phenolphthalein TS and dilute nitric acid until the color of this solution disappears, immediately add 5 mL of dilute acetic acid, shake, add water to make 100 mL, and obtain a clear liquid by centrifugation or filtration. To 25 mL of this liquid add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, immediately shake, and allow to stand for 10 minutes: the turbidity of this solution is not deeper than that of the following control solution.

Control solution: Dissolve 0.250 g of calcium carbonate, previously dried at 180°C for 4 hours, in 3 mL of dilute hydrochloric acid, and add water to make 100 mL. To 4 mL of this solution add 5 mL of dilute acetic acid and water to make 100 mL. To 25 mL of this solution add 1 mL of oxalic acid TS and ethanol (95) to make 50 mL, and shake.

(6) **Arsenic** <1.11>—Dissolve 0.40 g of Light Anhydrous Silicic Acid in 10 mL of sodium hydroxide TS by boiling in a porcelain crucible, cool, add 5 mL of water and 5 mL of dilute hydrochloric acid, shake, and perform the test with this solution as the test solution (not more than 5 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C,

4 hours).

Loss on ignition <2.43> Not more than 12.0% (1 g, 850–900°C, constant mass).

Volume test Weigh 5.0 g of Light Anhydrous Silicic Acid, transfer gradually to a 200-mL measuring cylinder, and allow to stand: the volume is not less than 70 mL.

Assay Weigh accurately about 1 g of Light Anhydrous Silicic Acid, add 20 mL of hydrochloric acid, and evaporate to dryness on a sand bath. Moisten the residue with hydrochloric acid, evaporate to dryness, and heat between 110°C and 120°C for 2 hours. Cool, add 5 mL of dilute hydrochloric acid, and heat. Allow to cool to room temperature, add 20 to 25 mL of hot water, filter rapidly, and wash the residue with warm water until the last washing becomes negative to the Qualitative Tests <1.09> (2) for chloride. Transfer the residue together with the filter paper to a platinum crucible, ignite to ash, and continue the ignition for 30 minutes. Cool, weigh the crucible, and designate the mass as *a* (g). Moisten the residue in the crucible with water, add 6 mL of hydrofluoric acid and 3 drops of sulfuric acid, and evaporate to dryness. Heat strongly for 5 minutes, cool, weigh the crucible, and designate the mass as *b* (g).

Content (g) of silicon dioxide (SiO₂) = *a* – *b*

Containers and storage Containers—Tight containers.

Silver Nitrate

硝酸銀

AgNO₃: 169.87

Silver Nitrate, when dried, contains not less than 99.8% of AgNO₃.

Description Silver Nitrate occurs as lustrous, colorless or white crystals.

It is very soluble in water, soluble in ethanol (95), and practically insoluble in diethyl ether.

It gradually turns grayish black by light.

Identification A solution of Silver Nitrate (1 in 50) responds to the Qualitative Tests <1.09> for silver salt and for nitrate.

Purity (1) Clarity and color of solution, and acidity or alkalinity—Dissolve 1.0 g of Silver Nitrate in 10 mL of freshly boiled and cooled water: the solution is clear and colorless. It is neutral.

(2) Bismuth, copper and lead—To 5 mL of a solution of Silver Nitrate (1 in 10) add 3 mL of ammonia TS: the solution is clear and colorless.

Loss on drying <2.41> Not more than 0.20% (2 g, silica gel, light resistant, 4 hours).

Assay Weigh accurately about 0.7 g of Silver Nitrate, previously powdered and dried, dissolve in 50 mL of water, add 2 mL of nitric acid, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS

= 16.99 mg of AgNO₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Silver Nitrate Ophthalmic Solution

硝酸銀点眼液

Silver Nitrate Ophthalmic Solution is an aqueous eye lotion.

It contains not less than 0.95 w/v% and not more than 1.05 w/v% of silver nitrate (AgNO₃: 169.87).

Method of preparation

Silver Nitrate	10 g
Purified Water	a sufficient quantity
<hr/>	
	To make 1000 mL

Prepare as directed under Ophthalmic Solution, with the above ingredients.

Description Silver Nitrate Ophthalmic Solution is a clear, colorless liquid.

Identification Silver Nitrate Ophthalmic Solution responds to the Qualitative Tests <1.09> for silver salt and for nitrate.

Assay Measure accurately 20 mL of Silver Nitrate Ophthalmic Solution, add 30 mL of water and 2 mL of nitric acid, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS = 16.99 mg of AgNO₃

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Silver Protein

プロテイン銀

Silver Protein is a compound of silver and proteins.

It contains not less than 7.5% and not more than 8.5% of silver (Ag: 107.87).

Description Silver Protein occurs as a light yellow-brown to brown powder. It is odorless. It (1 g) dissolves slowly in 2 mL of water. It is practically insoluble in ethanol (95), in diethyl ether and in chloroform.

The pH of a solution of Silver Protein (1 in 10) is between 7.0 and 8.5.

It is slightly hygroscopic.

It is affected by light.

Identification (1) To 10 mL of a solution of Silver Protein (1 in 100) add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. To the filtrate add 5 mL of a solution of sodium hydroxide (1 in 10), and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops.

(2) To 5 mL of a solution of Silver Protein (1 in 100) add dropwise iron (III) chloride TS: the color of the solution fades and a precipitate is gradually formed.

(3) Incinerate 0.2 g of Silver Protein by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: this solution responds to the Qualitative Tests <1.09> (1) for silver salt.

Purity Silver salt—Dissolve 0.10 g of Silver Protein in 10 mL of water, and filter. To the filtrate add 1 mL of potassium chromate TS: no turbidity is produced.

Assay Transfer about 1 g of Silver Protein, accurately weighed, to a 100-mL decomposition flask, add 10 mL of sulfuric acid, cover the flask with a funnel, and boil for 5 minutes. Cool, add dropwise 3 mL of nitric acid with caution, and heat for 30 minutes without boiling. Cool, add 1 mL of nitric acid, boil, and, if necessary, repeat this operation until the solution becomes colorless. After cooling, transfer the solution to a 250-mL conical flask with 100 mL of water, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 10.79 mg of Ag

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Silver Protein Solution

プロテイン銀液

Silver Protein Solution contains not less than 0.22 w/v% and not more than 0.26 w/v% of silver (Ag: 107.87).

Method of preparation

Silver Protein	30 g
Glycerin	100 mL
Mentha Water	a sufficient quantity
To make 1000 mL	

Dissolve and mix the above ingredients.

Description Silver Protein Solution is a clear, brown liquid, having the odor of mentha oil.

Identification (1) To 1 mL of Silver Protein Solution add 10 mL of ethanol (95), mix, and add 2 mL of sodium hydroxide TS. Add immediately 1 mL of a solution of copper (II) chloride dihydrate in ethanol (95) (1 in 10), shake, and filter: the filtrate is blue in color (glycerin).

(2) To 3 mL of Silver Protein Solution add water to make 10 mL, add 2 mL of dilute hydrochloric acid, shake frequently for 5 minutes, and filter. Add 5 mL of a solution of sodium hydroxide (1 in 10) to the filtrate, and add 2 mL of diluted copper (II) sulfate TS (2 in 25): a purple color develops (silver protein).

(3) To 5 mL of the sample solution obtained in (2) add iron (III) chloride TS dropwise: a brown precipitate is formed (silver protein).

(4) Place 3 mL of Silver Protein Solution in a crucible,

heat cautiously, and evaporate almost to dryness. Then incinerate gradually by strong heating, dissolve the residue in 1 mL of nitric acid by warming, and add 10 mL of water: the solution responds to the Qualitative Tests <1.09> (1) for silver salt.

Assay Pipet 25 mL of Silver Protein Solution into a 250-mL Kjeldahl flask, and heat cautiously until a white gas of glycerin is evolved. After cooling, add 25 mL of sulfuric acid, cover the flask with a funnel, and heat gently for 5 minutes. After cooling, drop gradually 5 mL of nitric acid, heat with occasional shaking in a water bath for 45 minutes, and cool. Add 2 mL of nitric acid, boil gently, and repeat this operation until the solution becomes colorless upon cooling. Transfer cautiously the cooled content in the flask into a 500-mL conical flask with 250 mL of water. Boil gently for 5 minutes, cool, and titrate <2.50> with 0.1 mol/L ammonium thiocyanate VS (indicator: 3 mL of ammonium iron (III) sulfate TS).

Each mL of 0.1 mol/L ammonium thiocyanate VS
= 10.79 mg of Ag

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Simple Ointment

単軟膏

Method of preparation

Yellow Beeswax	330 g
Fixed oil	a sufficient quantity
To make 1000 g	

Prepare as directed under Ointments, with the above ingredients.

Description Simple Ointment is yellow in color. It has a slight, characteristic odor.

Containers and storage Containers—Tight containers.

Simple Syrup

単シロップ

Simple Syrup is an aqueous solution of Sucrose.

Method of preparation

Sucrose	850 g
Purified Water	a sufficient quantity
To make 1000 mL	

Prepare as directed under Syrups, with the above materials.

Description Simple Syrup is a clear, colorless to pale yellow, viscous liquid.

It is odorless and has a sweet taste.

Identification (1) Evaporate Simple Syrup on a water bath

to dryness. 1 g of the residue so obtained, when ignited, melts to swell, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of the residue obtained in (1) add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark red precipitate is produced.

Specific gravity <2.56> d_{20}^{20} : 1.310 – 1.325

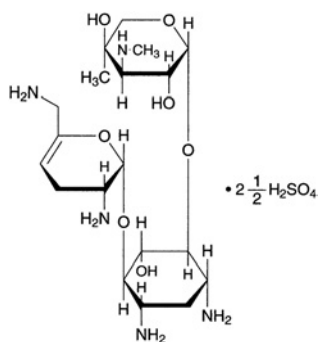
Purity (1) Artificial sweetening agents—To 100 mL of Simple Syrup add 100 mL of water, shake, acidify a 50-mL portion of the solution with dilute sulfuric acid, and make another 50-mL portion alkaline with sodium hydroxide TS. To each portion add 100 mL of diethyl ether, shake, separate the diethyl ether layer, and evaporate the combined diethyl ether extract on a water bath to dryness: the residue has no sweet taste.

(2) Salicylic acid—To the residue obtained in (1) add 2 to 3 drops of dilute iron (III) chloride TS: no purple color develops.

Containers and storage Containers—Tight containers.

Sisomicin Sulfate

シソマイシン硫酸塩



$C_{19}H_{37}N_5O_7 \cdot 2\frac{1}{2}H_2SO_4$: 692.72

3-Deoxy-4-C-methyl-3-methylamino- β -L-arabinopyranosyl-(1 \rightarrow 6)-[2,6-diamino-2,3,4,6-tetra-deoxy- α -D-glycero-hex-4-enopyranosyl-(1 \rightarrow 4)]-2-deoxy-D-streptamine hemipentasulfate [53179-09-2]

Sisomicin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Micromonospora inyoensis*.

It contains not less than 590 μ g (potency) and not more than 700 μ g (potency) per mg, calculated on the dried basis. The potency of Sisomicin Sulfate is expressed as mass (potency) of sisomicin ($C_{19}H_{37}N_5O_7$: 447.53).

Description Sisomicin Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95).

It is hygroscopic.

Identification (1) Dissolve 50 mg of Sisomicin Sulfate in 5 mL of water, and add 0.3 mL of bromine TS: the solution is immediately decolorized.

(2) Dissolve 15 mg each of Sisomicin Sulfate and Sisomicin Sulfate Reference Standard in 5 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 5 minutes: the principal spots from the sample solution and standard solution exhibit a red-purple to red-brown color and show the same R_f value.

(3) A solution of Sisomicin Sulfate (1 in 100) responds to the Qualitative Tests <1.09> (1) for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: +100 – +110° (0.25 g calculated on the dried basis, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of Sisomicin Sulfate in 5 mL of water: the pH of the solution is between 3.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sisomicin Sulfate in 5 mL of water: the solution is clear and colorless to light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sisomicin Sulfate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 50 mg of Sisomicin Sulfate, calculated on the dried basis, in water to make 10 mL, and use this solution as the sample solution. Pipet 0.5 mL, 1 mL and 1.5 mL of the sample solution, add water to each to make exactly 50 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform, ammonia water (28) and acetone (2:2:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 0.2% ninhydrin-water saturated 1-butanol TS on the plate, and heat at 100°C for 5 minutes. The spots corresponding to R_f about 0.35 and R_f about 0.30 are not more intense than that of the spot from the standard solution (3), and the spot of gallamine corresponding to R_f about 0.25 is not more intense than the spot from the standard solution (1). The total amount of the related substances is not more than 6%.

Loss on drying <2.41> Not more than 15.0% (0.15 g, in vacuum not exceeding 0.67 kPa, 110°C, 3 hours). Sampling should be carried out in a manner to avoid moisture absorption.

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus epidermidis* ATCC 12228

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to

8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Sisomicin Sulfate Reference Standard equivalent to about 25 mg (potency), add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 1 µg (potency) and 0.25 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sisomicin sulfate equivalent to about 25 mg (potency), add 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of the solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 1 µg (potency) and 0.25 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, not exceeding -20°C, under nitrogen or argon atmosphere.

Freeze-dried Smallpox Vaccine

乾燥痘そうワクチン

Freeze-dried Smallpox Vaccine is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine becomes a white to gray, turbid liquid on addition of solvent.

Freeze-dried Smallpox Vaccine Prepared in Cell Culture

乾燥細胞培養痘そうワクチン

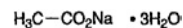
Freeze-dried Smallpox Vaccine Prepared in Cell Culture is a preparation for injection which is dissolved before use. It contains live vaccinia virus.

It conforms to the requirements of Freeze-dried Smallpox Vaccine Prepared in Cell Culture in the Minimum Requirements for Biological Products.

Description Freeze-dried Smallpox Vaccine Prepared in Cell Culture becomes a reddish clear liquid on addition of solvent.

Sodium Acetate Hydrate

酢酸ナトリウム水和物



$\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$: 136.08

Monosodium acetate trihydrate [6131-90-4]

Sodium Acetate Hydrate, when dried, contains not less than 99.5% of sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$: 82.03).

Description Sodium Acetate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless or has a slight, acetous odor. It has a cool, saline and slightly bitter taste.

It is very soluble in water, freely soluble in acetic acid (100), soluble in ethanol (95), and practically insoluble in diethyl ether.

It is efflorescent in warm, dry air.

Identification A solution of Sodium Acetate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for acetate and for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 2.0 g of Sodium Acetate Hydrate in 20 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 1.0 g of Sodium Acetate Hydrate in 20 mL of freshly boiled and cooled water, and add 3 drops of phenolphthalein TS: a red color develops. When cooled to 10°C, or 1.0 mL of 0.01 mol/L hydrochloric acid VS is added after cooling to 10°C, the red color disappears.

(3) Chloride <1.03>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011 %).

(4) Sulfate <1.14>—Perform the test with 1.0 g of Sodium Acetate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.017%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Acetate Hydrate according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Calcium and magnesium—Dissolve 4.0 g of Sodium Acetate Hydrate in 25 mL of water, add 6 g of ammonium chloride, 20 mL of ammonia solution (28) and 0.25 mL of a solution of sodium hydrogensulfite (1 in 10), and titrate <2.50 > with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the blue color changes to grayish blue (indicator: 0.1 g of methylthymol blue-potassium nitrate indicator): the amount of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS consumed is not more than 0.5 mL.

(7) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Acetate Hydrate, according to Method 1, and perform the test (not more than 2 ppm).

(8) Potassium permanganate-reducing substance—Dissolve 1.0 g of Sodium Acetate Hydrate in 100 mL of water, add 5 mL of dilute sulfuric acid, boil, add 0.50 mL of 0.002 mol/L potassium permanganate VS, and further boil for 5

minutes: the red color of the solution does not disappear.

Loss on drying <2.41> 39.0 – 40.5% (1 g, first at 80°C for 2 hours, and then at 130°C for 2 hours).

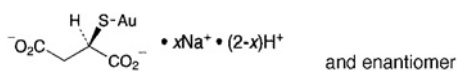
Assay Weigh accurately about 0.2 g of Sodium Acetate Hydrate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from yellow to green (indicator: 1 mL of *p*-naphtholbenzein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.203 mg of C₂H₃NaO₂

Containers and storage Containers—Tight containers.

Sodium Aurothiomalate

金チオリンゴ酸ナトリウム



Mixture of C₄H₃AuNa₂O₄S: 390.08 and
C₄H₄AuNaO₄S: 368.09

Monogold monosodium monohydrogen (2*RS*)-2-sulfidobutane-1,4-dioate

Monogold disodium (2*RS*)-2-sulfidobutane-1,4-dioate
[12244-57-4, Sodium Aurothiomalate]

Sodium Aurothiomalate contains not less than 49.0% and not more than 52.5% of gold (Au: 196.97), calculated on the anhydrous basis and corrected by the amount of ethanol.

Description Sodium Aurothiomalate occurs as white to light yellow, powder or granules.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It is hygroscopic.

It changes in color by light to greenish pale yellow.

Identification (1) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 1 mL of a solution of calcium nitrate tetrahydrate (1 in 10): a white precipitate is produced, and it dissolves in dilute nitric acid and reappears on the addition of ammonium acetate TS.

(2) To 2 mL of a solution of Sodium Aurothiomalate (1 in 10) add 3 mL of silver nitrate TS: a yellow precipitate is produced, and it dissolves in an excess of ammonia TS.

(3) Place 2 mL of a solution of Sodium Aurothiomalate (1 in 10) in a porcelain crucible, add 1 mL of ammonia TS and 1 mL of hydrogen peroxide (30), evaporate to dryness, and ignite. Add 20 mL of water to the residue, and filter: the residue on the filter paper occurs as a yellow or dark yellow, powder or granules.

(4) The filtrate obtained in (3) responds to the Qualitative Tests <1.09> for sodium salt.

(5) The filtrate obtained in (3) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 1.0 g of Sodium Aurothiomalate in 10 mL of water: the pH of this solution is between 5.8 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Sodium Aurothiomalate in 10 mL of water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sodium Aurothiomalate according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Aurothiomalate according to Method 3, and perform the test (not more than 2 ppm).

(4) Ethanol—Weigh accurately about 0.2 g of Sodium Aurothiomalate, add exactly 3 mL of the internal standard solution and 2 mL of water to dissolve, and use this solution as the sample solution. Separately, pipet 3 mL of ethanol (99.5), and add water to make exactly 1000 mL. Pipet 2 mL of this solution, add exactly 3 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the ratios of the peak area of ethanol to that of the internal standard, Q_T and Q_S : the amount of ethanol is not more than 3.0%.

Amount (mg) of ethanol = $(Q_T/Q_S) \times 6 \times 0.793$

0.793: Density (g/mL) of ethanol (99.5) at 20°C

Internal standard solution—A solution of 2-propanol (1 in 500).

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 3 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (particle diameter: 150 – 180 μm) (average pore size: 0.0085 μm; 300 – 400 m²/g).

Column temperature: A constant temperature of about 180°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 7 minutes.

System suitability—

System performance: When the procedure is run with 2 μL of the standard solution under the above operating conditions, ethanol and the internal standard are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 2 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of ethanol to that of the internal standard is not more than 2.0%.

Water <2.48> Not more than 5.0% (0.1 g, coulometric titration). Use a water vaporizer (heating temperature: 105°C; heating time: 30 minutes).

Assay Weigh accurately about 25 mg of Sodium Aurothiomalate, and dissolve in 2 mL of aqua regia by heating. After cooling, add water to make exactly 100 mL. Pipet 2 mL of the solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, pipet 5 mL, 10 mL and 15 mL of Standard Gold Solution for atomic absorption spectrophotometry, add water to make exactly 25 mL, and use these solutions as the standard solutions (1), (2) and (3), respectively. Perform the test with the sample solu-

tion and standard solutions (1), (2) and (3) as directed under Atomic Absorption Spectrophotometry <2.23> under the following conditions. Determine the amount of gold in the sample solution using the calibration curve obtained from the absorbances of the standard solutions.

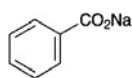
Gas: Combustible gas—Acetylene
Supporting gas—Air

Lamp: Gold hollow-cathode lamp
Wavelength: 242.8 nm

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sodium Benzoate

安息香酸ナトリウム



$C_7H_5NaO_2$: 144.10

Monosodium benzoate [532-32-1]

Sodium Benzoate, when dried, contains not less than 99.0% of $C_7H_5NaO_2$.

Description Sodium Benzoate occurs as white granules, crystals or crystalline powder. It is odorless, and has a sweet and saline taste.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification A solution of Sodium Benzoate (1 in 100) responds to the Qualitative Tests <1.09> for benzoate and the Qualitative Tests <1.09> (1) and (2) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Benzoate in 5 mL of water: the solution is clear and colorless.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Benzoate in 20 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS and 0.20 mL of 0.05 mol/L sulfuric acid VS: the solution remains colorless. To this solution add 0.40 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Sulfate <1.14>—Dissolve 0.40 g of Sodium Benzoate in 40 mL of water, add slowly 3.5 mL of dilute hydrochloric acid with thorough stirring, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, take the subsequent 20 mL of the filtrate, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.120%).

(4) Heavy metals <1.07>—Dissolve 2.0 g of Sodium Benzoate in 44 mL of water, add gradually 6 mL of dilute hydrochloric acid with thorough stirring, and filter. Discard the first 5 mL of the filtrate, take the subsequent 25 mL of the filtrate, neutralize with ammonia TS, add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(5) Arsenic <1.11>—Mix well 1.0 g of Sodium Benzoate with 0.40 g of calcium hydroxide, ignite, dissolve the residue in 10 mL of dilute hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

(6) Chlorinated compounds—Dissolve 1.0 g of Sodium Benzoate in 10 mL of water, add 10 mL of dilute sulfuric acid, and extract with two 20-mL portions of diethyl ether. Combine the diethyl ether extracts, and evaporate the diethyl ether on a water bath. Place 0.5 g of the residue and 0.7 g of calcium carbonate in a crucible, mix with a small amount of water, and dry. Ignite it at about 600°C, dissolve in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washing, add water to make 50 mL, and add 0.5 mL of silver nitrate TS: this solution has no more turbidity than the following control solution

Control solution: Dissolve 0.7 g of calcium carbonate in 20 mL of dilute nitric acid, and filter. Wash the residue with 15 mL of water, combine the filtrate and the washings, add 1.2 mL of 0.01 mol/L Hydrochloric acid VS and water to make 50 mL, and add 0.5 mL of silver nitrate TS.

(7) Phthalic acid—To 0.10 g of Sodium Benzoate add 1 mL of water and 1 mL of resorcinol-sulfuric acid TS, and heat the mixture in an oil bath heated at a temperature between 120°C and 125°C to evaporate the water, then heat the residue for further 90 minutes, cool, and dissolve in 5 mL of water. To 1 mL of the solution add 10 mL of a solution of sodium hydroxide (43 in 500), shake, then examine under light at a wavelength between 470 nm and 490 nm: the green fluorescence of the solution is not more intense than that of the following control solution.

Control solution: Dissolve 61 mg of potassium hydrogen phthalate in water to make exactly 1000 mL. Pipet exactly 1 mL of the solution, add 1 mL of resorcinol-sulfuric acid TS, and proceed as directed above.

Loss on drying <2.41> Not more than 1.5% (2 g, 110°C, 4 hours).

Assay Weigh accurately about 1.5 g of Sodium Benzoate, previously dried, and transfer to a 300-mL glass-stoppered flask. Dissolve in 25 mL of water, add 75 mL of diethyl ether and 10 drops of bromophenol blue TS, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS, while mixing the aqueous and diethyl ether layers by vigorous shaking, until a persistent, light green color is produced in the aqueous layer.

Each mL of 0.5 mol/L hydrochloric acid VS
= 72.05 mg of $C_7H_5NaO_2$

Containers and storage Containers—Well-closed containers.

Sodium Bicarbonate

炭酸水素ナトリウム

$NaHCO_3$: 84.01

Sodium Bicarbonate contains not less than 99.0% of $NaHCO_3$.

Description Sodium Bicarbonate occurs as white crystals or

crystalline powder. It is odorless, and has a characteristic, saline taste.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It slowly decomposes in moist air.

Identification A solution of Sodium Bicarbonate (1 in 30) responds to the Qualitative Tests <1.09> for sodium salt and for bicarbonate.

pH <2.54> Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of water: the pH of this solution is between 7.9 and 8.4.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—To 0.40 g of Sodium Bicarbonate add 4 mL of dilute nitric acid, heat to boil, cool, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.040%).

(3) Carbonate—Dissolve 1.0 g of Sodium Bicarbonate in 20 mL of freshly boiled and cooled water with very gentle swirling at a temperature not exceeding 15°C. Add 2.0 mL of 0.1 mol/L hydrochloric acid VS and 2 drops of phenolphthalein TS: no red color develops immediately.

(4) Ammonium—Heat 1.0 g of Sodium Bicarbonate: the gas evolved does not change moistened red litmus paper to blue.

(5) Heavy metals <1.07>—Dissolve 4.0 g of Sodium Bicarbonate in 5 mL of water and 4.5 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 2 mL of dilute acetic acid, 35 mL of water and 1 drop of ammonium TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 4.5 mL of hydrochloric acid to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 5 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of Sodium Bicarbonate in 3 mL of water and 2 mL of hydrochloric acid, and perform the test using this solution as the test solution (not more than 2 ppm).

Assay Weigh accurately about 2 g of Sodium Bicarbonate, dissolve in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS. When the color of the solution changes from blue to yellow-green, boil with caution, cool, and continue the titration <2.50> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 84.01 mg of NaHCO₃

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Bicarbonate Injection

炭酸水素ナトリウム注射液

Sodium Bicarbonate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of sodium hydrogen carbonate (NaHCO₃: 84.01).

Method of preparation Prepare as directed under Injections, with Sodium Bicarbonate.

Description Sodium Bicarbonate Injection is a clear, colorless liquid.

Identification To a volume of Sodium Bicarbonate Injection, equivalent to 1 g of Sodium Bicarbonate according to the labeled amount, add water to make 30 mL: the solution responds to the Qualitative Tests <1.09> for sodium salt and for bicarbonate.

pH <2.54> 7.0 – 8.5

Bacterial endotoxins <4.01> Less than 5.0 EU/mEq.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Sodium Bicarbonate Injection, equivalent to about 2 g of sodium hydrogen carbonate (NaHCO₃), titrate with 0.5 mol/L sulfuric acid VS, and proceed as directed in the Assay under Sodium Bicarbonate.

Each mL of 0.5 mol/L sulfuric acid VS
= 84.01 mg of NaHCO₃

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Bisulfite

Sodium Hydrogen Sulfite

亜硫酸水素ナトリウム

NaHSO₃: 104.06

Sodium Bisulfite is a mixture of sodium hydrogen-sulfite and sodium pyrosulfite.

It contains not less than 64.0% and not more than 67.4% of sulfur dioxide (SO₂: 64.06).

Description Sodium Bisulfite occurs as white granules or powder, having the odor of sulfur dioxide.

Sodium Bisulfite is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Bisulfite (1 in 20) is acid.

Sodium Bisulfite is slowly affected by air or by light.

Identification A solution of Sodium Bisulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for bisulfite.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water: the solution is clear and colorless.

(2) Thiosulfate—Dissolve 1.0 g of Sodium Bisulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Bisulfite in 10 mL of water, add 5 mL of hydrochloric acid, and

evaporate on a water bath to dryness. To the residue add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of hydrochloric acid on a water bath to dryness, and add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Iron <1.10>—Prepare the test solution with 1.0 g of Sodium Bisulfite according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 0.5 g of Sodium Bisulfite in 10 mL of water. Add 1 mL of sulfuric acid, heat on a sand bath until white fumes are evolved, add water to make 5 mL, and perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Bisulfite, and transfer immediately into an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 3.203 mg of SO₂

Containers and storage Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Borate

ホウ砂

Na₂B₄O₇·10H₂O: 381.37

Sodium Borate contains not less than 99.0% and not more than 103.0% of Na₂B₄O₇·10H₂O.

Description Sodium Borate occurs as colorless or white crystals or a white, crystalline powder. It is odorless, and has a slightly characteristic, saline taste.

It is freely soluble in glycerin, soluble in water, and practically insoluble in ethanol (95), in ethanol (99.5) and in diethyl ether.

When placed in dry air, Sodium Borate effloresces and is coated with a white powder.

Identification A solution of Sodium Borate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for borate.

pH <2.54> Dissolve 1.0 g of Sodium Borate in 20 mL of water: the pH of this solution is between 9.1 and 9.6.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Borate in 20 mL of water by warming slightly: the solution is clear and colorless.

(2) Carbonate or bicarbonate—Dissolve 1.0 g of powdered Sodium Borate in 20 mL of freshly boiled and cooled water, and add 3 mL of dilute hydrochloric acid: the solution

does not effervesce.

(3) Heavy metals <1.07>—Dissolve 1.5 g of Sodium Borate in 25 mL of water and 7 mL of 1 mol/L hydrochloric acid TS, add 1 drop of phenolphthalein TS, and add ammonia TS until a pale red color develops. Then add dilute acetic acid until the solution becomes colorless again, add 2 mL of dilute acetic acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 3.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.40 g of Sodium Borate according to Method 1, and perform the test (not more than 5 ppm).

Assay Weigh accurately about 2 g of Sodium Borate, dissolve in 50 mL of water, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS (indicator: 3 drops of methyl red TS).

Each mL of 0.5 mol/L hydrochloric acid VS
= 95.34 mg of Na₂B₄O₇·10H₂O

Containers and storage Containers—Tight containers.

Sodium Bromide

臭化ナトリウム

NaBr: 102.89

Sodium Bromide, when dried, contains not less than 99.0% of NaBr.

Description Sodium Bromide occurs as colorless or white crystals or crystalline powder. It is odorless.

It is freely soluble in water, and soluble in ethanol (95).

It is hygroscopic, but not deliquescent.

Identification A solution of Sodium Bromide (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt and for bromide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Bromide in 3 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Bromide in 10 mL of water, add 0.10 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS, heat to boil, and cool: the solution is colorless.

(3) Chloride—Make a calculation from the result obtained in the Assay. Not more than 97.9 mL of 0.1 mol/L silver nitrate VS is consumed for 1 g of Sodium Bromide.

(4) Sulfate <1.14>—Perform the test with 2.0 g of Sodium Bromide. Prepare the control solution with 1.0 mL of 0.005 mol/L sulfuric acid VS (not more than 0.024%).

(5) Iodide—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 2 to 3 drops of iron (III) chloride TS and 1 mL of chloroform, and shake: no red-purple color develops in the chloroform layer.

(6) Bromate—Dissolve 1.0 g of Sodium Bromide in 10 mL of freshly boiled and cooled water, and add 2 drops of potassium iodide TS, 1 mL of starch TS and 3 drops of dilute sulfuric acid. Shake the mixture gently, and allow to stand for 5 minutes: no blue color develops.

(7) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Bromide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Sodium Bromide in 10 mL of water, add 0.5 mL of dilute hydrochloric acid and 1 mL of potassium sulfate TS, and allow to stand for 10 minutes: no turbidity is produced.

(9) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Bromide according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 5.0% (1 g, 110°C, 4 hours).

Assay Weigh accurately about 0.4 g of Sodium Bromide, previously dried, and dissolve in 50 mL of water. Add 10 mL of dilute nitric acid and 50 mL of 0.1 mol/L silver nitrate VS, exactly measured, and titrate <2.50> the excess silver nitrate with 0.1 mol/L ammonium thiocyanate VS (indicator: 2 mL of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.1 mol/L silver nitrate VS
= 10.29 mg of NaBr

Containers and storage Containers—Tight containers.

Sodium Carbonate Hydrate

炭酸ナトリウム水和物

Na₂CO₃·10H₂O: 286.14

Sodium Carbonate Hydrate contains not less than 99.0% and not more than 103.0% of Na₂CO₃·10H₂O.

Description Sodium Carbonate Hydrate occurs as colorless or white crystals.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Sodium Carbonate Hydrate (1 in 10) is alkaline.

It is efflorescent in air.

It liquefies in its water of crystallization at 34°C, and becomes anhydrous at above 100°C.

Identification A solution of Sodium Carbonate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Carbonate Hydrate in 5 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Sodium Carbonate Hydrate in 10 mL of water, add 7 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals <1.07>—Dissolve 2.0 g of Sodium Carbonate Hydrate in 10 mL of water, add 8 mL of dilute hydrochloric acid, and evaporate to dryness on a water bath. Dissolve the residue in 35 mL of water and 2 mL of dilute a-

cetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 8 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.65 of Sodium Carbonate Hydrate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying <2.41> 61.0 ~ 63.0% (1 g, 105°C, 4 hours).

Assay Dissolve about 3 g of Sodium Carbonate Hydrate, weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Boil cautiously, cool, and further titrate <2.50> until a greenish yellow color appears (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 143.1 mg of Na₂CO₃·10H₂O

Containers and storage Containers—Tight containers.

Dried Sodium Carbonate

乾燥炭酸ナトリウム

Na₂CO₃: 105.99

Dried Sodium Carbonate, when dried, contains not less than 99.0% of Na₂CO₃.

Description Dried Sodium Carbonate occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

A solution of Dried Sodium Carbonate (1 in 10) is alkaline.

It is hygroscopic.

Identification A solution of Dried Sodium Carbonate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for carbonate.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Dried Sodium Carbonate in 10 mL of water, add 12 mL of dilute nitric acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.071%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Dried Sodium Carbonate in 10 mL of water, add 7.5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water and 2 mL of dilute acetic acid, dilute with water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 7.5 mL of dilute hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and dilute with water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.65 g

of Dried Sodium Carbonate according to Method 1, and perform the test (not more than 3.1 ppm).

Loss on drying <2.41> Not more than 2.0% (2 g, 106°C, 4 hours).

Assay Dissolve about 1.2 g of Dried Sodium Carbonate, weighed accurately, in 25 mL of water, and titrate with 0.5 mol/L sulfuric acid VS until the color of the solution changes from blue to yellow-green. Then boil cautiously, cool, and further titrate <2.50> until a greenish yellow color develops (indicator: 2 drops of bromocresol green TS).

Each mL of 0.5 mol/L sulfuric acid VS
= 52.99 mg of Na₂CO₃

Containers and storage Containers—Tight containers.

Sodium Chloride

塩化ナトリウム

NaCl: 58.44

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Sodium Chloride contains not less than 99.0% and not more than 100.5% of NaCl, calculated on the dried basis.

♦**Description** Sodium Chloride occurs as colorless or white, crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).♦

Identification (1) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

(2) A solution of Sodium Chloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

♦**Purity** (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Chloride in 5 mL of water: the solution is clear and colorless.♦

(2) Acidity or alkalinity—Dissolve 20.0 g of Sodium Chloride in 100.0 mL of freshly boiled and cooled water, and use this solution as the sample solution. To 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L hydrochloric acid VS: the color of the solution is yellow. Separately, to 20 mL of the sample solution add 0.1 mL of bromothymol blue TS and 0.5 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is blue.

(3) Sulfates—To 7.5 mL of the sample solution obtained in (2) add water to make 30 mL, and use this solution as the sample solution. Separately, dissolve 0.181 g of potassium sulfate in diluted ethanol (99.5) (3 in 10) to make exactly 500 mL. Pipet 5 mL of this solution, and add diluted ethanol (99.5) (3 in 10) to make exactly 100 mL. To 4.5 mL of this solution add 3 mL of a solution of barium chloride dihydrate (1 in 4), shake, and allow to stand for 1 minutes. To 2.5 mL of this solution add 15 mL of the sample solution and 0.5 mL of acetic acid (31), and allow to stand for 5 minutes: any turbidity produced does not more than that produced in the follow-

ing control solution.

Control solution: Dissolve 0.181 g of potassium sulfate in water to make exactly 500 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and proceed in the same manner as directed above using this solution instead of the sample solution.

(4) Phosphates—To 2.0 mL of the sample solution obtained in (2) add 5 mL of 2 mol/L sulfuric acid TS and water to make 100.0 mL, then add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes: the color of the solution is not darker than the following control solution.

Control solution: To 1.0 mL of Standard Phosphoric Acid Solution add 12.5 mL of 2 mol/L sulfuric acid TS and water to make exactly 250 mL. To 100 mL of this solution add 4 mL of ammonium molybdate-sulfuric acid TS and 0.1 mL of tin (II) chloride-hydrochloric acid TS, and allow to stand for 10 minutes.

(5) Bromides—To 0.50 mL of the sample solution obtained in (2) add 4.0 mL of water, 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. After allowing to stand for 2 minutes, add 0.15 mL of 0.1 mol/L sodium thiosulfate VS, mix, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, to 5.0 mL of a solution of potassium bromide (3 in 1,000,000) add 2.0 mL of dilute phenol red TS and 1.0 mL of a solution of sodium toluenesulfonchloramide trihydrate (1 in 10,000), and mix immediately. Proceed in the same manner as for the preparation of the sample solution, and use the solution so obtained as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the control: the absorbance at 590 nm of the sample solution is not more than that of the standard solution.

(6) Iodides—Wet 5 g of Sodium Chloride with drop-wisely added 0.15 mL of a freshly prepared mixture of starch TS, 0.5 mol/L sulfuric acid TS and sodium nitrite TS (1000:40:3), allow to stand for 5 minutes, and examine under daylight: a blue color does not appear.

(7) Ferrocyanides—Dissolve 2.0 g of Sodium Chloride in 6 mL of water, and add 0.5 mL of a mixture of a solution of iron (II) sulfate heptahydrate (1 in 100) and a solution of ammonium iron (III) sulfate dodecahydrate in diluted sulfuric acid (1 in 400) (1 in 100) (19:1): a blue color does not develop within 10 minutes.

♦(8) Heavy metals <1.07>—Proceed with 5.0 g of Sodium Chloride according to Method 1, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 3 ppm).♦

(9) Iron—To 10 mL of the sample solution obtained in (2) add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, alkalize with ammonia TS, add water to make 20 mL, and allow to stand for 5 minutes: the solution has not more color than the following control solution.

Control solution: Pipet 1 mL of Standard Iron Solution, and add water to make exactly 25 mL. To 10 mL of this solution add 2 mL of a solution of citric acid monohydrate (1 in 5) and 0.1 mL of mercapto acetic acid, and proceed in the same manner as directed for the sample solution.

(10) Barium—To 5.0 mL of the sample solution obtained

in (2) add 5.0 mL of water and 2.0 mL of dilute sulfuric acid, and allow to stand for 2 hours: the solution has not more turbidity than the following control solution.

Control solution: To 5.0 mL of the sample solution obtained in (2) add 7.0 mL of water, and allow to stand for 2 hours.

(11) Magnesium and alkaline-earth materials—To 200 mL of water add 0.1 g of hydroxylammonium chloride, 10 mL of ammonium chloride buffer solution, pH 10, 1 mL of 0.1 mol/L zinc sulfate VS and 0.2 g of eriochrome black T-sodium chloride indicator, and warm to 40°C. Add 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS dropwise until the red-purple color of the solution changes to blue-purple. To this solution add a solution prepared by dissolving 10.0 g of Sodium Chloride in 100 mL of water, and add 2.5 mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS: the color of the solution is a blue-purple.

♦(12) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Chloride according to Method 1, and perform the test (not more than 2 ppm).♦

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 50 mg of Sodium Chloride, dissolve in 50 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS (potentiometric titration).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

♦**Containers and storage** Containers—Tight containers.♦

10% Sodium Chloride Injection

10% 塩化ナトリウム注射液

10% Sodium Chloride Injection is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	100 g
Distilled Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

Description 10% Sodium Chloride Injection is a clear, colorless liquid. It has a saline taste.

It is neutral.

Identification 10% Sodium Chloride Injection responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

Bacterial endotoxins <4.01> Less than 3.6 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay Pipet 10 mL of 10% Sodium Chloride Injection, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 30 mL of water, and titrate <2.50>, with vigorous

shaking, with 0.1 mol/L silver nitrate VS (indicator: 3 drops of fluorescein sodium TS).

Each mL 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Isotonic Sodium Chloride Solution

0.9% Sodium Chloride Injection

Isotonic Salt Solution

Isotonic Sodium Chloride Injection

生理食塩液

Isotonic Sodium Chloride Solution is an aqueous solution for injection.

It contains not less than 0.85 w/v% and not more than 0.95 w/v% of sodium chloride (NaCl: 58.44).

Method of preparation

Sodium Chloride	9 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative is added.

Description Isotonic Sodium Chloride Solution is a clear, colorless liquid. It has a slightly saline taste.

Identification Isotonic Sodium Chloride Solution responds to the Qualitative Tests <1.09> for sodium salt and for chloride.

pH <2.54> 4.5 – 8.0

Purity (1) Heavy metals <1.07>—Concentrate 100 mL of Isotonic Sodium Chloride Solution to about 40 mL on a water bath, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 3.0 mL of Standard Lead Solution and 2 mL of dilute acetic acid, and add water to make 50 mL (not more than 0.3 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 20 mL of Isotonic Sodium Chloride Solution, and perform the test (not more than 0.1 ppm).

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly 20 mL of Isotonic Sodium Chloride Solution, add 30 mL of water, and titrate <2.50> with 0.1 mol/L silver nitrate VS with vigorous shaking (indicator: 3 drops of fluorescein sodium TS).

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Sodium Chromate (⁵¹Cr) Injection

クロム酸ナトリウム (⁵¹Cr) 注射液

Sodium Chromate (⁵¹Cr) Injection is an aqueous solution for injection containing chromium-51 (⁵¹Cr) in the form of sodium chromate.

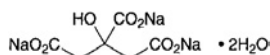
It conforms to the requirements of Sodium Chromate (⁵¹Cr) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Chromate (⁵¹Cr) Injection is a clear, light yellow liquid. It is odorless or has an odor of the preservatives.

Sodium Citrate Hydrate

クエン酸ナトリウム水和物



$C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10

Trisodium 2-hydroxypropane-1,2,3-tricarboxylate dihydrate [6132-04-3]

Sodium Citrate Hydrate, when dried, contains not less than 99.0% and not more than 101.0% of sodium citrate ($C_6H_5Na_3O_7$: 258.07).

Description Sodium Citrate Hydrate occurs as colorless crystals, or a white, crystalline powder. It is odorless, and has a cooling, saline taste.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification A solution of Sodium Citrate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for citrate and for sodium salt.

pH <2.54> Dissolve 1.0 g of Sodium Citrate Hydrate in 20 mL of water: the pH of this solution is between 7.5 and 8.5.

Purity (1) Clarity and color of solution—A solution of 1.0 g of Sodium Citrate Hydrate in 10 mL of water is clear and colorless.

(2) Chloride <1.03>—Take 0.6 g of Sodium Citrate Hydrate, and perform the test. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.015%).

(3) Sulfate <1.14>—To 0.5 g of Sodium Citrate Hydrate add water to make 40 mL, then add 3.0 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 2.5 g of Sodium Citrate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead

Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sodium Citrate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(6) Tartrate—To a solution of 1.0 g of Sodium Citrate Hydrate in 2 mL of water add 1 mL of potassium acetate TS and 1 mL of acetic acid (31): no crystalline precipitate is formed after the sides of the tube have been rubbed with a glass rod.

(7) Oxalate—Dissolve 1.0 g of Sodium Citrate Hydrate in a mixture of 1 mL of water and 3 mL of dilute hydrochloric acid, add 4 mL of ethanol (95) and 0.2 mL of calcium chloride TS, and allow to stand for 1 hour: the solution is clear.

(8) Readily carbonizable substances <1.15>—Take 0.5 g of Sodium Citrate Hydrate, and perform the test by heating at 90°C for 1 hour: the solution has no more color than Matching Fluid K.

Loss on drying <2.41> 10.0 – 13.0% (1 g, 180°C, 2 hours).

Assay Weigh accurately about 0.2 g of Sodium Citrate Hydrate, previously dried, add 30 mL of acetic acid for nonaqueous titration, warm to dissolve, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 8.602 mg of $C_6H_5Na_3O_7$

Containers and storage Containers—Tight containers.

Sodium Citrate Injection for Transfusion

輸血用クエン酸ナトリウム注射液

Sodium Citrate Injection for Transfusion is an aqueous solution for injection.

It contains not less than 9.5 w/v% and not more than 10.5 w/v% of sodium citrate hydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10).

Method of preparation

Sodium Citrate Hydrate	100 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

No preservatives may be added.

Description Sodium Citrate Injection for Transfusion is a clear, colorless liquid.

Identification Sodium Citrate Injection for Transfusion responds to the Qualitative Tests <1.09> for sodium salt and for citrate.

pH <2.54> 7.0 – 8.5

Bacterial endotoxins <4.01> Less than 5.6 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay Pipet 5 mL of Sodium Citrate Injection for Transfu-

sion, and add water to make exactly 25 mL. Evaporate 10 mL of this solution, exactly measured, on a water bath to dryness, dry the residue at 180°C for 2 hours, and dissolve in 30 mL of acetic acid (100) by warming. Cool, titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.803 mg of $C_6H_5Na_3O_7 \cdot 2H_2O$

Containers and storage Containers—Hermetic containers.

Diagnostic Sodium Citrate Solution

診断用クエン酸ナトリウム液

Diagnostic Sodium Citrate Solution contains not less than 3.3 w/v% and not more than 4.3 w/v% of sodium citrate hydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$: 294.10).

The requirements as described for aqueous injections under Injections are applicable.

Method of preparation

Sodium Citrate Hydrate	38 g
Water for Injection	a sufficient quantity
To make 1000 mL	

Prepare as directed under Injections, with the above ingredients.

No preservative may be added.

Description Diagnostic Sodium Citrate Solution is a clear, colorless liquid.

Identification Diagnostic Sodium Citrate Solution responds to the Qualitative Tests <1.09> for sodium salt and for citrate.

pH <2.54> 7.0 – 8.5

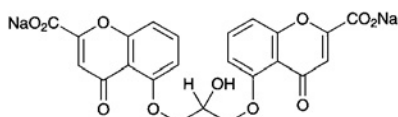
Assay Pipet 5 mL of Diagnostic Sodium Citrate Solution, evaporate on a water bath to dryness, dry the residue at 180°C for 2 hours, and dissolve in 30 mL of acetic acid (100) by warming. Cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 9.803 mg of $C_6H_5Na_3O_7 \cdot 2H_2O$

Containers and storage Containers—Hermetic containers.

Sodium Cromoglicate

クロモグリク酸ナトリウム



$C_{23}H_{14}Na_2O_{11}$: 512.33

Disodium 5,5'-(2-hydroxytrimethylenedioxy)bis(4-oxo-4H-

1-benzopyran-2-carboxylate) [15826-37-6]

Sodium Cromoglicate contains not less than 98.0% of $C_{23}H_{14}Na_2O_{11}$, calculated on the dried basis.

Description Sodium Cromoglicate occurs as a white, crystalline powder. It is odorless and tasteless at first, and later develops a slightly bitter taste.

It is freely soluble in water, sparingly soluble in propylene glycol, very slightly soluble in ethanol (95), and practically insoluble in 2-propanol and in diethyl ether.

It is hygroscopic.

It gradually acquires a yellow color by light.

Identification (1) Dissolve 0.1 g of Sodium Cromoglicate in 2 mL of water, add 2 mL of sodium hydroxide TS, and boil for 1 minute: a yellow color is produced. After cooling, add 0.5 mL of concentrated diazobenzene sulfonic acid TS: a dark red color is produced.

(2) Determine the absorption spectrum of a solution of Sodium Cromoglicate in phosphate buffer solution, pH 7.4 (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Sodium Cromoglicate responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 0.50 g of Sodium Cromoglicate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity or alkalinity—Dissolve 2.0 g of Sodium Cromoglicate in 40 mL of freshly boiled and cooled water, add 6 drops of bromothymol blue TS, and use this solution as the sample solution. To 20 mL of the sample solution add 0.25 mL of 0.1 mol/L sodium hydroxide VS: a blue color is produced. To another 20 mL of the sample solution add 0.25 mL of 0.1 mol/L hydrochloric acid VS: a yellow color is produced.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sodium Cromoglicate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Oxalate—Dissolve 0.25 g of Sodium Cromoglicate in water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 0.049 g of oxalic acid dihydrate, exactly weighed, in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution and standard solution, add exactly 5 mL of iron salicylate TS to each solution, and add water to make 50 mL. Determine the absorbances of these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank: the absorbance of the sample solution at 480 nm is not smaller than that of the standard solution.

(5) Related substances—Dissolve 0.20 g of Sodium Cromoglicate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 10 mL, pipet 1 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a

plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of methanol, chloroform and acetic acid (100) (9:9:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 10.0% (1 g, in vacuum, 105°C, 4 hours).

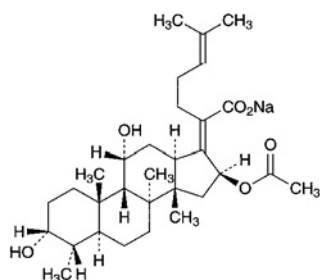
Assay Weigh accurately about 0.18 g of Sodium Cromoglicate, and dissolve in a mixture of 25 mL of propylene glycol and 5 mL of 2-propanol by warming. After cooling, add 30 mL of 1,4-dioxane, and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 25.62 mg of $C_{23}H_{14}Na_2O_{11}$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sodium Fusidate

フシジン酸ナトリウム



$C_{31}H_{47}NaO_6$: 538.69

Monosodium (17Z)-*ent*-16 α -acetoxy-3 β ,11 β -dihydroxy-4 β ,8 β ,14 α -trimethyl-18-nor-5 β ,10 α -cholesta-17(20),24-dien-21-oate [751-94-0]

Sodium Fusidate is the sodium salt of a substance having antibacterial activity produced by the growth of *Fusidium coccineum*.

It contains not less than 935 μ g (potency) and not more than 969 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Sodium Fusidate is expressed as mass (potency) of fusidic acid ($C_{31}H_{48}O_6$: 516.71).

Description Sodium Fusidate occurs as white, crystals of crystalline powder.

It is freely soluble in water, in methanol and in ethanol (99.5).

Identification (1) Determine the infrared absorption spectra of Sodium Fusidate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sodium Fusidate responds to the Qualitative Tests

<1.09> (1) for sodium salt.

Purity Heavy metals <1.07>—Proceed with 2.0 g of Sodium Fusidate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Water <2.48> Not more than 2.0% (1 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Staphylococcus aureus* ATCC 6538 P

(ii) Culture medium—Use the medium ii in 3) Medium for other organisms under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Diethanolamine Fusidate Reference Standard, equivalent to about 20 mg (potency), dissolve in 2 mL of ethanol (95), add water to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sodium Fusidate, equivalent to about 20 mg (potency), and dissolve in water to make exactly 20 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 4 μ g (potency) and 1 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and at a temperature 2 to 8°C.

Sodium Hydroxide

水酸化ナトリウム

NaOH: 40.00

Sodium Hydroxide contains not less than 95.0% of NaOH.

Description Sodium Hydroxide occurs as white fused masses, in small pellets, in flakes, in sticks, and in other forms. It is hard and brittle, and shows a crystalline fracture.

It is freely soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

It rapidly absorbs carbon dioxide in air.

It deliquesces in moist air.

Identification (1) A solution of Sodium Hydroxide (1 in 500) is alkaline.

(2) A solution of Sodium Hydroxide (1 in 25) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of

Sodium Hydroxide in 20 mL of water: the solution is clear and colorless.

(2) Chloride $\langle 1.03 \rangle$ —Dissolve 2.0 g of Sodium Hydroxide in water, and add water to make 100 mL. To 25 mL of the solution add 10 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.7 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.050%).

(3) Heavy metals $\langle 1.07 \rangle$ —Dissolve 1.0 g of Sodium Hydroxide in 5 mL of water, add 11 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 35 mL of water, add 2 mL of dilute acetic acid and 1 drop of ammonia TS, add water to make 50 mL, and perform the test using this solution as the test solution. Evaporate 11 mL of dilute hydrochloric acid on a water bath to dryness, dissolve the residue in 2 mL of dilute acetic acid and 3.0 mL of Standard Lead Solution, add water to make 50 mL, and use this solution as the control solution (not more than 30 ppm).

(4) Potassium—Dissolve 0.10 g of Sodium Hydroxide in water and dilute with water to make 40 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, and shake. Add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), shake immediately, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and dilute with water to make 1000 mL. Add 1.0 mL of dilute acetic acid to 4.0 mL of this solution, shake, and proceed as directed above.

(5) Sodium carbonate—The amount of sodium carbonate (Na_2CO_3 : 105.99) is not more than 2.0%, when calculated by the following equation using B (mL) which is obtained in the Assay.

$$\text{Amount (mg) of sodium carbonate} = 105.99 \times B$$

(6) Mercury—Dissolve 2.0 g of Sodium Hydroxide in 1 mL of a solution of potassium permanganate (3 in 50) and 30 mL of water, neutralize gradually with purified hydrochloric acid, and add 5 mL of diluted sulfuric acid (1 in 2). To this solution add a solution of hydroxylammonium chloride (1 in 5) until the precipitate of manganese dioxide disappears, add water to make exactly 100 mL, and use this solution as the sample solution. Perform the tests according to the Atomic Absorption Spectrophotometry $\langle 2.23 \rangle$ (Cold vapor type) with the sample solution. Place the sample solution in the sample bottle of an atomic absorption spectrophotometer, add 10 mL of tin (II) chloride-sulfuric acid TS, connect the bottle immediately to the atomic absorption spectrophotometer, and circulate air. Read the absorbance A_T of the sample solution when the indication of the recorder rises rapidly and becomes constant at the wavelength of 253.7 nm. On the other hand, to 2.0 mL of Standard Mercury Solution add 1 mL of a solution of potassium permanganate (3 in 50), 30 mL of water and a volume of purified hydrochloric acid equal to that used in the preparation of the sample solution, and read the absorbance A_S of the solution obtained by the same procedure as used for the sample solution: A_T is smaller than A_S .

Assay Weigh accurately about 1.5 g of Sodium Hydroxide, and dissolve in 40 mL of freshly boiled and cooled water. Cool the solution to 15°C, add 2 drops of phenolphthalein

TS, and titrate with 0.5 mol/L sulfuric acid VS until the red color of the solution disappears. Record the amount, A (mL), of 0.5 mol/L sulfuric acid VS consumed. Then add 2 drops of methyl orange TS to the solution, and further titrate $\langle 2.50 \rangle$ with 0.5 mol/L sulfuric acid VS until the solution shows a persistent light red color. Record the amount, B (mL), of 0.5 mol/L sulfuric acid VS consumed. Calculate the amount of NaOH from the difference, A (mL) – B (mL).

$$\begin{aligned} \text{Each mL of 0.5 mol/L sulfuric acid VS} \\ = 40.00 \text{ mg of NaOH} \end{aligned}$$

Containers and storage Containers—Tight containers.

Sodium Iodide

ヨウ化ナトリウム

NaI: 149.89

Sodium Iodide, when dried, contains not less than 99.0% of NaI.

Description Sodium Iodide occurs as colorless crystals or a white, crystalline powder. It is odorless.

Sodium Iodide is very soluble in water, and freely soluble in glycerin and in ethanol (95).

It deliquesces in moist air.

Identification A solution of Sodium Iodide (1 in 20) responds to the Qualitative Tests $\langle 1.09 \rangle$ for sodium salt and for iodide.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Iodide in 2 mL of water: the solution is clear and colorless.

(2) Alkalinity—Dissolve 1.0 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 1.0 mL of 0.005 mol/L sulfuric acid VS and 1 drop of phenolphthalein TS: no color is produced.

(3) Chloride, bromide and thiosulfate—Dissolve 0.20 g of Sodium Iodide in 5 mL of ammonia TS, add 15.0 mL of 0.1 mol/L silver nitrate VS, shake for a few minutes, and filter. To 10 mL of the filtrate add 15 mL of dilute nitric acid: no brown color appears. The solution has no more turbidity than the following control solution.

Control solution: To 0.30 mL of 0.01 mol/L hydrochloric acid VS add 2.5 mL of ammonia TS, 7.5 mL of 0.1 mol/L silver nitrate VS and 15 mL of dilute nitric acid.

(4) Nitrate, nitrite and ammonium—Place 1.0 g of Sodium Iodide in a 40-mL test tube, and add 5 mL of water, 5 mL of sodium hydroxide TS and 0.2 g of aluminum wire. Insert a pledget of absorbent cotton in the mouth of the test tube, and place a piece of moistened red litmus paper on the cotton. Heat the test tube on a water bath for 15 minutes: the evolved gas does not turn moistened red litmus paper to blue.

(5) Cyanide—Dissolve 0.5 g of Sodium Iodide in 10 mL of water. To 5 mL of this solution add 1 drop of iron (II) sulfate TS and 2 mL of sodium hydroxide TS, warm, and add 4 mL of hydrochloric acid: no green color develops.

(6) Iodate—Dissolve 0.5 g of Sodium Iodide in 10 mL of freshly boiled and cooled water, and add 2 drops of dilute sulfuric acid and 1 drop of starch TS: no blue color develops

immediately.

(7) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Iodide according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(8) Barium—Dissolve 0.5 g of Sodium Iodide in 10 mL of water, add 1 mL of dilute sulfuric acid, and allow to stand for 5 minutes: no turbidity is produced.

(9) Potassium—Dissolve 1.0 g of Sodium Iodide in water, and add water to make 100 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, add 5.0 mL of a solution of sodium tetraphenylboron (1 in 30), immediately shake, and allow to stand for 10 minutes: the solution has no more turbidity than the following control solution.

Control solution: Dissolve 9.5 mg of potassium chloride in water, and add water to make 1000 mL. To 4.0 mL of this solution add 1.0 mL of dilute acetic acid, shake, and then proceed as directed above.

(10) Arsenic <1.11>—Prepare the test solution with 0.40 g of Sodium Iodide according to Method 1, and perform the test (not more than 5 ppm).

Loss on drying <2.41> Not more than 5.0% (2 g, 120°C, 2 hours).

Assay Weigh accurately about 0.4 g of Sodium Iodide, previously dried, in an iodine flask, dissolve in 10 mL of water, add 35 mL of hydrochloric acid and 5 mL of chloroform, and titrate <2.50> with 0.05 mol/L potassium iodate VS while shaking vigorously until the red-purple color of the chloroform layer disappears. The end point is attained when the red-purple color does not reappear in the chloroform layer within 5 minutes after the layer has been decolorized.

Each mL of 0.05 mol/L potassium iodate VS
= 14.99 mg of NaI

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Sodium Iodide (¹²³I) Capsules

ヨウ化ナトリウム (¹²³I) カプセル

Sodium Iodide (¹²³I) Capsules contain iodine-123 in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (¹²³I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (¹³¹I) Capsules

ヨウ化ナトリウム (¹³¹I) カプセル

Sodium Iodide (¹³¹I) Capsules are prepared by dispensing Sodium Iodide (¹³¹I) Solution into capsules and drying it.

Sodium Iodide (¹³¹I) Capsules conform to the requirements of Sodium Iodide (¹³¹I) Capsules in the Minimum Requirements for Radiopharmaceuticals.

Sodium Iodide (¹³¹I) Solution

ヨウ化ナトリウム (¹³¹I) 液

Sodium Iodide (¹³¹I) Solution contains iodine-131 (¹³¹I) in the form of sodium iodide.

It conforms to the requirements of Sodium Iodide (¹³¹I) Solution in the Minimum Requirements for Radiopharmaceuticals.

Description Sodium Iodide (¹³¹I) Solution is a clear, colorless liquid. It is odorless, or has an odor due to the preservatives or stabilizers.

Sodium Iodohippurate (¹³¹I) Injection

ヨウ化ヒプル酸ナトリウム (¹³¹I) 注射液

Sodium Iodohippurate (¹³¹I) Injection is an aqueous solution for injection containing iodine-131 (¹³¹I) in the form of sodium *o*-iodohippurate.

It conforms to the requirements of Sodium Iodohippurate (¹³¹I) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Iodohippurate (¹³¹I) Injection is a clear, colorless liquid. It is odorless or has an odor of the preservatives or stabilizers.

Sodium Iotalamate Injection

イオタラム酸ナトリウム注射液

Sodium Iotalamate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105% of the labeled amount of iotalamic acid (C₁₁H₉I₃N₂O₄; 613.91).

Method of preparation

(1)	
Iotalamic Acid	645 g
Sodium Hydroxide	42 g
Water for Injection	a sufficient quantity
	To make 1000 mL

(2)	
Iotalamic Acid	772.5 g
Sodium Hydroxide	50.5 g
Water for Injection	a sufficient quantity
	To make 1000 mL

Prepare as directed under Injections, with the above in-

redients (1) or (2).

Description Sodium Iotalamate Injection is a clear, colorless or pale yellow, slightly viscous liquid.

It is gradually colored by light.

Identification (1) To a volume of Sodium Iotalamate Injection, equivalent to 1 g of Iotalamic Acid according to the labeled amount, add 25 mL of water, and add 2.5 mL of dilute hydrochloric acid with thorough stirring: a white precipitate is produced. Filter the precipitate by suction through a glass filter (G4), wash the precipitate with two 10-mL portions of water, and dry at 105°C for 1 hour. Proceed with the precipitate as directed in the Identification (2) under Iotalamic Acid.

(2) Sodium Iotalamate Injection responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> 6.5 – 7.7

Purity (1) Primary aromatic amines—To a volume of Sodium Iotalamate Injection, equivalent to 0.20 g of Iotalamic Acid according to the labeled amount, add 15 mL of water, shake, add 4 mL of a solution of sodium nitrite (1 in 100) under ice-cooling, and proceed as directed in the Purity (2) under Iotalamic Acid: the absorbance is not more than 0.17.

(2) Iodine and iodide—To a volume of Sodium Iotalamate Injection, equivalent to 1.5 g of Iotalamic Acid according to the labeled amount, add 20 mL of water and 5 mL of dilute sulfuric acid, shake well, and filter the precipitate by suction through a glass filter (G4). To the filtrate add 5 mL of toluene, and shake vigorously: the toluene layer is colorless. Then add 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously: the toluene layer has no more color than the following control solution.

Control solution: Dissolve 0.25 g of potassium iodide in water to make 1000 mL. To 2.0 mL of this solution add 20 mL of water, 5 mL of dilute sulfuric acid, 5 mL of toluene and 2 mL of a solution of sodium nitrite (1 in 100), and shake vigorously.

Bacterial endotoxins <4.01> Less than 3.4 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay Pipet a volume of Sodium Iotalamate Injection, equivalent to about 4 g of iotalamic acid ($C_{11}H_9I_3N_2O_4$), add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of iotalamic acid for assay, previously dried at 105°C for 4 hours, dissolve in 100 mL of water and 1 mL of sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 5 mL of this solution, add water to make exactly 50 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution, add the mobile phase to make 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of iotalamic acid to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of iotalamic acid } (C_{11}H_9I_3N_2O_4) \\ & = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of iotalamic acid for assay

Internal standard solution—A solution of L-tryptophan in the mobile phase (3 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: To 3.9 g of phosphoric acid and 2.8 mL of triethylamine add water to make 2000 mL. To this solution add 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of iotalamic acid is about 6 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, iotalamic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of iotalamic acid to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant.

Sodium Lauryl Sulfate

ラウリル硫酸ナトリウム

Sodium Lauryl Sulfate is a mixture of sodium alkyl sulfate consisting chiefly of sodium lauryl sulfate ($C_{12}H_{25}NaO_4S$: 288.38).

Description Sodium Lauryl Sulfate occurs as white to light yellow crystals or powder. It has a slightly characteristic odor.

It is sparingly soluble in methanol and in ethanol (95).

A solution of Sodium Lauryl Sulfate (1 in 10) is a clear or an opalescent solution, which foams on agitation.

Identification (1) To 0.2 g of the residue obtained in Total alcohol content add 4 mL of bromine-cyclohexane TS with vigorous shaking, add 0.3 g of *N*-bromosuccinimide, and heat in a water bath at 80°C for 5 minutes: a red color develops.

(2) A solution of Sodium Lauryl Sulfate (1 in 10) responds to the Qualitative Tests <1.09> (1) for sodium salt.

(3) To a solution of Sodium Lauryl Sulfate (1 in 10) add dilute hydrochloric acid to make acid, boil gently, and cool: the solution responds to the Qualitative Tests <1.09> for sulfate.

Purity (1) Alkalinity—Dissolve 1.0 g of Sodium Lauryl Sulfate in 100 mL of water, add 2 drops of phenol red TS and

0.60 mL of 0.1 mol/L hydrochloric acid VS: the solution remains yellow.

(2) Sodium chloride—Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 50 mL of water, neutralize the solution with dilute nitric acid, if necessary, add exactly 5 mL of 0.1 mol/L sodium chloride TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 2 drops of fluorescein sodium TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L silver nitrate VS
= 5.844 mg of NaCl

The combined content of sodium chloride (NaCl: 58.44) and sodium sulfate (Na₂SO₄: 142.04) obtained in the next paragraph (3) is not more than 8.0%.

(3) Sodium sulfate—Dissolve about 1 g of Sodium Lauryl Sulfate, accurately weighed, in 10 mL of water, add 100 mL of ethanol (95), and heat at a temperature just below the boiling point for 2 hours. Filter through a glass filter (G4) while hot, and wash with 100 mL of boiling ethanol (95). Dissolve the precipitate by washing with 150 mL of water, collecting the washings in a beaker. Add 10 mL of hydrochloric acid, heat to boiling, add 25 mL of barium chloride TS, and allow to stand overnight. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, ignite to a constant mass between 500°C and 600°C by raising the temperature gradually, and weigh as barium sulfate (BaSO₄: 233.39).

Amount (mg) of sodium sulfate (Na₂SO₄)
= amount (mg) of barium sulfate (BaSO₄) × 0.6086

(4) Unsulfated alcohols—Dissolve about 10 g of Sodium Lauryl Sulfate, accurately weighed, in 100 mL of water, add 100 mL of ethanol (95), and transfer to a separator. Extract the solution with three 50-mL portions of petroleum benzin. If an emulsion forms, sodium chloride may be added to promote separation of the two layers. Combine the petroleum benzin extracts and wash with three 50-mL portions of water. Evaporate the petroleum benzin on a water bath, and dry the residue at 105°C for 30 minutes. The mass of the dried residue is not more than 4.0% of the mass of the Sodium Lauryl Sulfate taken.

Water <2.48> Not more than 5.0% (0.5 g, direct titration).

Total alcohol content Dissolve about 5 g of Sodium Lauryl Sulfate, accurately weighed, in 150 mL of water and 50 mL of hydrochloric acid, and boil under a reflux condenser for 4 hours. Cool, extract with two 75-mL portions of diethyl ether, and evaporate the combined diethyl ether extracts on a water bath. Dry the residue at 105°C for 30 minutes. The mass of the residue is not less than 59.0%.

Containers and storage Containers—Well-closed containers.

Sodium Pertechnetate (^{99m}Tc) Injection

過テクネチウム酸ナトリウム (^{99m}Tc) 注射液

Sodium Pertechnetate (^{99m}Tc) Injection is an aque-

ous solution for injection containing technetium-99m (^{99m}Tc) in the form of sodium pertechnetate.

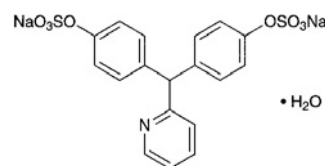
It conforms to the requirements of Sodium Pertechnetate (^{99m}Tc) Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Sodium Pertechnetate (^{99m}Tc) Injection is a clear, colorless liquid.

Sodium Picosulfate Hydrate

ピコスルファートナトリウム水和物



C₁₈H₁₃NNa₂O₈S₂·H₂O: 499.42
Disodium 4,4'-(pyridin-2-ylmethylene)bis(phenyl sulfate)
monohydrate [10040-45-6, anhydride]

Sodium Picosulfate Hydrate contains not less than 98.5% of sodium picosulfate (C₁₈H₁₃NNa₂O₈S₂: 481.41), calculated on the anhydrous basis.

Description Sodium Picosulfate Hydrate occurs as a white, crystalline powder. It is odorless and tasteless.

It is very soluble in water, soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in diethyl ether.

It is gradually colored by light.

The pH of a solution of Sodium Picosulfate Hydrate (1 in 20) is between 7.4 and 9.4.

Identification (1) Mix 5 mg of Sodium Picosulfate Hydrate with 0.01 g of 1-chloro-2,4-dinitrobenzene, and melt by gentle heating for 5 to 6 seconds. After cooling, add 4 mL of potassium hydroxide-ethanol TS: an orange-red color develops.

(2) To 0.2 g of Sodium Picosulfate Hydrate add 5 mL of dilute hydrochloric acid, boil for 5 minutes, cool, and add 1 mL of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sodium Picosulfate Hydrate (1 in 25,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Sodium Picosulfate Hydrate, previously dried at 105°C in vacuum for 4 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(5) A solution of Sodium Picosulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Absorbance <2.24> E_{1cm}^{1%} (263 nm): 120 – 130 (calculated on

the anhydrous basis, 4 mg, water, 100 mL).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Picosulfate Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—Perform the test with 0.5 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.028%).

(3) Sulfate <1.14>—Perform the test with 0.40 g of Sodium Picosulfate Hydrate. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.042%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Picosulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 2.0 g of Sodium Picosulfate Hydrate according to Method 3, and perform the test (not more than 1 ppm).

(6) Related substances—Dissolve 0.25 g of Sodium Picosulfate Hydrate in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 500 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (74:20:19) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 3.0 – 4.5% (0.5 g, direct titration).

Assay Weigh accurately about 0.4 g of Sodium Picosulfate Hydrate, dissolve in 50 mL of methanol, add 7 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 48.14 mg of $C_{18}H_{13}NNa_2O_8S_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sodium Polystyrene Sulfonate

ポリスチレンスルホン酸ナトリウム

Sodium Polystyrene Sulfonate is a cation exchange resin prepared as the sodium form of the sulfonated styrene divinylbenzene copolymer.

It contains not less than 9.4% and not more than 11.0% of sodium (Na: 22.99), calculated on the anhydrous basis.

Each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, exchanges with not less than 0.110 g and not more than 0.135 g of potassium (K: 39.10).

Description Sodium Polystyrene Sulfonate occurs as a yellow-brown powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetone and in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Sodium Polystyrene Sulfonate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 1 g of Sodium Polystyrene Sulfonate add 10 mL of dilute hydrochloric acid, stir, and filter. Add ammonia TS to the filtrate to neutralize: the solution responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Ammonium—Place 1.0 g of Sodium Polystyrene Sulfonate in a flask, add 5 mL of sodium hydroxide TS, cover the flask with a watch glass having a moistened strip of red litmus paper on the underside, and boil for 15 minutes: the gas evolved does not change the red litmus paper to blue.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Polystyrene Sulfonate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Sodium Polystyrene Sulfonate according to Method 3, and perform the test (not more than 1 ppm).

(4) Styrene—To 10.0 g of Sodium Polystyrene Sulfonate add 10 mL of acetone, shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of styrene in acetone to make exactly 100 mL. Pipet 1 mL of this solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine peak areas, A_T and A_S , of styrene in each solution: A_T is not larger than A_S .

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of styrene is about 8 minutes.

System suitability—

System performance: Dissolve 20 mg each of styrene and butyl parahydroxybenzoate in 100 mL of acetone. To 5 mL of this solution add acetone to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, butyl parahydroxybenzoate and styrene are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of styrene is not more than 2.0%.

Water <2.48> Not more than 10.0% (0.2 g, direct titration).

Assay (1) Sodium—Weigh accurately about 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 50 mL of 3 mol/L hydrochloric acid TS, shake for 60 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Sodium Stock Solution, dilute exactly with water so that each mL of the solution contains 1 to 3 μg of sodium (Na: 22.99), and use these solutions as the standard solution. Perform the test with the sample solution and the standard solution according to the Atomic Absorption Spectrophotometry <2.23> under the following conditions, and determine the amount of sodium in the sample solution using the calibration curve obtained from the standard solutions.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: A sodium hollow-cathode lamp

Wavelength: 589.0 nm

(2) Potassium exchange capacity—Weigh accurately about 1.5 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, in a glass-stoppered flask, add exactly 100 mL of Standard Potassium Stock Solution, shake for 15 minutes, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 1000 mL, and use this solution as the sample solution. Separately, pipet a suitable quantity of Standard Potassium Stock Solution, dilute with water so that each mL of the solution contains 1 to 5 μg of potassium (K: 39.10), and use the solution as the standard solution. Perform the test with these solutions as directed under Atomic Absorption Spectrophotometry <2.23>, and determine the amount Y (mg) of potassium in 1000 mL of the sample solution using the calibration curve obtained from the standard solution. The quantity of potassium absorbed on each g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis, is calculated from the following equation: it is between 0.110 g and 0.135 g.

Quantity (mg) of potassium (K) absorbed on 1 g of Sodium Polystyrene Sulfonate, calculated on the anhydrous basis

$$= (X - 100Y)/W$$

X : Amount (mg) of potassium in 100 mL of the Standard Potassium Stock Solution before exchange.

W : Mass (g) of Sodium Polystyrene Sulfonate taken, calculated on the anhydrous basis.

Gas: Combustible gas—Acetylene

Supporting gas—Air

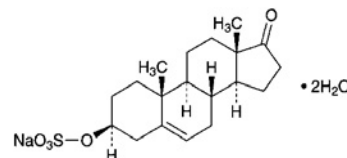
Lamp: A potassium hollow-cathode lamp

Wavelength: 766.5 nm

Containers and storage Containers—Tight containers.

Sodium Prasterone Sulfate Hydrate

プラステロン硫酸エステルナトリウム水和物



$\text{C}_{19}\text{H}_{27}\text{NaO}_5 \cdot 2\text{H}_2\text{O}$: 426.50

Monosodium 17-oxoandrost-5-en-3 β -yl sulfate dihydrate
 [1099-87-2, anhydride]

Sodium Prasterone Sulfate Hydrate contains not less than 98.0% of sodium prasterone sulfate ($\text{C}_{19}\text{H}_{27}\text{NaO}_5$ S: 390.47), calculated on the dried basis.

Description Sodium Prasterone Sulfate Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is soluble in methanol, sparingly soluble in water and in ethanol (95), and practically insoluble in acetone and in diethyl ether.

The pH of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) is between 4.5 and 6.5.

Melting point: about 160°C (with decomposition, after drying).

Identification (1) Dissolve 0.01 g of Sodium Prasterone Sulfate Hydrate in 4 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of a solution of sodium hydroxide (1 in 8): a red-purple color develops, and gradually changes to brown.

(2) To 10 mL of a solution of Sodium Prasterone Sulfate Hydrate (1 in 200) add 0.5 mL of bromine TS: the color of bromine TS immediately disappears.

(3) Determine the infrared absorption spectrum of Sodium Prasterone Sulfate Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Sodium Prasterone Sulfate Hydrate (1 in 200) responds to the Qualitative Tests <1.09> for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +10.7 – +12.1° (0.73 g, calculated on the dried basis, methanol, 20 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.25 g of Sodium Prasterone Sulfate Hydrate in 50 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 1.0 g of Sodium Prasterone Sulfate Hydrate in 20 mL of acetone and 20 mL of water, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 20 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.011%).

(3) Sulfate <1.14>—To 1.2 g of Sodium Prasterone Sulfate Hydrate add 20 mL of water, shake vigorously for 5 minutes, and filter. To 10 mL of the filtrate add 20 mL of ace-

tone, 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 20 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.032%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Sodium Prasterone Sulfate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(5) Related substances—Dissolve 0.10 g of Sodium Prasterone Sulfate Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and water (75:22:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 8.0–9.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Assay Weigh accurately about 0.25 g of Sodium Prasterone Sulfate Hydrate, dissolve in 30 mL of water. Apply this solution to a chromatographic column 10 mm in inside diameter, previously prepared by pouring 5 mL of strongly acidic ion-exchange resin (H type) for column chromatography, and elute at the rate of 4 mL per minute. Wash the chromatographic column with 100 mL of water, combine the washings with above effluent solution, and titrate <2.50> with 0.05 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L sodium hydroxide VS
= 19.52 mg of $C_{19}H_{27}NaO_5S$

Containers and storage Containers—Tight containers.

Sodium Pyrosulfite

Sodium Metabisulfite

ピロ亜硫酸ナトリウム

$Na_2S_2O_5$: 190.11

Sodium Pyrosulfite contains not less than 95.0% of $Na_2S_2O_5$.

Description Sodium Pyrosulfite occurs as white crystals or crystalline powder. It has the odor of sulfur dioxide.

It is freely soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Sodium Pyrosulfite (1 in 20) is acid.

It is hygroscopic.

It decomposes slowly on exposure to air.

Identification A solution of Sodium Pyrosulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and for bisulfite.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water: the solution is clear and colorless.

(2) Thiosulfate—Dissolve 1.0 g of Sodium Pyrosulfite in 15 mL of water, add slowly 5 mL of dilute hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Pyrosulfite in 10 mL of water, and evaporate with 5 mL of hydrochloric acid on a water bath to dryness. Dissolve the residue in 10 mL of water, add 1 drop of phenolphthalein TS, and add ammonia TS until the solution becomes slightly red. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 5 mL of hydrochloric acid on a water bath to dryness, and to the residue add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Iron <1.10>—Prepare the test solution with 1.0 g of Sodium Pyrosulfite according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 0.5 g of Sodium Pyrosulfite in 10 mL of water, heat with 1 mL of sulfuric acid on a sand bath until white fumes are evolved, and add water to make 5 mL. Perform the test with this solution as the test solution (not more than 4 ppm).

Assay Weigh accurately about 0.15 g of Sodium Pyrosulfite, and transfer to an iodine flask containing an exactly measured 50 mL of 0.05 mol/L iodine VS. Stopper tightly, shake well, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid VS, and titrate <2.50> the excess of iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

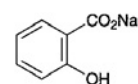
Each mL of 0.05 mol/L iodine VS = 4.753 mg of $Na_2S_2O_5$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, preferably well-filled, and not exceeding 30°C.

Sodium Salicylate

サリチル酸ナトリウム



$C_7H_5NaO_3$: 160.10

Monosodium 2-hydroxybenzoate [54-21-7]

Sodium Salicylate, when dried, contains not less than 99.5% of $C_7H_5NaO_3$.

Description Sodium Salicylate occurs as white, crystals or

crystalline powder.

It is very soluble in water, freely soluble in acetic acid (100), and soluble in ethanol (95).

It is gradually colored by light.

Identification (1) Determine the infrared absorption spectrum of Sodium Salicylate, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Sodium Salicylate (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54> The pH of a solution of 2.0 g of Sodium Salicylate in 20 mL of water is between 6.0 and 8.0.

Purity (1) Clarity of solution—Dissolve 1.0 g of Sodium Salicylate in 10 mL of water: the solution is clear, and its absorbance at 420 nm determined as directed under Ultraviolet-visible Spectrophotometry <2.24> is not more than 0.02.

(2) Chloride <1.03>—Dissolve 0.5 g of Sodium Salicylate in 15 mL of water, add 6 mL of dilute nitric acid and ethanol (95) to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS, 28 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.021%).

(3) Sulfate—Dissolve 0.25 g of Sodium Salicylate in 5 mL of water, and add 0.5 mL of barium chloride TS: the solution shows no change.

(4) Sulfite and thiosulfate—Dissolve 1.0 g of Sodium Salicylate in 20 mL of water, add 1 mL of hydrochloric acid, and filter. Add 0.15 mL of 0.05 mol/L iodine VS to the filtrate: a yellow color develops.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Sodium Salicylate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—To 1.0 g of Sodium Salicylate in a decomposition flask add 5 mL of nitric acid and 2 mL of sulfuric acid, and heat carefully until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat. After cooling, add several 2-mL portions of hydrogen peroxide (30), and heat until the solution is colorless to pale yellow. Repeat the procedure of adding nitric acid and hydrogen peroxide (30) and heating, if necessary. After cooling, add 2 mL of a saturated solution of ammonium oxalate monohydrate, and heat until white fumes are evolved. After cooling, add water to make 5 mL, and perform the test with this solution (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Assay Weigh accurately about 0.3 g of Sodium Salicylate, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.01 mg of C₇H₅NaO₃

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dried Sodium Sulfite

乾燥亜硫酸ナトリウム

Na₂SO₃: 126.04

Dried Sodium Sulfite contains not less than 97.0% of Na₂SO₃.

Description Dried Sodium Sulfite is white crystals or powder. It is odorless.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Dried Sodium Sulfite (1 in 10) is about 10.

It gradually changes in moist air.

Identification An aqueous solution of Dried Sodium Sulfite (1 in 20) responds to the Qualitative Tests <1.09> for sodium salt and sulfite.

Purity (1) Thiosulfate—Dissolve 1.0 g of Dried Sodium Sulfite in 15 mL of water, add gradually 5 mL of hydrochloric acid, shake, and allow to stand for 5 minutes: no turbidity is produced.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Dried Sodium Sulfite in 5 mL of water, add 2 mL of hydrochloric acid gradually, and evaporate the mixture on a water bath to dryness. Add 3 mL of boiling water and 1 mL of hydrochloric acid to the residue, and again evaporate to dryness on a water bath. Dissolve the residue in 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 3 mL of hydrochloric acid to dryness, and add 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 0.5 g of Dried Sodium Sulfite in 5 mL of water, add 1 mL of sulfuric acid, and evaporate on a sand bath until white fumes are evolved. Add water to make 5 mL, take this solution as the sample solution, and perform the test (not more than 4 ppm).

Assay Weigh accurately about 0.2 g of Dried Sodium Sulfite, transfer immediately to an iodine flask containing exactly 50 mL of 0.05 mol/L iodine VS, stopper, shake, and allow to stand for 5 minutes in a dark place. Add 1 mL of hydrochloric acid, and titrate <2.50> the excess iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 1 mL of starch TS). Perform a blank determination.

Each mL of 0.05 mol/L iodine VS = 6.302 mg of Na₂SO₃

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Hydrate

チオ硫酸ナトリウム水和物

Na₂S₂O₃·5H₂O: 248.18

Sodium Thiosulfate Hydrate, when dried, contains

not less than 99.0% and not more than 101.0% of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$: 158.11).

Description Sodium Thiosulfate Hydrate occurs as colorless, crystals or crystalline powder. It is odorless.

It is very soluble in water, and practically insoluble in ethanol (99.5).

It effloresces in dry air, and is deliquescent in moist air.

Identification (1) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for thiosulfate.

(2) A solution of Sodium Thiosulfate Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54> Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the pH of the solution is between 6.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Sodium Thiosulfate Hydrate in 10 mL of water, add slowly 5 mL of dilute hydrochloric acid, and evaporate on a water bath to dryness. Add 15 mL of water to the residue, boil gently for 2 minutes, and filter. Heat the filtrate to boil, and add bromine TS to the hot filtrate to produce a clear solution and provide a slight excess of bromine. Boil the solution to expel the bromine. Cool, add 1 drop of phenolphthalein TS, and add dropwise sodium hydroxide TS until a slight red color is produced. Add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Calcium—Dissolve 1.0 g of Sodium Thiosulfate in 10 mL of water, add 2 mL of ammonium oxalate TS, and allow to stand for 4 minutes: no turbidity is produced.

(4) Arsenic <1.11>—To 0.40 g of Sodium Thiosulfate add 3 mL of nitric acid and 5 mL of water, evaporate on a water bath to dryness, and perform the test with the residue. Prepare the test solution according to Method 2, and perform the test (not more than 5 ppm).

Loss on drying <2.41> 32.0 – 37.0% (1 g, in vacuum, 40 – 45°C, 16 hours).

Assay Weigh accurately about 0.4 g of Sodium Thiosulfate, previously dried, dissolve in 30 mL of water, and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 15.81 mg of $\text{Na}_2\text{S}_2\text{O}_3$

Containers and storage Containers—Tight containers.

Sodium Thiosulfate Injection

チオ硫酸ナトリウム注射液

Sodium Thiosulfate Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 105

% of the labeled amount of sodium thiosulfate hydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$: 248.18).

Method of preparation Prepare as directed under Injections, with Sodium Thiosulfate Hydrate.

Description Sodium Thiosulfate Injection is a clear, colorless liquid.

Identification Sodium Thiosulfate Injection responds to the Qualitative Tests <1.09> for sodium salt and for thiosulfate.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Perform the test with Sodium Thiosulfate Injection stored in a container in a volume exceeding 10 mL: it meets the requirements.

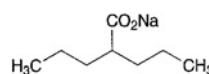
Assay Measure exactly a volume of Sodium Thiosulfate Injection, equivalent to about 0.5 g of sodium thiosulfate hydrate ($\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$), add water to make 30 mL, and titrate <2.50> with 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

Each mL of 0.05 mol/L iodine VS
= 24.82 mg of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$

Containers and storage Containers—Hermetic containers.

Sodium Valproate

バルプロ酸ナトリウム



$\text{C}_8\text{H}_{15}\text{NaO}_2$: 166.19

Monosodium 2-propylpentanoate [1069-66-5]

Sodium Valproate, when dried, contains not less than 98.5% of $\text{C}_8\text{H}_{15}\text{NaO}_2$.

Description Sodium Valproate occurs as a white, crystalline powder. It has a characteristic odor and a slightly bitter taste.

It is very soluble in water, freely soluble in formic acid, in ethanol (95), in ethanol (99.5) and in acetic acid (100), and practically insoluble in chloroform and in diethyl ether.

It is hygroscopic.

Identification (1) To 1 mL of a solution of Sodium Valproate in ethanol (99.5) (1 in 200) add 4 mL of hydroxylamine perchlorate-dehydrated ethanol TS and 1 mL of *N,N'*-dicyclohexylcarbodiimide-dehydrated ethanol TS, shake well, and allow to stand in lukewarm water for 20 minutes. After cooling, add 1 mL of iron (III) perchlorate-dehydrated ethanol TS, and shake: a purple color develops.

(2) To 5 mL of a solution of Sodium Valproate (1 in 20) add 1 mL of a solution of cobalt (II) nitrate hexahydrate (1 in 20), and warm on a water bath: a purple precipitate is formed.

(3) Dissolve 0.5 g of Sodium Valproate in 5 mL of water, add 5 mL of chloroform and 1 mL of 2 mol/L hydrochloric acid TS, and shake vigorously for 1 minute. After allowing to stand, separate the chloroform layer, dehydrate the chloroform with anhydrous sodium sulfate, then filter, and evaporate the filtrate to dryness. Determine the infrared absorption spectrum of the residue as directed in the liquid film

method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Sodium Valproate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

pH <2.54> Dissolve 1.0 g of Sodium Valproate in 20 mL of water: the pH of this solution is between 7.0 and 8.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sodium Valproate in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Sodium Valproate in 25 mL of ethanol (95), and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.70 mL of 0.01 mol/L hydrochloric acid VS add 25 mL of ethanol (95), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.050%).

(3) Sulfate <1.14>—Dissolve 0.5 g of Sodium Valproate in 25 mL of ethanol (95), and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.50 mL of 0.005 mol/L sulfuric acid VS add 25 mL of ethanol (95), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Dissolve 2.0 g of Sodium Valproate in 44 mL of water, shake with 6 mL of dilute hydrochloric acid, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, neutralize the subsequent 25 mL with ammonia TS, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(5) Arsenic <1.11>—Dissolve 2.0 g of Sodium Valproate in 10 mL of water, shake with 10 mL of dilute hydrochloric acid, allow to stand for 5 minutes, and filter. Discard the first 5 mL of the filtrate, and perform the test with the subsequent 10 mL (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of Sodium Valproate in 10 mL of a mixture of formic acid and chloroform (1:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of formic acid and chloroform (1:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 μ L each of the sample solution and the standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the valproic acid from the sample solution is not larger than the peak area of the valproic acid from the standard solution.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 2 m in length, packed with siliceous earth for gas chromatography (150 to 180 μ m in particle diameter) coated with diethylene glycol adipate ester for gas chromatography and phosphoric acid at the ratios of 5% and 1%, respectively.

Column temperature: A constant temperature of about 145°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of valproic acid is between 6 and 10 minutes.

Selection of column: Mix 1 mL of the sample solution and 4 mL of a solution of *n*-valerianic acid in a mixture of formic acid and chloroform (1:1) (1 in 1000). Proceed with 2 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of *n*-valerianic acid and valproic acid in this order with the resolution between these peaks being not less than 3.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of valproic acid obtained from 2 μ L of the standard solution is between 4 mm and 10 mm.

Time span of measurement: About twice as long as the retention time of valproic acid, beginning after the solvent peak.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 0.2 g of Sodium Valproate, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 16.62 mg of C₈H₁₅NaO₂

Containers and storage Containers—Tight containers.

Sorbitan Sesquioleate

ソルビタンセスキオレイン酸エステル

Sorbitan Sesquioleate is a mixture of monoester and diester of sorbitol anhydride, partially esterified with oleic acid.

Description Sorbitan Sesquioleate is a pale yellow to light yellow-brown, viscous oily liquid. It has a faint, characteristic odor and a slightly bitter taste.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and very slightly soluble in methanol.

It is dispersed as fine oily drops in water.

Identification (1) To 0.5 g of Sorbitan Sesquioleate add 5 mL of ethanol (95) and 5 mL of dilute sulfuric acid, and heat on a water bath for 30 minutes. Cool, shake with 5 mL of petroleum ether, and allow to stand, and separate the upper layer and the lower layer. Shake 2 mL of the lower layer with 2 mL of freshly prepared catechol solution (1 in 10), then with 5 mL of sulfuric acid: a red to red-brown color develops.

(2) Heat the upper layer obtained in (1) on a water bath, and evaporate petroleum ether. To the residue add 2 mL of diluted nitric acid (1 in 2), and then add 0.5 g of potassium nitrite between 30°C and 35°C with stirring: the solution develops an opalescence, and, when cooled, crystals are formed.

Specific gravity <1.13> d_{25}^{25} : 0.960 – 1.020

Saponification value <1.13> 150 – 168

Purity (1) Acidity—To 2.0 g of Sorbitan Sesquioleate add

50 mL of neutralized ethanol, and heat on a water bath nearly to boiling with stirring once or twice. Cool, add 4.3 mL of 0.1 mol/L sodium hydroxide VS and 5 drops of phenolphthalein TS: a red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sorbitan Sesquioleate according to Method 2, and perform the test (not more than 2 ppm).

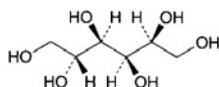
Water <2.48> Not more than 3.0% (1 g, direct titration, stir for 30 minutes).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Containers and storage Containers—Tight containers.

D-Sorbitol

D-ソルビトール



$C_6H_{14}O_6$: 182.17
D-Glucitol [50-70-4]

D-Sorbitol, when dried, contains not less than 97.0% of $C_6H_{14}O_6$.

Description D-Sorbitol occurs as white granules, powder, or crystalline masses. It is odorless, and has a sweet taste with a cold sensation.

It is very soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) To 1 mL of a solution of D-Sorbitol (7 in 10) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

(2) Shake thoroughly 1 mL of a solution of D-Sorbitol (1 in 20) with 1 mL of a freshly prepared solution of catechol (1 in 10), add rapidly 2 mL of sulfuric acid, and shake: a reddish purple to red-purple color immediately develops.

(3) Boil 0.5 g of D-Sorbitol with 10 mL of acetic anhydride and 1 mL of pyridine under a reflux condenser for 10 minutes, cool, shake with 25 mL of water, and allow to stand in a cold place. Transfer the solution to a separator, extract with 30 mL of chloroform, and evaporate the extract on a water bath. Add 80 mL of water to the oily residue, heat for 10 minutes on a water bath, then filter the hot mixture. After cooling, collect the produced precipitate through a glass filter (G3), wash with water, recrystallize once from ethanol (95), and dry in a desiccator (in vacuum, silica gel) for 4 hours: the precipitate melts between 97°C and 101°C.

Purity (1) Clarity and color of solution, and acidity or alkalinity—Dissolve 5 g of D-Sorbitol in 20 mL of water by warming with shaking: the solution is clear, colorless, and neutral.

(2) Chloride <1.03>—Perform the test with 2.0 g of D-

Sorbitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <1.14>—Perform the test with 4.0 g of D-Sorbitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <1.07>—Proceed with 5.0 g of D-Sorbitol according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Dissolve 0.5 g of D-Sorbitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(6) Arsenic <1.11>—Prepare the test solution with 1.5 g of D-Sorbitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Glucose—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and boil gently with 40 mL of Fehling's TS for 3 minutes. After cooling, filter the supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show an alkali reaction, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash with water. Combine the filtrate and the washings, heat at 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of volume for titration consumed or consumption is required.

(8) Sugars—Dissolve 20.0 g of D-Sorbitol in 25 mL of water, and heat with 8 mL of dilute hydrochloric acid under a reflux condenser in a water bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops, and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 80°C, 3 hours).

Residue on ignition <2.44> Not more than 0.02% (5 g).

Assay Weigh accurately about 0.2 g of D-Sorbitol, previously dried, dissolve in water and add water to make exactly 100 mL. Pipet 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Tight containers.

D-Sorbitol Solution

D-ソルビトール液

D-Sorbitol Solution contains not less than 97%

and not more than 103% of the labeled amount of D-sorbitol ($C_6H_{14}O_6$; 182.17).

Description D-Sorbitol Solution is a clear, colorless liquid. It is odorless, and has a sweet taste.

It is miscible with water, with ethanol (95), with glycerin and with propylene glycol.

It sometimes separates crystalline masses.

Identification (1) To a volume of D-Sorbitol Solution, equivalent to 0.7 g of D-Sorbitol according to the labeled amount, add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): a blue-green color develops, but no turbidity is produced.

(2) To a volume of D-Sorbitol Solution, equivalent to 1 g of D-Sorbitol according to the labeled amount, add water to make 20 mL. To 1 mL of this solution add 1 mL of a freshly prepared solution of catechol (1 in 10), mix well, then add rapidly 2 mL of sulfuric acid, and mix: a reddish purple to red-purple color immediately develops.

Purity (1) Acidity or alkalinity—D-Sorbitol Solution is neutral.

(2) Chloride <1.03>—Proceed with a volume of D-Sorbitol Solution, equivalent to 2.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <1.14>—To a volume of D-Sorbitol Solution, equivalent to 4.0 g of D-Sorbitol according to the labeled amount, and perform the test. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <1.07>—Proceed with a volume of D-Sorbitol Solution, equivalent to 5.0 g of D-Sorbitol according to the labeled amount, and according to Method 1, perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Take a volume of D-Sorbitol Solution, equivalent to 0.5 g of D-Sorbitol according to the labeled amount, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color develops.

(6) Arsenic <1.11>—Take a volume of D-Sorbitol Solution, equivalent to 1.5 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 5 mL on a water bath, if necessary, cool, and perform the test using this solution as the test solution (not more than 1.3 ppm).

(7) Glucose—Take a volume of D-Sorbitol Solution, equivalent to 20.0 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 40 mL on a water bath, if necessary, add 40 mL of Fehling's TS, and boil gently for 3 minutes. After cooling, filter the supernatant liquid cautiously through a glass filter (G4), leaving the precipitate in the flask as much as possible, wash the precipitate with hot water until the last washings no longer show alkalinity, and filter the washings through the glass filter. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter through the glass filter, and wash the filter with water. Combine the filtrate and the washings, heat at 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS: not more than 6.3 mL of 0.02 mol/L potassium permanganate VS is required.

(8) Sugars—Take a volume of D-Sorbitol Solution,

equivalent to 20.0 g of D-Sorbitol according to the labeled amount, dilute with water or concentrate to 40 mL of a water bath, if necessary, add 8 mL of dilute hydrochloric acid, and heat under a reflux condenser in a water bath for 3 hours. After cooling, add 2 drops of methyl orange TS, followed by sodium hydroxide TS until an orange color develops, and add water to make 100 mL. Boil gently 10 mL of this solution with 10 mL of water and 40 mL of Fehling's TS for 3 minutes and proceed as directed in (7).

Residue on ignition <2.44> Measure exactly a volume of D-Sorbitol Solution, equivalent to 5 g of D-Sorbitol according to the labeled amount, add 3 to 4 drops of sulfuric acid, and heat gently to evaporate. Ignite to burn, cool, and perform the test with the residue: not more than 1 mg.

Assay Measure exactly a volume of D-Sorbitol Solution, equivalent to about 5 g of D-sorbitol ($C_6H_{14}O_6$) according to the labeled amount, and add water to make exactly 250 mL. Pipet 10 mL of this solution, add water to make exactly 100 mL. Pipet exactly 10 mL of the solution into an iodine flask, add exactly 50 mL of potassium periodate TS, and heat for 15 minutes in a water bath. Cool, add 2.5 g of potassium iodide, immediately stopper tightly, and shake well. Allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.822 mg of $C_6H_{14}O_6$

Containers and storage Containers—Tight containers.

Soybean Oil

Oleum Sojae

ダイズ油

Soybean Oil is the fixed oil obtained from the seeds of *Glycine max* merrill (*Leguminosae*).

Description Soybean Oil is a clear, pale yellow oil. It is odorless or has a slight odor, and has a bland taste.

It is miscible with diethyl ether and with petroleum ether. It is slightly soluble in ethanol (95), and practically insoluble in water.

It congeals between -10°C and -17°C .

Congealing point of the fatty acids: 22 – 27°C

Specific gravity <1.13> d_{25}^{25} : 0.916 – 0.922

Acid value <1.13> Not more than 0.2.

Saponification value <1.13> 188 – 195

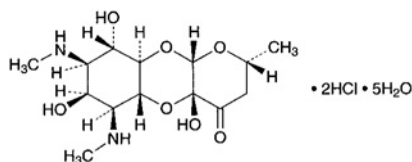
Unsaponifiable matter <1.13> Not more than 1.0%.

Iodine value <1.13> 126 – 140

Containers and storage Containers—Tight containers.

Spectinomycin Hydrochloride Hydrate

スペクチノマイシン塩酸塩水和物



$C_{14}H_{24}N_2O_7 \cdot 2HCl \cdot 5H_2O$: 495.35
(2*R*,4*aR*,5*aR*,6*S*,7*S*,8*R*,9*S*,9*aR*,10*aS*)-
4*a*,7,9-Trihydroxy-2-methyl-6,8-
bis(methylamino)-2,3,4*a*,5*a*,6,7,8,9,9*a*,10*a*-decahydro-
4*H*-pyrano[2,3-*b*][1,4]benzodioxin-4-one dihydrochloride
pentahydrate [22189-32-8]

Spectinomycin Hydrochloride Hydrate is the hydrochloride of a substance having antibacterial activity produced by the growth of *Streptomyces spectabilis*.

It contains not less than 603 μ g (potency) and not more than 713 μ g (potency) per mg. The potency of Spectinomycin Hydrochloride Hydrate is expressed as mass (potency) of spectinomycin ($C_{14}H_{24}N_2O_7$: 332.35).

Description Spectinomycin Hydrochloride Hydrate occurs as a white to light yellowish white crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (95).

Identification (1) To 5 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 100) add gently anthrone TS: a blue to blue-green color is produced at the zone of contact.

(2) Determine the infrared absorption spectra of Spectinomycin Hydrochloride Hydrate and Spectinomycin Hydrochloride Reference Standard as directed in the paste method under the Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 3 mL of a solution of Spectinomycin Hydrochloride Hydrate (1 in 150) add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +15 – +21° (2.1 g calculated on the anhydrous basis, water, 25 mL, 200 mm).

pH <2.54> Dissolve 0.10 g of Spectinomycin Hydrochloride Hydrate in 10 mL of water: the pH of the solution is between 4.0 and 5.6.

Water <2.48> Not less than 16.0% and not more than 20.0% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

- Test organism—*Klebsiella pneumoniae* ATCC 10031
- Culture medium—Use the medium i in 3) under (1)

Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.8 to 8.0 after sterilization.

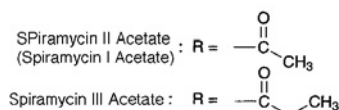
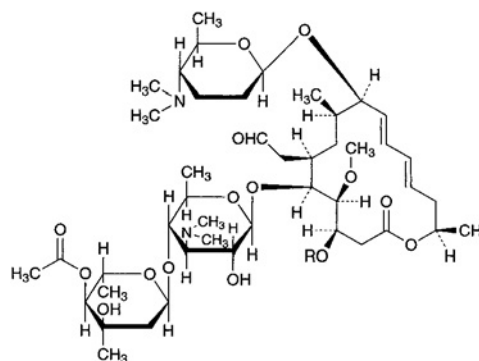
(iii) Standard solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL, and use this solution as the standard stock solution. Keep the standard stock solution at a temperature not exceeding 5°C and use within 10 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spectinomycin Hydrochloride Hydrate, equivalent to about 20 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 20 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 200 μ g (potency) and 50 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spiramycin Acetate

スピラマイシン酢酸エステル



(Spiramycin II Acetate (Spiramycin I Acetate))
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-3-
Acetoxy-5-[4-*O*-acetyl-2,6-dideoxy-3-*C*-methyl- α -
L-ribo-hexopyranosyl-(1→4)-3,6-dideoxy-3-
dimethylamino- β -*D*-glucopyranosyloxy]-9-(2,3,4,6-
tetra-deoxy-4-dimethylamino- β -*D*-erythro-
hexopyranosyloxy)-6-formylmethyl-9-hydroxy-4-methoxy-
8-methylhexadeca-10,12-dien-15-olide
(Spiramycin III Acetate)
(3*R*,4*R*,5*S*,6*R*,8*R*,9*R*,10*E*,12*E*,15*R*)-5-
[4-*O*-Acetyl-2,6-dideoxy-3-*C*-methyl- α -*L*-ribo-
hexopyranosyl-(1→4)-3,6-dideoxy-3-dimethylamino- β -
D-glucopyranosyloxy]-9-(2,3,4,6-tetra-deoxy-4-
dimethylamino- β -*D*-erythro-hexopyranosyloxy)-6-
formylmethyl-9-hydroxy-4-methoxy-8-methyl-3-

propionyloxyhexadeca-10,12-dien-15-olide
[74014-51-0, Spiramycin Acetate]

Spiramycin Acetate is a derivative of a mixture of macrolide substances having antibacterial activity produced by the growth of *Streptomyces ambofaciens*.

It contains not less than 900 μg (potency) and not more than 1450 μg (potency) per mg, calculated on the dried basis. The potency of Spiramycin Acetate is expressed as mass (potency) of spiramycin acetate II ($\text{C}_{47}\text{H}_{78}\text{N}_2\text{O}_{16}$; 927.13). One mg (potency) of Spiramycin Acetate is equivalent to 0.7225 mg of spiramycin acetate II ($\text{C}_{47}\text{H}_{78}\text{N}_2\text{O}_{16}$).

Description Spiramycin Acetate occurs as a white to light yellowish white powder.

It is very soluble in acetonitrile and in methanol, freely soluble in ethanol (99.5), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Spiramycin Acetate in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spiramycin Acetate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Content ratio of the active principle Dissolve 25 mg of Spiramycin Acetate in 25 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 5 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the areas, A_{II} , A_{III} , A_{IV} , A_{V} , A_{VI} and A_{VII} , of the peaks of spiramycin acetate II, spiramycin acetate III, spiramycin acetate IV, spiramycin acetate V, spiramycin acetate VI and spiramycin acetate VII, respectively, by the automatic integration method, and calculate the ratios of the amounts of A_{II} , A_{IV} and the total of A_{III} and A_{V} to the total amount of all these peaks: the amount of A_{II} is 30 – 45%, A_{IV} is 30 – 45%, and the total of A_{III} and A_{V} is not more than 25%. The relative retention times of spiramycin acetate III, spiramycin acetate IV, spiramycin acetate V, spiramycin acetate VI and spiramycin acetate VII with respect to spiramycin acetate II are 1.3, 1.7, 2.3, 0.85 and 1.4, respectively.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (3 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile, 0.02 mol/L potassium dihydrogen phosphate TS and a solution of dipotassium hydrogen phosphate (87 in 25,000) (26:7:7).

Flow rate: Adjust the flow rate so that the retention time of acetylspiramycin II is about 10 minutes.

System suitability—

System performance: Dissolve 25 mg of Spiramycin Acetate II Reference Standard in the mobile phase to make 100 mL. When the procedure is run with 5 μL of this solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of spiramycin acetate II are not less than 14,500 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 5 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of spiramycin acetate II is not more than 2.0%.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Spiramycin Acetate according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Spiramycin Acetate according to Method 3, and perform the test (not more than 1 ppm).

Loss on drying <2.41> Not more than 3.0% (1 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.5% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

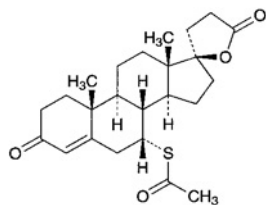
(iii) Standard solutions—Weigh accurately an amount of Spiramycin Acetate II Reference Standard, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C, and use within 3 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Spiramycin Acetate, equivalent to about 50 mg (potency), dissolve in 20 mL of methanol, and add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution for antibiotics, pH 8.0 to make solutions so that each mL contains 80 μg (potency) and 20 μg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Spirolactone

スピロノラクトン



$C_{24}H_{32}O_4S$: 416.57
7 α -Acetylsulfanyl-3-oxo-17 α -pregn-4-ene-21,17 β -
carbolactone [52-01-7]

Spirolactone, when dried, contains not less than 97.0% and not more than 103.0% of $C_{24}H_{32}O_4S$.

Description Spirolactone occurs as a white to light yellow-brown, fine powder.

It is freely soluble in chloroform, soluble in ethanol (95), slightly soluble in methanol, and practically insoluble in water.

Melting point: 198 – 207°C (Insert the capillary tube into a bath at about 125°C, and continue the heating so that the temperature rises at a rate of about 10°C per minute in the range between 140°C and 185°C, and when the temperature is near the expected melting range, reduce the heating so that the temperature rises at a rate of about 3°C per minute.)

Identification (1) Determine the absorption spectrum of a solution of Spirolactone in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Spirolactone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Spirolactone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Spirolactone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve Spirolactone and Spirolactone Reference Standard in methanol, respectively, then evaporate methanol to dryness, and repeat the test on the residues.

Optical rotation <2.49> $[\alpha]_D^{20}$: –33 – –37° (after drying, 0.25 g, chloroform, 25 mL, 200 mm).

Purity (1) Mercapto compounds—Shake 2.0 g of Spirolactone with 20 mL of water, and filter. To 10 mL of the filtrate add 1 mL of starch TS and 0.05 mL of 0.01 mol/L iodine VS, and mix: a blue color develops.

(2) Related substances—Dissolve 0.20 g of Spirolactone in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of

the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with *n*-butyl acetate to a distance of about 15 cm, and air-dry the plate. Spray evenly a solution of sulfuric acid in methanol (1 in 10) on the plate, and heat the plate at 105°C for 10 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Spirolactone and Spirolactone Reference Standard, previously dried at 105°C for 2 hours, dissolve in methanol to make exactly 250 mL. Pipet 5 mL each of these solutions, add methanol to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 238 nm.

$$\begin{aligned} &\text{Amount (mg) of } C_{24}H_{32}O_4S \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : amount (mg) of Spirolactone Reference Standard

Containers and storage Containers—Tight containers.

Stearic Acid

ステアリン酸

Stearic Acid is solid fatty acids obtained from fats, and it consists chiefly of stearic acid ($C_{18}H_{36}O_2$: 284.48) and palmitic acid ($C_{16}H_{32}O_2$: 256.42).

Description Stearic Acid occurs as white, unctuous or crystalline masses or powder. It has a faint, fatty odor.

It is freely soluble in diethyl ether, soluble in ethanol (95), and practically insoluble in water.

Melting point: 56 – 72°C.

Acid value <1.13> 194 – 210

Iodine value <1.13> Not more than 4.0.

Purity (1) Mineral acid—Melt 5 g of Stearic Acid by warming, shake with 5 mL of boiling water for 2 minutes, filter after cooling, and add 1 drop of methyl orange TS to the filtrate: no red color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Stearic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Fat and paraffin—Boil 1.0 g of Stearic Acid with 0.5 g of anhydrous sodium carbonate and 30 mL of water: the solution, while hot, is clear or not more turbid than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 30 mL, and add 1 mL of silver nitrate TS.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Containers and storage Containers—Well-closed containers.

Stearyl Alcohol

ステアリルアルコール

Stearyl Alcohol is a mixture of solid alcohols, and consists chiefly of stearyl alcohol ($C_{18}H_{38}O$: 270.49).

Description Stearyl Alcohol occurs as a white, unctuous matter. It has a faint, characteristic odor. It is tasteless.

It is freely soluble in ethanol (95), in ethanol (99.5), in diethyl ether, and practically insoluble in water.

Melting point <1.13> 56 – 62°C Prepare the sample according to Method 2, then attach tightly a capillary tube to the bottom of the thermometer by means of a rubber band or by any suitable means, and make the bottom of the capillary tube equal in position to the lower end of the thermometer. Insert this thermometer into a test tube 17 mm in inside diameter and about 170 mm in height, fasten the thermometer with cork stopper so that the lower end of the thermometer is about 25 mm distant from the bottom of the test tube. Suspend the test tube in a beaker containing water, and heat the beaker with constant stirring until the temperature rises to 5°C below the expected melting point. Then regulate the rate of increase to 1°C per minute. The temperature at which the sample is transparent and no turbidity is produced is taken as the melting point.

Acid value <1.13> Not more than 1.0.

Ester value <1.13> Not more than 3.0.

Hydroxyl value <1.13> 200 – 220

Iodine value <1.13> Not more than 2.0.

Purity (1) Clarity of solution—Dissolve 3.0 g of Stearyl Alcohol in 25 mL of ethanol (99.5) by warming: the solution is clear.

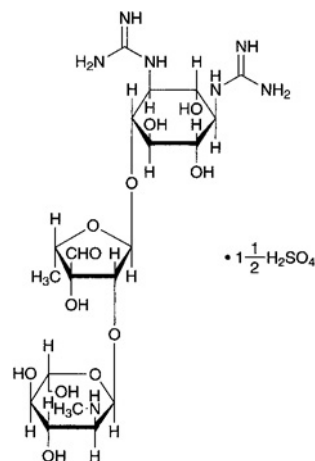
(2) Alkalinity—To the solution obtained in (1) add 2 drops of phenolphthalein TS: no red color develops.

Residue on ignition <2.44> Not more than 0.05% (2 g).

Containers and storage Containers—Well-closed containers.

Streptomycin Sulfate

ストレプトマイシン硫酸塩



$C_{21}O_{39}N_7O_{12} \cdot 1\frac{1}{2}H_2SO_4$: 728.69

2-Deoxy-2-methylamino- α -L-glucopyranosyl-(1 \rightarrow 2)-5-deoxy-3-C-formyl- α -L-lyxofuranosyl-(1 \rightarrow 4)- N,N' -diamidino-D-streptamine sesquisulfate [3810-74-0]

Streptomycin Sulfate is the sulfate of an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces griseus*.

It contains not less than 740 μ g (potency) and not more than 820 μ g (potency) per mg, calculated on the dried basis. The potency of Streptomycin Sulfate is expressed as mass (potency) of streptomycin ($C_{21}H_{39}N_7O_{12}$: 581.57).

Description Streptomycin Sulfate occurs as a white to light yellowish white powder.

It is freely soluble in water, and very slightly soluble in ethanol (95).

Identification (1) Dissolve 50 mg of Streptomycin Sulfate in 5 mL of water, add 1 mL of ninhydrin TS and 0.5 mL of pyridine, and heat for 10 minutes: a purple color is developed.

(2) Dissolve 10 mg each of Streptomycin Sulfate and Streptomycin Sulfate Reference Standard in 10 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a solution of potassium dihydrogen phosphate (7 in 100) to a distance of about 12 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at about 150°C for about 5 minutes: the principal spots from the sample solution and the standard solution show the same in color tone and R_f value.

(3) A solution of Streptomycin Sulfate (1 in 5) responds to the Qualitative Tests <1.09> for sulfate.

Optical rotation <2.49> $[\alpha]_D^{20}$: -79 – -88° (0.5 g calculated on the dried basis, water, 50 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 2.0 g of Streptomycin Sulfate in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Streptomycin Sulfate in 5 mL of water is clear, and colorless or pale yellow.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Streptomycin Sulfate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Streptomycin Sulfate according to Method 3 and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve exactly 0.20 g of Streptomycin Sulfate in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), add a mixture of methanol and sulfuric acid (97:3) to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve exactly 36 mg of D(+)-mannose in a mixture of methanol and sulfuric acid (97:3) to make 5 mL, and heat under a reflux condenser for 1 hour. After cooling, wash the inside of the condenser with a suitable amount of a mixture of methanol and sulfuric acid (97:3), and add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL. Pipet 5 mL of this solution, add a mixture of methanol and sulfuric acid (97:3) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and acetic acid (100) (2:1:1) to a distance of 13 to 15 cm, and air-dry the plate. Spray evenly a mixture of a solution of 1,3-dihydroxynaphthalene in ethanol (95) (1 in 500) and diluted sulfuric acid (1 in 5) (1:1) on the plate, and heat at 110°C for 5 minutes: the spot from the sample solution corresponding to the spot from the standard solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 5.0% (0.5 g, reduced pressure not exceeding 0.67 kPa, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer, having pH 7.8 – 8.0 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Streptomycin Sulfate Reference Standard, previously dried, equivalent to about 20 mg (potency), dissolve in diluted phosphate buffer solution, pH 6.0 (1 in 2) to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as

the high concentration standard solution and low concentration standard solution, respectively.

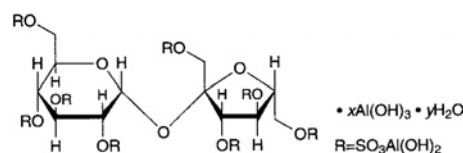
(iv) Sample solutions—Weigh accurately an amount of Streptomycin Sulfate, equivalent to about 20 mg (potency), dissolve in water to make exactly 50 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Sucralfate Hydrate

Aluminum Sucrose Sulfate Ester

スクラルファート水和物



$C_{12}H_{30}Al_8O_{51}S_8 \cdot xAl(OH)_3 \cdot yH_2O$
[54182-58-0]

Sucralfate Hydrate contains not less than 17.0% and not more than 21.0% of aluminum (Al: 26.98) and not less than 34.0% and not more than 43.0% of sucrose octasulfate ester ($C_{12}H_{22}O_{35}S_8$: 982.80), calculated on the dried basis.

Description Sucralfate Hydrate occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in hot water, in ethanol (95) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sulfuric acid-sodium hydroxide TS.

Identification (1) To 0.05 g of Sucralfate Hydrate in a small test tube add 0.05 g of fresh pieces of sodium, and melt by careful heating. Immerse the test tube immediately in 100 mL of water, break the test tube, shake well, and filter. To 5 mL of the filtrate add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a red-purple color develops.

(2) Dissolve 40 mg of Sucralfate Hydrate in 2 mL of dilute sulfuric acid, and add gently 2 mL of anthrone TS to make 2 layers: a blue color develops at the zone of contact, and gradually changes to blue-green.

(3) Dissolve 0.5 g of Sucralfate Hydrate in 10 mL of dilute hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for aluminum.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sucralfate Hydrate in 10 mL of dilute sulfuric acid: the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.5 g of Sucralfate Hydrate in 30 mL of dilute nitric acid, and heat gently to boiling. After cooling, add water to make 100 mL, and to 10 mL of this solution add 3 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.70 mL of 0.01

mol/L hydrochloric acid VS (not more than 0.50%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Sucralfate Hydrate in 20 mL of a solution of sodium chloride (1 in 5) and 1 mL of dilute hydrochloric acid, and to this solution add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: evaporate 1 mL of dilute hydrochloric acid on a water bath to dryness, and add 20 mL of a solution of sodium chloride (1 in 5), 2 mL of dilute acetic acid, 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(4) Arsenic <1.11>—Dissolve 1.0 g of Sucralfate Hydrate in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 2 ppm).

(5) Free aluminum—To 3.0 g of Sucralfate Hydrate add 50 mL of water, heat in a water bath for 5 minutes, cool, and filter. Wash the residue with four 5-mL portions of water, combine the filtrate with the washings, add 2 mL of dilute hydrochloric acid, and heat in a water bath for 30 minutes. After cooling, neutralize the solution with sodium hydroxide TS, add water to make exactly 100 mL, and use this solution as the sample solution. Pipet 50 mL of the sample solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination (not more than 0.2%).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.349 mg of Al

(6) Related substances—Proceed with 50 μ L of the sample solution obtained in the Assay (2) Sucrose octasulfate ester as directed in the Assay (2) Sucrose octasulfate ester, and perform the test as directed under Liquid Chromatography <2.01>. Determine the peak area of sucrose octasulfate ester from the sample solution and that of a related substance with the relative retention time about 0.7 to the peak of sucrose octasulfate ester by the automatic integration method, and calculate the ratio of the peak area of the related substance to that of sucrose octasulfate ester: it is not more than 0.1.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of sucrose octasulfate ester from 50 μ L of the standard solution obtained in the Assay (2) Sucrose octasulfate ester composes 60% to 100% of the full scale.

Loss on drying <2.41> Not more than 14.0% (1 g, 105°C, 3 hours).

Acid-consuming capacity Weigh accurately about 0.25 g of Sucralfate Hydrate, previously dried, place in a 200-mL glass-stoppered conical flask, add exactly 100 mL of 0.1 mol/L hydrochloric acid VS, stopper the flask tightly, and shake at $37 \pm 2^\circ\text{C}$ for exactly 1 hour (150 shakings per minute, amplitude: 20 mm). After cooling in water for 5 minutes, pipet 10 mL of the supernatant liquid, and titrate <2.50> the excess acid with 0.1 mol/L sodium hydroxide VS until the pH becomes 3.5. Perform a blank determination. The amount of 0.1 mol/L hydrochloric acid VS consumed per g of Sucralfate is not less than 130 mL.

Assay (1) Aluminum—Weigh accurately about 1 g of Sucralfate Hydrate, dissolve in 10 mL of dilute hydrochloric acid by warming on a water bath, cool, and add water to make exactly 250 mL. Pipet 25 mL of this solution, add exactly 25 mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS and 20 mL of acetic acid-ammonium acetate buffer solution, pH 4.5, and boil for 5 minutes. After cooling, add 50 mL of ethanol (95), and titrate <2.50> the excess disodium dihydrogen ethylenediamine tetraacetate with 0.05 mol/L zinc acetate VS until the color of the solution changes from green-purple through purple to red (indicator: 3 mL of dithizone TS). Perform a blank determination.

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.349 mg of Al

(2) Sucrose octasulfate ester—Weigh accurately about 0.55 g of Sucralfate Hydrate, add exactly 10 mL of sulfuric acid-sodium hydroxide TS, shake vigorously, and dissolve with ultrasonic wave at below 30°C for 5 minutes. To this solution add 0.1 mol/L sodium hydroxide VS to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.25 g of Potassium Sucrose Octasulfate Reference Standard, add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Prepare rapidly the sample solution and the standard solution, and perform the test immediately. Pipet 50 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of sucrose octasulfate ester from each solution.

Amount (mg) of sucrose octasulfate ester ($\text{C}_{12}\text{H}_{22}\text{O}_{35}\text{S}_8$)
= $W_S \times (A_T/A_S) \times 0.7633$

W_S : Amount (mg) of Potassium Sucrose Octasulfate Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: A differential refractometer.

Column: A stainless steel column about 4 mm in inside diameter and about 30 cm in length, packed with aminopropylsilylated silica gel for liquid chromatography (about 8 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve a suitable amount (26 to 132 g) of ammonium sulfate in 1000 mL of water, and adjust with phosphoric acid to a pH of 3.5. Allow a solution of Potassium Sucrose Octasulfate Reference Standard in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and perform the test immediately. Adjust the amount of ammonium sulfate in the mobile phase so that the peak of a related substance with the relative retention time being about 0.7 to that of sucrose octasulfate ester almost returns to the base line, and the peak of sucrose octasulfate ester elutes most rapidly.

Flow rate: Adjust the flow rate so that the retention time of sucrose octasulfate ester is between 6 and 11 minutes.

Selection of column: Allow a solution of Potassium Sucrose Octasulfate Reference Standard in dilute hydrochloric acid (1 in 100) to stand at 60°C for 10 minutes, cool, and proceed immediately with 50 μ L of this solution under the above operating conditions. Use a column with a resolution being

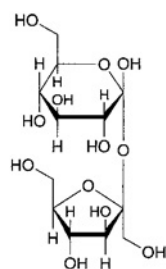
not less than 1.5 between sucrose octasulfate ester and a related substance with the relative retention time being about 0.7 to sucrose octasulfate ester.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of sucrose octasulfate ester is not more than 2.0%.

Containers and storage Containers—Tight containers.

Sucrose

精製白糖



$C_{12}H_{22}O_{11}$: 342.30

β -D-Fructofuranosyl α -D-glucopyranoside [57-50-1]

Sucrose contains no additives.

For Sucrose used for preparation of the large volume infusions, the label states the purpose.

Description Sucrose is a white crystalline powder, or lustrous colorless or white crystals.

It is very soluble in water, and slightly soluble in ethanol (95).

Identification (1) To 10 mg each of Sucrose and white soft sugar add diluted methanol (3 in 5) to make 20 mL each, and use these solutions as the sample solution and the standard solution (a), respectively. Separately, to 10 mg each of glucose, lactose hydrate, fructose and white soft sugar add methanol (3 in 5) to make 20 mL, and use this solution as the standard solution (b). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (a) and (b) on a plate of silica gel for thin-layer chromatography, and dry the plate completely. Develop the plate with a mixture of 1,2-dichloroethane, acetic acid (100), methanol and water (10:5:3:2) to a distance of about 15 cm, and dry the plate with a hot air. And immediately repeat the development with replaced developing mixture, and dry the plate in the same way. Spray evenly a solution of 0.5 g of thymol in 100 mL of a mixture of ethanol (95) and sulfuric acid (19:1), heat at 130°C for 10 minutes: the principal spot from the sample solution is the same with the principal spot from the standard solution (a) in the R_f , color and size, and four spots from the standard solution (b) are apparently distinguishable.

(2) Dissolve 50.0 g of Sucrose in recently boiled and cooled water to make 100 mL, and use this solution as the sample solution. To 1 mL of the sample solution add water to make 100 mL, then to 5 mL of this solution add 0.15 mL of freshly prepared copper (II) sulfate TS and 2 mL of freshly prepared 2 mol/L sodium hydroxide TS: the solution is clear

and blue, and not changes on boiling. Then to this solution add 4 mL of dilute hydrochloric acid, boil, and add 4 mL of 2 mol/L sodium hydroxide TS: orange precipitates are immediately produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +66.3 – +67.0° (26 g, water, 100 mL, 100 mm).

Purity (1) Clarity and color of solution—The sample solution obtained in the Identification (2) is clear, and has no more color than the following control solution.

Control solution: To exactly 2.4 mL of iron (III) chloride colorimetric stock solution and exactly 0.6 mL of cobalt (II) chloride colorimetric stock solution add 7.0 mL of diluted hydrochloric acid (7 in 250). To 5.0 mL of this solution add 95.0 mL of diluted hydrochloric acid (7 in 250).

(2) Acidity or alkalinity—To 10 mL of the sample solution obtained in the Identification (2) add 0.3 mL of phenolphthalein TS: the solution is colorless, and develops a red color on addition of 0.3 mL of 0.01 mol/L sodium hydroxide VS.

(3) Sulfite—Dissolve 5.0 g of Sucrose in 40 mL of water, add 2.0 mL of dilute sodium hydroxide TS and water to make exactly 50 mL, and use this solution as the sample solution. Separately, dissolve 0.076 g of sodium disulfite in water to make exactly 50 mL, then pipet 5 mL of this solution, add water to make exactly 100 mL. Pipet 3 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Immediately, pipet 10 mL each of the sample solution and the standard solution, add 1.0 mL of 3 mol/L hydrochloric acid, 2.0 mL of decolorized fuchsin TS and 2.0 mL of formaldehyde solution TS, and allow to stand for 30 minutes. Determine the absorbance at 583 nm of these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24> using the control solution obtained by proceeding with 10.0 mL of water in the same manner as above: the absorbance of the sample solution is not larger than that of the standard solution (not more than 15 ppm as SO_2). When the standard solution does not show a red-purple to blue-purple color, result of the test is invalid.

(4) Lead—Put exactly 50 mg of Sucrose in a polytetrafluoroethylene decomposition-vessel, add 0.5 mL of nitric acid to dissolve, seal up the vessel, and heat at 150°C for 5 hours. After cooling, add water to make exactly 5 mL, and use this solution as the sample solution. Perform the test with more than 3 parts of the sample solution as directed in the standard addition method under Atomic Absorption Spectrophotometry <2.23> (electrothermal type) according to the following conditions. The standard solution is prepared by adding water to a suitable volume of Standard Lead Solution exactly volumed, and perform a blank determination with a solution prepared by adding water to 10.0 mL of nitric acid to make exactly 100 mL, and make any necessary correction (not more than 0.5 ppm).

Operating conditions—

Lamp: A hollow cathode lamp

Wavelength: 283.3 nm

Temperature for drying: 110°C

Temperature for incineration: 600°C

Temperature for atomization: 2100°C

(5) Invert sugar—Transfer 5 mL of the sample solution obtained in the Identification (2) to a test-tube about 150 mm long and about 16 mm in diameter, add 5 mL of water, 1.0 mL of 1 mol/L sodium hydroxide VS and 1.0 mL of methy-

lene blue TS, mix, and place in a water bath. After exactly 2 minutes, take the tube out of the bath, and examine the solution immediately: the blue color does not disappear completely (0.04%). Ignore any blue color at the air and solution interface.

Conductivity

(i) Potassium chloride conductivity calibration standard solution—Dissolve powdered potassium chloride, previously dried at 500–600°C for 4 hours, in newly distilled water having less conductivity than $2 \mu\text{S}\cdot\text{cm}^{-1}$ to get three kinds of the standard solution containing 0.7455 g, 0.0746 g and 0.0149 g of potassium chloride in 1000.0 g, respectively. The conductivities of these solutions at 20°C are shown in the following table.

Standard solution (g/1000.0 g)	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	Resistivity ($\Omega\cdot\text{cm}$)
0.7455	1330	752
0.0746	133.0	7519
0.0149	26.6	37594

(ii) Apparatus—Use an appropriate conductivity meter. The conductivity is determined to measure the electrical resistance of the column of liquid between the electrodes of the immersed measuring device (conductivity cell). The apparatus is supplied with alternative current to avoid the effects of electrode polarization. It is usually equipped with a temperature compensation device. The conductivity cell contains of two parallel platinum electrodes coated with platinum black, and both electrodes are generally protected by a glass tube which allows good exchange between the solution and the electrodes. Use a cell giving the cell constant of 0.01 to 1 cm^{-1} .

(iii) Procedure—Use the suitable potassium chloride conductivity calibration standard solution to the measurement. After washing the well with water, rinse 2 to 3 times with the calibration standard solution, fill up the cell with the calibration standard solution, and determine the conductivity of the calibration standard solution kept at $20 \pm 0.1^\circ\text{C}$. Repeat the determination, and measure the conductivity of the calibration standard solution, G_{χ_0} (μS), after a stable reading of $\pm 3\%$ is obtained. The cell constant, J , is calculated by the following:

$$J = \frac{\chi_{\text{KCl}}}{G_{\chi_0}}$$

J : Cell constant (cm^{-1})

χ_{KCl} : Conductivity constant of the potassium chloride conductivity calibration standard solution ($\mu\text{S}\cdot\text{cm}^{-1}$) (20°C)

G_{χ_0} : Conductivity measured (μS)

Dissolve 31.3 g of Sucrose in newly distilled water to make exactly 100 mL, and use this solution as the sample solution. After washing well the cell with water, rinse the cell with the sample solution 2 to 3 times, fill up with the sample solution, and determine the conductivity of the sample solution, G_T (μS), kept at $20 \pm 0.1^\circ\text{C}$, while stirring. Determine the conductivity of the water used for preparation of the sample solution, G_0 (μS), in the same manner as above, and calculate the conductivity, χ_T ($\mu\text{S}\cdot\text{cm}^{-1}$) and χ_0 ($\mu\text{S}\cdot\text{cm}^{-1}$), by the following expressions:

$$\chi_T (\mu\text{S}\cdot\text{cm}^{-1}) = JG_T$$

$$\chi_0 (\mu\text{S}\cdot\text{cm}^{-1}) = JG_0$$

Determine the corrected conductivity, χ_C , of the sample solution by the following expression: not more than $35 \mu\text{S}\cdot\text{cm}^{-1}$.

$$\chi_C (\mu\text{S}\cdot\text{cm}^{-1}) = \chi_T - 0.35 \chi_0$$

Loss on drying <2.41> Not more than 0.1% (2 g, 105°C, 3 hours).

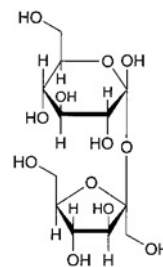
Dextrins For Sucrose used to prepare large volume aqueous infusions, to 2 mL of the sample solution obtained in the Identification (2) add 8 mL of water, 0.05 mL of dilute hydrochloric acid and 0.05 mL of iodine TS: the solution remains yellow.

Bacterial endotoxins <4.01> Less than 0.25 EU/mg, for Sucrose exclusively to be used to prepare Injections for intravenous infusion of larger volume.

Containers and storage Containers—Well-closed containers.

White Soft Sugar

白糖



$\text{C}_{12}\text{H}_{22}\text{O}_{11}$: 342.30

β -D-Fructofuranosyl α -D-glucopyranoside [57-50-1]

Description White Soft Sugar is colorless or white crystals or crystalline powder. It is odorless and has a sweet taste.

It is very soluble in water, very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of White Soft Sugar (1 in 10) is neutral.

Identification (1) When 1 g of White Soft Sugar is ignited, it melts and swells, and decomposes, emitting an odor of caramel, to bulky charcoal.

(2) To 0.1 g of White Soft Sugar add 2 mL of dilute sulfuric acid, boil, add 4 mL of sodium hydroxide TS and 3 mL of Fehling's TS, and heat to boiling: a red to dark red precipitate is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: +65.0 – +67.0° (after drying, 13 g, water, 50 mL, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 100 g of White Soft Sugar in 100 mL of water, take 50 mL of this solution in a Nessler tube, and view transversely the Nessler tube against a white background: the solution is colorless or only slightly yellow and has no blue color. Fill the solution in the Nessler tube, stopper, and allow to stand for 2 days: no precipitate is produced.

(2) Chloride <1.03>—To 10.0 g of White Soft Sugar add water to make 100 mL, and use this solution as the sample so-

lution. To 20 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <1.14>—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Calcium—To 10 mL of the sample solution obtained in (2) add 1 mL of ammonium oxalate TS: this solution shows immediately no change.

(5) Heavy metals <1.07>—Proceed with 5.0 g of White Soft Sugar according to Method 1, and perform the test. Prepare the control solution with 2.5 mL of Standard Lead Solution (not more than 5 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of White Soft Sugar according to Method 1, and perform the test (not more than 2 ppm).

(7) Invert sugar—Dissolve 5.0 g of White Soft Sugar in water to make 100 mL, filter if necessary, and use this solution as the sample solution. Separately place 100 mL of alkaline copper (II) sulfate solution in a 300-mL beaker, cover the beaker with a watch glass, and boil. Immediately add 50.0 mL of the sample solution, boil the mixture exactly for 5 minutes, add at once 50 mL of freshly boiled and cooled water, dip it in a water bath of a temperature below 10°C for 5 minutes, and collect the precipitate in a tared glass filter (G4). Wash the residue on the filter with water until the last washing is neutral, then wash with 10 mL of ethanol (95), add 10 mL of diethyl ether, and dry at 105°C for 30 minutes: the mass of the residual precipitate is not more than 0.120 g.

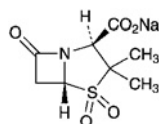
Loss on drying <2.41> Not more than 1.30% (15 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (2 g).

Containers and storage Containers—Well-closed containers.

Sulbactam Sodium

スルバクタムナトリウム



$C_8H_{10}NNaO_5S$: 255.22

Monosodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate 4,4-dioxide [69388-84-7]

Sulbactam Sodium contains not less than 875 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Sulbactam Sodium is expressed as mass (potency) of sulbactam ($C_8H_{11}NO_5S$: 233.24).

Description Sulbactam Sodium occurs as a white to yellowish white crystalline powder.

It is freely soluble in water, sparingly soluble in methanol,

very slightly soluble in ethanol (99.5), and practically insoluble in acetonitrile.

Identification (1) Determine the infrared absorption spectrum of Sulbactam Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbactam Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +219 – +233° (1 g, water, 100 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the pH of the solution is between 5.2 and 7.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulbactam Sodium in 20 mL of water: the solution is clear, and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sulbactam Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulbactam Sodium as directed in Method 3, and perform the test (not more than 2 ppm).

(4) Sulbactam penicillamine—Weigh accurately about 0.2 g of Sulbactam Sodium, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of sulbactam sodium for sulbactam penicillamine, dissolve in 2 mL of water, add 0.5 mL of sodium hydroxide TS, allow to stand for 10 minutes at a room temperature, and add 0.5 mL of 1 mol/L hydrochloric acid TS, then add the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of sulbactam penicillamine by the automatic integration method: the amount of sulbactam penicillamine is not more than 1.0%.

$$\begin{aligned} &\text{Amount (\%)} \text{ of sulbactam penicillamine} \\ &= (W_S/W_T) \times (A_T/A_S) \times 5 \end{aligned}$$

W_S : Amount (mg) of sulbactam sodium for sulbactam penicillamine

W_T : Amount (mg) of the sample

Operating conditions—

Column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

System suitability—

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam penicillamine is not more than 2.0%.

Water <2.48> Not more than 1.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Sulbactam Sodium and Sulbactam Reference Standard, equivalent to about 0.1 g (potency), dissolve each in a suitable amount of the mobile phase, add exactly 10 mL of the internal standard solution, then add the mobile phase to make 100 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of sulbactam to that of the internal standard.

$$\text{Amount } [\mu\text{g (potency)}] \text{ of sulbactam } (\text{C}_8\text{H}_{11}\text{NO}_5\text{S}) \\ = W_S \times (Q_T/Q_S) \times 1000$$

W_S : Amount [mg (potency)] of Sulbactam Reference Standard

Internal standard solution—A solution of ethyl parahydroxybenzoate in the mobile phase (7 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: To 750 mL of 0.005 mol/L tetrabutylammonium hydroxide TS add 250 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of sulbactam is about 6 minutes.

System suitability—

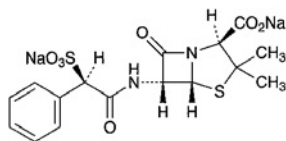
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, sulbactam and the internal standard are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of sulbactam is not more than 1.0%.

Containers and storage Containers—Tight containers.

Sulbenicillin Sodium

スルベニシリンナトリウム



$\text{C}_{16}\text{H}_{16}\text{N}_2\text{Na}_2\text{O}_7\text{S}_2$: 458.42

Disodium (2*S*,5*R*,6*R*)-3,3-dimethyl-7-oxo-6-[(2*R*)-2-phenyl-2-sulfonatoacetyl-amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate [28002-18-8]

Sulbenicillin Sodium contains not less than 900 μ g (potency) and not more than 970 μ g (potency) per mg, calculated on the anhydrous basis. The potency of Sulbenicillin Sodium is expressed as mass (potency) of sulbenicillin ($\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_7\text{S}_2$: 414.45).

Description Sulbenicillin Sodium occurs as white to light yellowish white powder.

It is very soluble in water, freely soluble in methanol, and slightly soluble in ethanol (99.5).

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Sulbenicillin Sodium as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sulbenicillin Sodium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Sulbenicillin Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

Optical rotation <2.49> $[\alpha]_D^{20}$: +167 – +182° (1 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.20 g of Sulbenicillin Sodium in 10 mL of water is between 4.5 and 7.0.

Purity (1) Clarity and color of solution—Dissolve 2.5 g of Sulbenicillin Sodium in 5 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulbenicillin Sodium according to Method 1, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulbenicillin Sodium in 15 mL of the mobile phase, and use this solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of these peaks by the area percentage method: the amount of the each peak other than the two peaks of sulbenicillin is not more than 2.0%, and the total amount of the peaks other than the two peaks of sulbenicillin is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 10 g of potassium dihydrogen phosphate in 750 mL of water, adjust the pH to 6.0 \pm 0.1 with sodium hydroxide TS, and add water to make 1000 mL. To 940 mL of this solution add 60 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of

the lately eluted peak of sulbenicillin is about 18 minutes.

Time span of measurement: About 1.5 times as long as the retention time of the lately eluted peak of sulbenicillin beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add the mobile phase to make exactly 10 mL. Confirm that the total area of the two peaks of sulbenicillin obtained from 10 μ L of this solution is equivalent to 7 to 13% of that from 10 μ L of the solution for system suitability test.

System performance: When the procedure is run with 10 μ L of the sample solution under the above operating conditions, the resolution between the two peaks of sulbenicillin is not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the total areas of the two peaks of sulbenicillin is not more than 5.0%.

Water <2.48> Not more than 6.0% (0.5 g, volumetric titration, direct titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.4 to 6.6 after sterilization.

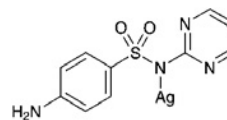
(iii) Standard solutions—Weigh accurately an amount of Sulbenicillin Sodium Reference Standard, equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution in a freezer, and use within 4 days. Take exactly a suitable amount of the standard stock solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μ g (potency) and 10 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Sulbenicillin Sodium, equivalent to about 50 mg (potency), and dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 40 μ g (potency) and 10 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Sulfadiazine Silver

スルファジアジン銀



$C_{10}H_9AgN_4O_2S$: 357.14

Monosilver 4-amino-*N*-(pyrimidin-2-yl)-benzenesulfonamide [22199-08-2]

Sulfadiazine Silver, when dried, contains not less than 99.0% and not more than 102.0% of $C_{10}H_9AgN_4O_2S$.

Description Sulfadiazine Silver occurs as a white to pale yellow, crystalline powder. It is odorless.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

It dissolves in ammonia TS.

It is gradually colored by light.

Melting point: about 275°C (with decomposition).

Identification Determine the infrared absorption spectrum of Sulfadiazine Silver, previously dried, as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Sulfadiazine Silver Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Nitrate—To 250 mL of water add 1.0 g of Sulfadiazine Silver, shake well for 50 minutes, filter, and use this filtrate as the sample solution. Separately, weigh accurately 0.25 g of potassium nitrate, and dissolve in water to make exactly 200 mL. Pipet 5 mL of this solution and add water to make exactly 200 mL, and use this solution as the standard solution. Pipet 2.0 mL each of the sample solution and the standard solution, and add 5 mL of a solution of cromotropic acid in sulfuric acid (1 in 10,000) and sulfuric acid to make exactly 10 mL. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with exactly 2.0 mL of water in the same manner, as the blank: A_T is not larger than A_S (not more than 0.05%).

(2) Related substances—Dissolve 50 mg of Sulfadiazine Silver in 5 mL of a mixture of ethanol (95) and ammonia solution (28) (3:2), and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add a mixture of ethanol (95) and ammonia solution (28) (3:2) to make exactly 20 mL. Pipet 2 mL of this solution, add a mixture of ethanol (95) and ammonia solution (28) (3:2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (10:5:2) to a distance of about 15 cm, and air-dry the

plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, phosphorus (V) oxide, 80%, 4 hours).

Residue on ignition <2.44> 41.0 – 45.0% (1 g).

Silver content Weigh accurately about 50 mg of Sulfadiazine Silver, previously dried, dissolve in 2 mL of nitric acid, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 100 mL, and use this solution as the sample solution. Measure accurately a suitable quantity of Standard Silver Solution for Atomic Absorption Spectrophotometry <2.23>, dilute with water to make a solution containing 1.0 to 2.0 μg of silver (Ag:107.87) per ml, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions, and calculate the silver content of the sample solution from the calibration curve obtained from the absorbance of the standard solution: it contains not less than 28.7% and not more than 30.8% of silver.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: A silver hollow cathode lamp

Wavelength: 328.1 nm

Assay Weigh accurately about 0.1 g each of Sulfadiazine Silver and Sulfadiazine Silver Reference Standard, each previously dried, and add ammonia TS to make exactly 100 mL, respectively. Pipet 1 mL each of these solutions, add water to make exactly 100 mL, and use these solutions as the sample solution and the standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 255 nm, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution, prepared with exactly 1 mL of ammonia TS and a sufficient water to make exactly 100 mL, as the blank.

$$\begin{aligned} &\text{Amount (mg) of } C_{10}H_9AgN_4O_2S \\ &= W_S \times (A_T/A_S) \end{aligned}$$

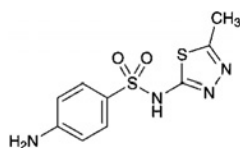
W_S : Amount (mg) of Sulfadiazine Silver Reference Standard

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamethizole

スルファメチゾール



$C_9H_{10}N_4O_2S_2$: 270.33

4-Amino-*N*-(5-methyl-1,3,4-thiadiazol-2-yl)-benzenesulfonamide [144-82-1]

Sulfamethizole, when dried, contains not less than 99.0% of $C_9H_{10}N_4O_2S_2$.

Description Sulfamethizole occurs as white to yellowish white crystals or crystalline powder. It is odorless.

It is slightly soluble in ethanol (95), and in acetic acid (100) and practical insoluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethizole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 208 – 211°C

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sulfamethizole in 3 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethizole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand for 1 hour in an ice bath, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfamethizole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulfamethizole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sulfamethizole in acetone to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 50 mL, then pipet 5 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and acetic acid (100) (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethizole, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS according to the potentiometric titration method or the amperometric titration method.

Each mL of 0.1 mol/L sodium nitrite VS
= 27.03 mg of $C_9H_{10}N_4O_2S_2$

Containers and storage Containers—Well-closed contain-

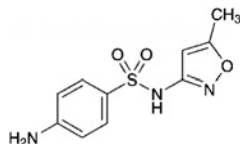
ers.

Storage—Light-resistant.

Sulfamethoxazole

Sulfisomezole

スルファメトキサゾール



$C_{10}H_{11}N_3O_3S$: 253.28

4-Amino-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide
[723-46-6]

Sulfamethoxazole, when dried, contains not less than 99.0% of $C_{10}H_{11}N_3O_3S$.

Description Sulfamethoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in *N,N*-dimethylformamide, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and very slightly soluble in water.

It dissolves in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamethoxazole, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 169 – 172°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamethoxazole in 5 mL of sodium hydroxide TS, and add 20 mL of water: the solution is clear and colorless.

(2) Acidity—To 1.0 g of Sulfamethoxazole add 50 mL of water, heat at 70°C for 5 minutes, allow to stand in ice water for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfamethoxazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulfamethoxazole according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Sulfamethoxazole in 10 mL of a solution of ammonia solution (28) in methanol (1 in 50), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 10 mL. Pipet 1 mL of this solution, add a solution of ammonia solution (28) in methanol (1 in 50) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetonitrile and diluted ammonia solution (28) (7 in 100) (10:8:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Sulfamethoxazole, previously dried, dissolve in 30 mL of *N,N*-dimethylformamide, add 10 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until a light blue color is produced (indicator: 0.5 mL of thymolphthalein TS). Separately, perform a blank determination in the same manner with a mixture of 30 mL of *N,N*-dimethylformamide and 26 mL of water, and make any necessary correction.

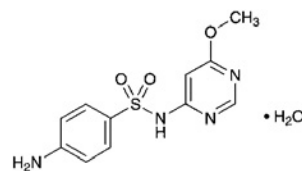
Each mL of 0.1 mol/L sodium hydroxide VS
= 25.33 mg of $C_{10}H_{11}N_3O_3S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfamonomethoxine Hydrate

スルファモノメトキシ水合物



$C_{11}H_{12}N_4O_3S \cdot H_2O$: 298.32

4-Amino-*N*-(6-methoxypyrimidin-4-yl)benzenesulfonamide monohydrate [1220-83-3, anhydride]

Sulfamonomethoxine Hydrate, when dried, contains not less than 99.0% of sulfamonomethoxine ($C_{11}H_{12}N_4O_3S$: 280.31).

Description Sulfamonomethoxine Hydrate occurs as white to pale yellow crystals, granules or crystalline powder. It is odorless.

It is soluble in acetone, slightly soluble in ethanol (95), very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It is gradually colored by light.

Identification Determine the infrared absorption spectrum of Sulfamonomethoxine Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 204 – 206°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow. Dissolve 0.5 g of Sulfamonomethoxine Hydrate in 5 mL of sodium hydroxide TS, and heat: no turbidity is produced. After cooling, add 5 mL of acetone: the solution is clear.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Sulfamonomethoxine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulfamonomethoxine Hydrate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.02 g of Sulfamonomethoxine Hydrate in ethanol (95) to make 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol and ammonia solution (28) (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> 4.5 – 6.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

Assay Weigh accurately about 0.5 g of Sulfamonomethoxine Hydrate, previously dried, dissolve in 5 mL of hydrochloric acid and 50 mL of water, add 10 mL of a solution of potassium bromide (3 in 10), cool below 15°C, and titrate <2.50> with 0.1 mol/L sodium nitrite VS (potentiometric titration or amperometric titration).

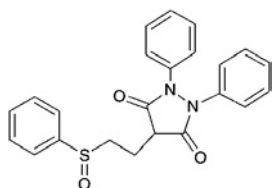
Each mL of 0.1 mol/L sodium nitrite VS
= 28.03 mg of $C_{11}H_{12}N_4O_3S$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Sulfinpyrazone

スルフィンピラゾン



$C_{23}H_{20}N_2O_3S$: 404.48
1,2-Diphenyl-4-[2-(phenylsulfinyl)ethyl]pyrazolidine-3,5-dione [57-96-5]

Sulfinpyrazone, when dried, contains not less than 98.5% of $C_{23}H_{20}N_2O_3S$.

Description Sulfinpyrazone occurs as a white to pale yellowish white powder. It is odorless, and has a bitter taste.

It is freely soluble in acetic acid (100) and in acetone, soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Melting point: about 138°C (with decomposition).

Identification (1) Dissolve 2 mg of Sulfinpyrazone in 1 mL of acetic acid (100), add 1 mL of palladium (II) chloride TS and 2 mL of chloroform, and shake: a yellow color develops in the chloroform layer.

(2) Determine the absorption spectrum of a solution of Sulfinpyrazone in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Sulfinpyrazone Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Sulfinpyrazone, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sulfinpyrazone in 10 mL of acetone: the solution is clear and colorless. Dissolve 0.5 g of Sulfinpyrazone in 10 mL of sodium hydroxide TS: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Sulfinpyrazone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sulfinpyrazone according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Sulfinpyrazone in 5 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution (1). Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot rapidly 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography under a stream of nitrogen. Develop the plate with a mixture of chloroform and acetic acid (100) (4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the most intense spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution (1), and the spots other than the principal and above spots from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Sulfinpyrazone, previously dried, dissolve in 40 mL of acetone, add 40 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 40.45 mg of C₂₃H₂₀N₂O₃S

Containers and storage Containers—Well-closed containers.

Sulfinpyrazone Tablets

スルフィンピラゾン錠

Sulfinpyrazone Tablets contain not less than 93% and not more than 107% of the labeled amount of sulfinpyrazone (C₂₃H₂₀N₂O₃S: 404.48).

Method of preparation Prepare as directed under Tablets, with Sulfinpyrazone.

Identification (1) Weigh a portion of powdered Sulfinpyrazone Tablets, equivalent to 2 mg of Sulfinpyrazone according to the labeled amount, add 1 mL of acetic acid (100), and shake. To this solution add 1 mL of palladium (II) chloride TS and 2 mL of chloroform, and shake: a yellow color develops in the chloroform layer.

(2) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 257 nm and 261 nm.

Dissolution Being specified separately.

Assay Weigh accurately, and powder not less than 20 Sulfinpyrazone Tablets. Weigh accurately a portion of the powder, equivalent to about 50 mg of sulfinpyrazone (C₂₃H₂₀N₂O₃S), add 25 mL of methanol, and shake for 15 minutes. To this solution add 50 mL of sodium hydroxide TS, shake, cool, and add water to make exactly 200 mL. After centrifuging, pipet 4 mL of the supernatant liquid, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.05 g of Sulfinpyrazone Reference Standard, previously dried at 105°C for 2 hours, dissolve in 25 mL of methanol, add 50 mL of sodium hydroxide TS, and cool. To this solution add water to make exactly 200 mL, pipet 4 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S, of these solutions at 260 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\text{Amount (mg) of sulfinpyrazone (C}_{23}\text{H}_{20}\text{N}_{2}\text{O}_{3}\text{S)} \\ = W_S \times (A_T/A_S)$$

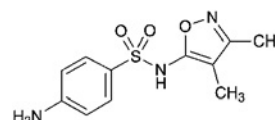
W_S: Amount (mg) of Sulfinpyrazone
Reference Standard

Containers and storage Containers—Well-closed containers.

Sulfisoxazole

Sulfafurazole

スルフイソキサゾール



C₁₁H₁₃N₃O₃S: 267.30

4-Amino-*N*-(3,4-dimethylisoxazol-5-yl)benzenesulfonamide [127-69-5]

Sulfisoxazole, when dried, contains not less than 99.0% of C₁₁H₁₃N₃O₃S.

Description Sulfisoxazole occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in pyridine and in *n*-butylamine, soluble in methanol, sparingly soluble in ethanol (95), slightly soluble in acetic acid (100), and very slightly soluble in water and in diethyl ether.

It dissolves in dilute hydrochloric acid, in sodium hydroxide TS and in ammonia TS.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Sulfisoxazole in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(2) Dissolve 0.02 g of Sulfisoxazole in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Add 5 mL of chloroform, shake, and allow to stand: a blue-green color develops in the chloroform layer.

(3) Dissolve 0.01 g of Sulfisoxazole in 1 mL of pyridine, add 2 drops of copper (II) sulfate TS, and shake. Add 3 mL of water and 5 mL of chloroform, shake, and allow to stand: a light yellow-brown color develops in the chloroform layer.

(4) To 0.5 g of Sulfisoxazole add 2 mL of acetic acid (100), dissolve by heating under a reflux condenser, add 1 mL of acetic anhydride, and boil for 10 minutes. Add 10 mL of water, cool, and alkalize with about 7 mL of a solution of sodium hydroxide (3 in 10). Filter, if necessary, immediately acidify by adding acetic acid (100) dropwise, collect the produced precipitate, recrystallize from methanol, and dry at 105°C for 1 hour: the crystals melt <2.60> between 208°C and 210°C.

Melting point <2.60> 192 – 196°C (with decomposition).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Sulfisoxazole in 5 mL of sodium hydroxide TS and 20 mL of water: the solution is clear and colorless to pale yellow.

(2) Acidity—To 1.0 g of Sulfisoxazole add 50 mL of water, warm at 70°C for 5 minutes, allow to stand in an ice bath for 1 hour, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulfisoxazole according to Method 2, and perform the test. Prepare

the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 0.5% (2 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Sulfisoxazole, previously dried, dissolve in 50 mL of methanol by warming, cool and titrate <2.50> with 0.2 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS). Perform a blank determination using a mixture of 50 mL of methanol and 18 mL of water, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.2 mol/L sodium hydroxide VS} \\ = 53.46 \text{ mg of } C_{11}H_{13}N_3O_3S \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Sulfobromophthalein Sodium

スルホブロモフタレインナトリウム



$C_{20}H_8Br_4Na_2O_{10}S_2$: 838.00

Disodium 5,5'-(4,5,6,7-tetrabromo-3-oxo-1,3-dihydroisobenzofuran-1,1-diyl)bis(2-hydroxybenzenesulfonate) [71-67-0]

Sulfobromophthalein Sodium, when dried, contains not less than 96.0% and not more than 104.0% of $C_{20}H_8Br_4Na_2O_{10}S_2$.

Description Sulfobromophthalein Sodium occurs as a white, crystalline powder. It is odorless.

It is soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 0.02 g of Sulfobromophthalein Sodium in 10 mL of water, and add 1 mL of sodium carbonate TS: a blue-purple color is produced. Add 1 mL of dilute hydrochloric acid to the solution: the color of the solution disappears.

(2) Transfer 0.2 g of Sulfobromophthalein Sodium to a porcelain crucible, mix well with 0.5 g of anhydrous sodium carbonate, and ignite until the mixture is charred. After cooling, add 15 mL of hot water to the residue, heat for 5 minutes on a water bath, filter, and render the filtrate slightly acid with hydrochloric acid: the solution responds to the Qualitative Tests <1.09> for bromide, and the Qualitative Tests <1.09> (1) and (2) for sulfate.

(3) Sulfobromophthalein Sodium responds to the Qualitative Tests <1.09> (1) for sodium salt.

pH <2.54> The pH of a solution of 1.0 g of Sul-

fobromophthalein Sodium in 20 mL of water is between 4.0 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Sulfobromophthalein Sodium in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—Perform the test with 2.0 g of Sulfobromophthalein Sodium. Prepare the control solution with 0.10 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.002%).

(3) Sulfate—To 10 mL of a solution of Sulfobromophthalein Sodium (1 in 500) add 5 drops of dilute hydrochloric acid, heat to boil, and add 1 mL of hot barium chloride TS: the solution is clear when observed 1 minute after the addition of the barium chloride TS.

(4) Calcium—Weigh accurately about 5 g of Sulfobromophthalein Sodium, transfer to a porcelain dish, heat gently to char, and heat strongly between 700°C and 750°C until the residue is incinerated. After cooling, add 10 mL of dilute hydrochloric acid, and heat for 5 minutes on a water bath. Transfer the contents to a flask with 50 mL of water, and add 5 mL of 8 mol/L potassium hydroxide TS and 0.1 g of NN indicator. Titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS until the red-purple color of the solution changes to blue.

$$\begin{aligned} \text{Each mL of 0.01 mol/L disodium dihydrogen} \\ \text{ethylenediamine tetraacetate VS} \\ = 0.4008 \text{ mg of Ca} \end{aligned}$$

The content of calcium (Ca: 40.08) is not more than 0.05%.

(5) Heavy metals <1.07>—Proceed with 1.0 g of Sulfobromophthalein Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Transfer 0.65 g of Sulfobromophthalein Sodium to a crucible, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 50), fire to burn, then heat gently until the residue is incinerated. If any carbon remains, moisten the residue with a small amount of nitric acid, and incinerate again by ignition. After cooling, add 10 mL of dilute sulfuric acid, and heat until white fumes are evolved. After cooling, add 5 mL of water to the residue, and perform the test with this solution as the test solution (not more than 3.1 ppm).

Loss on drying <2.41> Not more than 5.0% (0.5 g, 105°C, 3 hours).

Residue on ignition <2.44> 14 – 19% (after drying, 0.5 g, 700 – 750°C).

Assay Dissolve about 0.1 g of Sulfobromophthalein Sodium, previously dried and accurately weighed, in water to make exactly 500 mL. Pipet 5 mL of this solution, and add a solution of anhydrous sodium carbonate (1 in 100) to make exactly 200 mL. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. Determine the absorbance A of this solution at the wavelength of maximum absorption at about 580 nm, using water as the blank.

$$\begin{aligned} \text{Amount (mg) of } C_{20}H_8Br_4Na_2O_{10}S_2 \\ = (A/881) \times 200,000 \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Sulfobromophthalein Sodium Injection

スルホブロモフタレインナトリウム注射液

Sulfobromophthalein Sodium Injection is an aqueous solution for Injection. It contains not less than 94% and not more than 106% of the labeled amount of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$; 838.00).

Method of preparation Prepare as directed under Injections, with Sulfobromophthalein Sodium.

Description Sulfobromophthalein Sodium Injection is a clear and colorless or pale yellow liquid.

pH: 5.0 – 6.0

Identification (1) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.02 g of Sulfobromophthalein Sodium according to the labeled amount, and proceed as directed in the Identification (1) under Sulfobromophthalein Sodium.

(2) Measure a volume of Sulfobromophthalein Sodium Injection, equivalent to 0.1 g of Sulfobromophthalein Sodium according to the labeled amount, add 0.5 g of anhydrous sodium carbonate, and evaporate on a water bath to dryness. Ignite the residue until it is charred. Proceed as directed in the Identification (2) under Sulfobromophthalein Sodium.

Extractable volume <6.05> It meets the requirement.

Pyrogen <4.04> Add isotonic sodium chloride solution to Sulfobromophthalein Sodium Injection to make a 0.5 w/v% solution of Sulfobromophthalein Sodium according to the labeled amount. Inject into each of the rabbits 5 mL of this solution per kg of body mass: it meets the requirements.

Assay Measure exactly a volume of Sulfobromophthalein Sodium Injection, equivalent to about 0.1 g of sulfobromophthalein sodium ($C_{20}H_8Br_4Na_2O_{10}S_2$), add water to make exactly 500 mL, and proceed as directed in the Assay under Sulfobromophthalein Sodium.

$$\begin{aligned} &\text{Amount (mg) of sulfobromophthalein sodium} \\ & (C_{20}H_8Br_4Na_2O_{10}S_2) \\ & = (A/881) \times 200,000 \end{aligned}$$

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant.

Sulfur

イオウ

S: 32.07

Sulfur, when dried, contains not less than 99.5% of S.

Description Sulfur occurs as a light yellow to yellow pow-

der. It is odorless and tasteless.

It is freely soluble in carbon disulfide, and practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Ignite Sulfur: it burns with a blue flame and gives a pungent odor of sulfur dioxide.

(2) Dissolve 5 mg of Sulfur in 5 mL of sodium hydroxide TS by heating in a water bath, cool, and add 1 drop of sodium pentacyanonitrosylferrate (III) TS: a blue-purple color develops.

(3) Boil 1 mg of sulfur with 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS: a blue color develops.

Purity (1) Clarity of solution—Dissolve 1.0 g of Sulfur in a mixture of 20 mL of a solution of sodium hydroxide (1 in 6) and 2 mL of ethanol (95) by boiling: the solution is clear. Dissolve 2.0 g of Sulfur in 10 mL of carbon disulfide: the solution is almost clear or slightly opalescent.

(2) Acidity or alkalinity—Shake 2.0 g of Sulfur with 50 mL of freshly boiled and cooled water, and add 2 drops of phenolphthalein TS: no red color develops. Further add 1.0 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(3) Arsenic <1.11>—Prepare the test solution with 0.20 g of Sulfur according to Method 3, and perform the test (not more than 10 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, not more than 0.67 kPa, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.4 g of Sulfur, previously dried, dissolve in 20 mL of potassium hydroxide-ethanol TS and 10 mL of water by boiling, cool, and add water to make exactly 100 mL. Transfer exactly 25 mL of the solution to a 400-mL beaker, add 50-mL of hydrogen peroxide TS, and heat on a water bath for 1 hour. Acidify the solution with dilute hydrochloric acid, add 200 mL of water, heat to boil, add hot barium chloride TS dropwise until no more precipitate is formed, and heat on a water bath for 1 hour. Collect the precipitate, and wash with water until the last washing shows no opalescence with silver nitrate TS. Dry the precipitate, heat strongly to constant mass, and weigh as barium sulfate ($BaSO_4$; 233.39). Perform a blank determination, and make any necessary correction.

Amount (mg) of S

$$= \text{amount (mg) of barium sulfate (BaSO}_4) \times 0.13739$$

Containers and storage Containers—Well-closed containers.

Sulfur and Camphor Lotion

イオウ・カンフルローション

Method of preparation

Sulfur	60 g
<i>d</i> -Camphor or <i>dl</i> -Camphor	5 g
Hydroxypropylcellulose	4 g
Calcium Hydroxide	1 g
Ethanol	4 mL
Water or Purified Water	a sufficient quantity

To make 1000 mL

Dissolve Hydroxypropylcellulose in 200 mL of Water or Purified Water. Add this solution in small portions to the triturate of Sulfur with the Ethanol solution of *d*-Camphor or *dl*-Camphor, and triturate again the mixture. Separately, dissolve Calcium Hydroxide in 500 mL of Water or Purified Water, stopper tightly, shake, and allow to stand. Add 300 mL of this supernatant liquid to the above mixture, then add Water or Purified Water to make 1000 mL, and shake thoroughly.

Description Sulfur and Camphor Lotion is a light yellow suspension.

A part of the components separates out on standing.

Identification (1) To 5 mL of well shaken Sulfur and Camphor Lotion add 25 mL of water, and centrifuge [use this supernatant liquid for test (3)]. To 0.02 g of the precipitate add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a blue color develops (sulfur).

(2) To 10 mL of well shaken Sulfur and Camphor Lotion add 5 mL of diethyl ether, and mix. Separate the diethyl ether layer, and filter through a pledget of cotton. Wash the cotton with a small portion of diethyl ether, combine the washings with the filtrate, and distil cautiously on a water bath to remove the diethyl ether. Dissolve the residue in 1 mL of methanol, add 1 mL of 2,4-dinitrophenylhydrazine TS, and heat for about 2 minutes on a water bath. Cool, dilute with water to make about 5 mL, and allow to stand. Filter the produced precipitate through a glass filter (G4), and wash the residue on the filter with water until the last washing is colorless. Dissolve the residue in 10 mL of ethanol (95), add 5 mL of sodium hydroxide TS, and allow to stand for 2 minutes: a red color develops (*d*-camphor or *dl*-camphor).

(3) The supernatant liquid obtained in (1) responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt.

Containers and storage Containers—Tight containers.

Sulfur, Salicylic Acid and Thianthol Ointment

イオウ・サリチル酸・チアントール軟膏

Method of preparation

Sulfur	100 g
Salicylic Acid, finely powdered	30 g
Thianthol	100 mL
Zinc Oxide, very finely powdered	100 g
Simple Ointment or a suitable ointment base	a sufficient quantity
To make 1000 g	

Prepare as directed under Ointments, with above ingredients.

Description Sulfur, Salicylic Acid and Thianthol Ointment is light yellow in color.

Identification (1) Stir well 0.5 g of Sulfur, Salicylic Acid and Thianthol Ointment with 10 mL of water while heating, cool, and filter. To 1 mL of the filtrate add 5 mL of iron (III)

nitrate TS: a purple color is produced (salicylic acid).

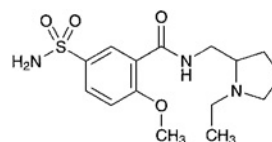
(2) Shake 1 g of Sulfur, Salicylic Acid and Thianthol Ointment with 20 mL of diethyl ether, remove the supernatant liquid and floating materials. Wash the residue with 10 mL of diethyl ether, and remove the diethyl ether by suction. To the residue add 2 mL of pyridine and 0.2 mL of sodium hydrogen carbonate TS, and boil: a light blue to blue color is produced (sulfur).

(3) To 1 g of Sulfur, Salicylic Acid and Thianthol Ointment add 15 mL of ethanol (95), stir well while warming on a water bath, cool, and filter. Use the filtrate as the sample solution. Dissolve 0.01 g each of salicylic acid and thianthol in 5 mL of ethanol (95), and use these solutions as the standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots of each component obtained from the sample solution and standard solutions (1) and (2) show the same *R_f* value. Spray iron (III) chloride TS upon the plate evenly: the spot from the standard solution (1) and that from the corresponding sample solution reveal a purple color.

Containers and storage Containers—Tight containers.

Sulpiride

スルピリド



$C_{15}H_{23}N_3O_4S$: 341.43

N-(1-Ethylpyrrolidin-2-ylmethyl)-2-methoxy-5-sulfamoylbenzamide [15676-16-1]

Sulpiride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{15}H_{23}N_3O_4S$.

Description Sulpiride is a white, crystalline powder.

It is freely soluble in acetic acid (100) and in dilute acetic acid, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and practically insoluble in water.

It is soluble in 0.05 mol/L sulfuric acid TS.

A solution of Sulpiride in methanol (1 in 100) shows no optical rotation.

Melting point: about 178°C (with decomposition).

Identification (1) Dissolve 0.1 g of Sulpiride in 0.05 mol/L sulfuric acid TS to make 100 mL. Dilute 5 mL of the solution with water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Sulpi-

ride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity of solution—Dissolve 2.0 g of Sulpiride in 7 mL of dilute acetic acid, and add water to make 20 mL: the solution is clear. Perform the test with the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: the absorbance at a wavelength of 450 nm does not exceed 0.020.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Sulpiride as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 50 mg of Sulpiride in 10 mL of methanol, and use this solution as the sample solution. Dilute 1 mL of the sample solution, accurately measured, with methanol to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more color than the spot from the standard solution. When the plate is exposed to iodine vapor for 30 minutes, the number of the spots other than the principal spot from the sample solution is not more than 2, and they have no more color than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Dissolve about 0.4 g of Sulpiride, previously dried and accurately weighed, in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS) until the color of the solution changes from violet through blue to bluish green. Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 34.14 \text{ mg of } C_{15}H_{23}N_3O_4S \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Sulpiride Capsules

スルピリドカプセル

Sulpiride Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Capsules, with Sulpiride.

Identification Determine the absorption spectrum of the

sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 capsule of Sulpiride Capsules add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride ($C_{15}H_{23}N_3O_4S$), and filter the solution. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} \text{Amount (mg) of sulpiride } (C_{15}H_{23}N_3O_4S) \\ = W_S \times (A_T/A_S) \times (V/50) \end{aligned}$$

W_S : Amount (mg) of sulpiride for assay

Dissolution Being specified separately.

Assay Cut the capsule of not less than 20 Sulpiride Capsules, weigh accurately the mass of the contents, and powder. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride ($C_{15}H_{23}N_3O_4S$), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mol/L sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

$$\text{Amount (mg) of sulpiride } (C_{15}H_{23}N_3O_4S) = W_S \times (A_T/A_S) \times 2$$

W_S : Amount (mg) of sulpiride for assay

Containers and storage Containers—Tight containers.

Sulpiride Tablets

スルピリド錠

Sulpiride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$: 341.43).

Method of preparation Prepare as directed under Tablets, with Sulpiride.

Identification Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank: it exhibits a maximum between 289 nm and 293 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the

Content uniformity test.

To 1 tablet of Sulpiride Tablets add 30 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, add 0.05 mol/L sulfuric acid TS to make exactly V mL so that each mL of the solution contains about 1 mg of sulpiride ($C_{15}H_{23}N_3O_4S$), and filter the solution. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} \text{Amount (mg) of sulpiride (C}_{15}\text{H}_{23}\text{N}_3\text{O}_4\text{S)} \\ = W_S \times (A_T/A_S) \times (V/50) \end{aligned}$$

W_S : Amount (mg) of sulpiride for assay

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Sulpiride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 30 minutes after starting the test for a 50-mg tablet and 45 minutes after for a 100-mg or a 200-mg tablet, and filter through a membrane filter with pore size of not more than 0.5 μm . Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add 2nd fluid for dissolution test to make exactly V' mL so that each mL contains about 56 μg of sulpiride ($C_{15}H_{23}N_3O_4S$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 5 mL of this solution, add 2nd fluid for dissolution test to make exactly 25 mL, and use this solution as the standard solution. Perform the test with the sample solution and the standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S , at 291 nm. The dissolution rate of a 50-mg tablet in 30 minutes is not less than 80%, that of a 100-mg tablet in 45 minutes is not less than 75%, and that of a 200-mg tablet in 45 minutes is not less than 70%.

Dissolution rate (%) with respect to the labeled amount of sulpiride ($C_{15}H_{23}N_3O_4S$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$$

W_S : Amount (mg) of sulpiride for assay

C: Labeled amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$) in 1 tablet

Assay Weigh accurately, and powder not less than 20 Sulpiride Tablets. Weigh accurately a portion of the powder, equivalent to about 0.1 g of sulpiride ($C_{15}H_{23}N_3O_4S$), add 70 mL of 0.05 mol/L sulfuric acid TS, shake for 30 minutes, and add 0.05 mol/L sulfuric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 5 mL of the filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of sulpiride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.05 mL sulfuric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 291 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank.

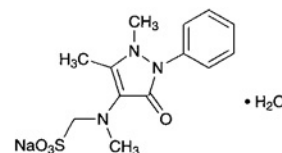
Amount (mg) of sulpiride ($C_{15}H_{23}N_3O_4S$) = $W_S \times (A_T/A_S) \times 2$

W_S : Amount (mg) of sulpiride for assay

Containers and storage Containers—Tight containers.

Sulpirine Hydrate

スルピリン水和物



$C_{13}H_{16}N_3NaO_4S \cdot H_2O$: 351.35

Monosodium [(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)(methyl)amino]methanesulfonate monohydrate [5907-38-0]

Sulpirine Hydrate contains not less than 98.5% of sulpirine ($C_{13}H_{16}N_3NaO_4S$: 333.34), calculated on the dried basis.

Description Sulpirine Hydrate occurs as white to light yellow crystals or crystalline powder. It is odorless, and has a bitter taste.

It is very soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is colored by light.

Identification (1) Add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS to 3 mL of a solution of Sulpirine Hydrate (1 in 15): a deep blue color develops at first, but the color immediately turns red, then gradually changes to yellow.

(2) Boil 5 mL of a solution of Sulpirine Hydrate (1 in 25) with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling, the odor of formaldehyde is perceptible.

(3) A solution of Sulpirine Hydrate (1 in 10) responds to the Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity of solution, and acidity or alkalinity—Dissolve 1.0 g of Sulpirine Hydrate in 10 mL of water: the solution is clear and neutral.

(2) Sulfate <1.14>—Dissolve 0.20 g of Sulpirine Hydrate in 0.05 mol/L hydrochloric acid VS to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS and 0.05 mol/L hydrochloric acid VS to make 50 mL (not more than 0.120%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Sulpirine Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Merbuline—Transfer 0.10 g of Sulpirine Hydrate with 2 mL of water and 1 mL of dilute sulfuric acid into a flask, cover with a funnel, and boil gently for 15 minutes. Cool, add 2 mL of a solution of sodium acetate trihydrate (1 in 2) and water to make 5 mL, shake this solution with 5 mL of benzaldehyde-saturated solution, and allow to stand for 5 minutes: the solution is clear.

(5) Chloroform-soluble substances—Mix, by frequent shaking, 1.0 g of Sulpyrine Hydrate and 10 mL of chloroform for 30 minutes. Collect the precipitate, wash with two 5-mL portions of chloroform, combine the washings with the filtrate, and evaporate on a water bath to dryness. Dry the residue at 105°C for 4 hours: the mass of the residue is not more than 5.0 mg.

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, 4 hours).

Assay Weigh accurately about 0.25 g of Sulpyrine Hydrate, dissolve in 100 mL of diluted hydrochloric acid (1 in 20), previously cooled below 10°C. Titrate <2.50> immediately with 0.05 mol/L iodine VS while keeping the temperature between 5°C and 10°C, until the color of the solution remains blue upon shaking vigorously for 1 minute after the addition of 0.05 mol/L iodine VS (indicator: 1 mL of starch TS).

$$\begin{aligned} \text{Each mL of 0.05 mol/L iodine VS} \\ = 16.67 \text{ mg of } C_{13}H_{16}N_3NaO_4S \end{aligned}$$

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Sulpyrine Injection

スルピリン注射液

Sulpyrine Injection is an aqueous solution for injection. It contains not less than 95% and not more than 105% of the labeled amount of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S \cdot H_2O$: 351.35).

Method of preparation Prepare as directed under Injections, with Sulpyrine Hydrate.

Description Sulpyrine Injection is a clear, colorless or pale yellow liquid.

pH: 5.0 – 8.5

Identification (1) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine Hydrate according to the labeled amount, add water to make 3 mL, then add 2 drops of dilute sulfuric acid and 1 mL of chlorinated lime TS: a deep blue color develops at first, and the color immediately turns red and gradually changes to yellow.

(2) To a volume of Sulpyrine Injection, equivalent to 0.2 g of Sulpyrine according to the labeled amount, add water to make 5 mL, and boil this solution with 3 mL of dilute hydrochloric acid: the odor of sulfur dioxide is perceptible at first, and on further boiling the odor of formaldehyde is perceptible.

Assay Pipet 2 mL of Sulpyrine Injection, dilute with water to exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 0.05 g of sulpyrine hydrate ($C_{13}H_{16}N_3NaO_4S \cdot H_2O$), and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the sample solution. Weigh accurately about 0.05 g of sulpyrine for assay (previously determine the loss on drying <2.41> in the same manner as Sulpyrine Hydrate), and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to exactly 100 mL, and use this solution as the standard solution. Pipet 2 mL each of

the sample solution and standard solution into separate 25-mL volumetric flasks, add 5 mL of ethanol (95), 2 mL of a solution of 4-dimethylaminocinnamaldehyde in ethanol (95) (1 in 250) and 2 mL of acetic acid (100) to each of these solutions, shake well, allow to stand for 15 minutes, and add water to exactly 25 mL. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 2 mL of water in the same manner as the blank. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and the standard solution at 510 nm.

$$\begin{aligned} \text{Amount (mg) of sulpyrine hydrate } (C_{13}H_{16}N_3NaO_4S \cdot H_2O) \\ \text{in 1 mL of Sulpyrine Injection} \\ = W_S \times (A_T/A_S) \times (50/V) \times 1.0540 \end{aligned}$$

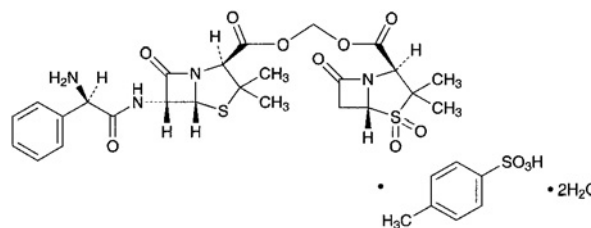
W_S : Amount (mg) of sulpyrine for assay, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, and under nitrogen atmosphere.

Sultamicillin Tosilate Hydrate

スルタミシリントシル酸塩水和物



$C_{25}H_{30}N_4O_9S_2 \cdot C_7H_8O_3S \cdot 2H_2O$: 802.89
(2*S*,5*R*)-(3,3-Dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept-2-ylcarbonyloxy)methyl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monotosylate dihydrate [83105-70-8, anhydride]

Sultamicillin Tosilate Hydrate contains not less than 698 μ g (potency) per mg, calculated on the anhydrous basis and corrected by the amount of residual solvent. The potency of Sultamicillin Tosilate is expressed as mass (potency) of sultamicillin ($C_{25}H_{30}N_4O_9S_2$: 594.66).

Description Sultamicillin Tosilate Hydrate occurs as a white to yellowish white crystalline powder.

It is freely soluble in acetonitrile, in methanol and in ethanol (99.5), and very slightly soluble in water.

Identification Determine the infrared absorption spectrum of Sultamicillin Tosilate Hydrate as directed in the paste method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Sultamicillin Tosilate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +173 – +187° (0.5 g calculated on the anhydrous bases, a mixture of water and acetonitrile)

trile (3:2), 25 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Sultamicillin Tosilate Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sultamicillin Tosilate Hydrate, according to Method 3, and perform the test (not more than 2 ppm).

(3) Ampicillin—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg (potency) of Ampicillin Reference Standard, dissolve in the mobile phase to make exactly 100 mL. Pipet 6 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the area of the peak of ampicillin by the automatic integration method: the peak area from the sample solution is not more than that from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: Dissolve 3.12 g of sodium dihydrogen phosphate dihydrate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make

1000 mL. To 80 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of ampicillin is about 14 minutes.

System suitability—

System performance: Dissolve 12 mg of Ampicillin Reference Standard, 4 mg of Sulbactam Reference Standard and 4 mg of *p*-toluenesulfonic acid monohydrate in 1000 mL of the mobile phase. When the procedure is run with 25 μ L of this solution under the above operating conditions, sulbactam, *p*-toluenesulfonic acid and ampicillin are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ampicillin is not more than 2.0%.

(4) Sulbactam—Perform the procedure rapidly. Weigh accurately about 20 mg of Sultamicillin Tosilate Hydrate, dissolve in the mobile phase to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately an amount of Sulbactam Reference Standard, equivalent to about 20 mg (potency), dissolve in the mobile phase to make exactly 100 mL. Pipet 2 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the area of the peak of sulbactam by the automatic integration method: the peak area from the sample solution is not more than that from the standard solution.

Operating conditions—

Proceed as directed in the operating conditions in the Purity (3).

System suitability—

Proceed as directed in the system suitability in the Purity (3).

(5) Penicilloic acids—Weigh accurately about 25 mg of Sultamicillin Tosilate Hydrate, dissolve in 1 mL of acetonitrile, and add 25 mL of 0.02 mol/L phosphate buffer solution, pH 3.0, in a 100-mL flask with stopper. Add exactly 5 mL of 0.005 mol/L iodine VS, and allow to stand the stoppered flask for 5 minutes. Titrate <2.50> with 0.005 mol/L sodium thiosulfate VS (indicator: 1.0 mL of starch TS). Perform a blank determination in the same manner, and make any necessary correction. Calculate the amount of penicilloic acid (C₂₅H₃₄N₄O₁₁S₂: 630.70) by using the following equation: it is not more than 3.0%.

$$\begin{aligned} \text{Each mL of 0.005 mol/L sodium thiosulfate VS} \\ = 0.2585 \text{ mg of C}_{25}\text{H}_{34}\text{N}_4\text{O}_{11}\text{S}_2 \end{aligned}$$

(6) Residual solvent <2.46>—Weigh accurately about 0.1 g of Sultamicillin Tosilate Hydrate, dissolve in 2 mL of methanol, add water to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g of ethyl acetate, and mix with water to make exactly 200 mL. Pipet 2 mL of this solution, add 10 mL of methanol and water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the peak areas, A_T and A_S , of ethyl acetate of these solutions. Calculate the amount of ethyl acetate by the following equation: not more than 2.0%.

$$\begin{aligned} \text{Amount (\%)} \text{ of ethyl acetate} \\ = (W_S/W_T) \times (A_T/A_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of ethyl acetate

W_T : Amount (mg) of the sample

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A column 3 mm in inside diameter and 1 m in length, packed with porous styrene-divinylbenzene copolymer for gas chromatography (0.0085 μ m in average pore size and 300–400 m²/g in specific surface area) (150 to 180 μ m in particle diameter).

Column temperature: A constant temperature of about 155°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethyl acetate is about 6 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of ethyl acetate are not less than 500 steps and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of ethyl acetate is not more than 5%.

Water <2.48> 4.0–6.0% (0.5 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Perform the procedure rapidly. Weigh accurately an amount of Sultamicillin Tosilate Hydrate and Sultamicillin Tosilate Reference Standard, equivalent to about 50 mg (potency), dissolve each in the mobile phase to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL of the internal standard solution, add the mobile phase to make 25 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of sultamicillin to that of the internal standard of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of sultamicillin (C}_{25}\text{H}_{30}\text{N}_4\text{O}_9\text{S}_2) \\ = W_S \times (Q_T/Q_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Sultamicillin Tosilate Reference Standard

Internal standard solution—A solution of isopropyl-4-aminobenzoate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 215 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Dissolve 3.12 g of sodium dihydrogenphosphate in about 750 mL of water, adjust to pH 3.0 with diluted phosphoric acid (1 in 10), and add water to make 1000 mL. To 400 mL of acetonitrile for liquid chromatography add this solution to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of sultamicillin is about 4 minutes.

System suitability—

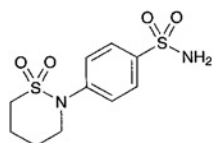
System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, *p*-toluenesulfonic acid, sultamicillin and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sultamicillin is not more than 2.0%.

Containers and storage Containers—Tight containers.

Sultiame

スルチアム



C₁₀H₁₄N₂O₄S₂: 290.36

4-(3,4,5,6-Tetrahydro-2H-1,2-thiazin-

2-yl)benzenesulfonamide S,S-dioxide [61-56-3]

Sultiame, when dried, contains not less than 98.5% of C₁₀H₁₄N₂O₄S₂.

Description Sultiame occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is very soluble in *N,N*-dimethylformamide, freely soluble in *n*-butylamine, slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

It dissolves in sodium hydroxide TS.

Identification (1) Dissolve 0.02 g of Sultiame in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. To this solution add 5 mL of chloroform, shake, and allow to stand: a green color develops in the chloroform layer.

(2) Mix 0.1 g of Sultiame with 0.5 g of sodium carbonate decahydrate, and melt carefully: the gas evolved changes moistened red litmus paper to blue. After cooling, crush the fused substance with a glass rod, stir with 10 mL of water, and filter. To 4 mL of the filtrate add 2 drops of hydrogen peroxide (30), 5 mL of diluted hydrochloric acid (1 in 5) and 2 to 3 drops of barium chloride TS: a white precipitate is formed.

(3) Determine the absorption spectrum of a solution of Sultiame in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Melting point <2.60> 185 – 188°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 2 mL of acetic acid (100) and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To the subsequent 40 mL add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 8 mL of sodium hydroxide TS, 0.8 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Sultiame in 20 mL of sodium hydroxide TS by warming, cool, and add 8 mL of dilute hydrochloric acid and water to make 100 mL. After shaking, filter, and discard the first 10 mL of the filtrate. To the subsequent 40 mL add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 8 mL of sodium hydroxide TS, 4.2 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 2.0 g of Sultiame according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Sultiame according to Method 3, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Sultiame in methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of sulfanilamide

in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and ammonia solution (28) (30:8:1) to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

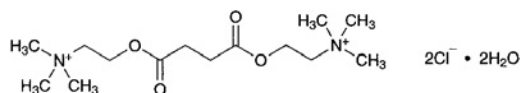
Assay Weigh accurately about 0.8 g of Sultiamine, previously dried, dissolve in 70 mL of *N,N*-dimethylformamide, and titrate <2.50> with 0.2 mol/L tetramethylammonium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.2 mol/L tetramethylammonium hydroxide VS
= 58.07 mg of $C_{10}H_{14}N_2O_4S_2$

Containers and storage Containers—Well-closed containers.

Suxamethonium Chloride Hydrate

スキサメトニウム塩化物水和物



$C_{14}H_{30}Cl_2N_2O_4 \cdot 2H_2O$: 397.34
2,2'-Succinyldioxybis(*N,N,N*-trimethylethylammonium) dichloride dihydrate [6101-15-1]

Suxamethonium Chloride Hydrate contains not less than 98.0% of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31), calculated on the anhydrous basis.

Description Suxamethonium Chloride Hydrate occurs as a white, crystalline powder.

It is freely soluble in water, in methanol and in acetic acid (100), slightly soluble in ethanol (95), very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Suxamethonium Chloride Hydrate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Suxamethonium Chloride Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> The pH of a solution of Suxamethonium Chloride Hydrate (1 in 100) is between 4.0 and 5.0.

Melting point <2.60> 159 – 164°C (hydrate form).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Suxamethonium Chloride Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 0.25 g of Suxamethonium Chloride Hydrate in 5 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, *n*-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly platinum chloride-potassium iodide TS on the plate, and allow to stand for 15 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 8.0 – 10.0% (0.4 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Suxamethonium Chloride Hydrate, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration).

Each mL of 0.1 mol/L perchloric acid VS
= 18.07 mg of $C_{14}H_{30}Cl_2N_2O_4$

Containers and storage Containers—Tight containers.

Suxamethonium Chloride for Injection

注射用スキサメトニウム塩化物

Suxamethonium Chloride for Injection is a preparation for injection which is dissolved before use. It contains not less than 93% and not more than 107% of the labeled amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$: 361.31).

The concentration of Suxamethonium Chloride for Injection should be stated as the amount of suxamethonium chloride ($C_{14}H_{30}Cl_2N_2O_4$).

Method of preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride for Injection occurs as a white, crystalline powder or mass.

Identification Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, dissolve in water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μ L each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solu-

tion of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly platinum chloride-potassium iodide TS on the plate: the spots obtained from the sample solution and standard solution are blue-purple in color and have similar *R_f*.

pH <2.54> The pH of a solution of Suxamethonium Chloride for Injection (1 in 100) is between 4.0 and 5.0.

Purity Related substances—Take an amount of Suxamethonium Chloride for Injection, equivalent to 0.25 g of Suxamethonium Chloride Hydrate according to the labeled amount, and proceed as directed in the Purity (2) under Suxamethonium Chloride Hydrate.

Assay Weigh accurately the contents of not less than 10 preparations of Suxamethonium Chloride for Injection. Weigh accurately about 0.5 g of the contents, and proceed as directed in the Assay under Suxamethonium Chloride Hydrate.

Each mL of 0.1 mol/L perchloric acid VS
= 18.07 mg of C₁₄H₃₀Cl₂N₂O₄

Containers and storage Containers—Hermetic containers.

Suxamethonium Chloride Injection

スキサメトニウム塩化物注射液

Suxamethonium Chloride Injection is an aqueous solution for injection. It contains not less than 93% and not more than 107% of the labeled amount of suxamethonium chloride (C₁₄H₃₀Cl₂N₂O₄: 361.31).

The concentration of Suxamethonium Chloride Injection should be stated as the amount of suxamethonium chloride (C₁₄H₃₀Cl₂N₂O₄).

Method of preparation Prepare as directed under Injections, with Suxamethonium Chloride Hydrate.

Description Suxamethonium Chloride Injection is a clear, colorless liquid.

Identification Take a volume of Suxamethonium Chloride Injection, equivalent to 0.05 g of Suxamethonium Chloride Hydrate according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of suxamethonium chloride for thin-layer chromatography in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 1 μL each of the sample solution and standard solution on a plate of cellulose for thin-layer chromatography. Develop the plate with a mixture of a solution of ammonium acetate (1 in 100), acetone, 1-butanol and formic acid (20:20:20:1) to a distance of about 10 cm, and dry the plate at 105°C for 15 minutes. Spray evenly platinum chloride-potassium iodide TS on the plate: the spots obtained from the sample solution and the standard solution are blue-purple in color and have similar *R_f*.

pH <2.54> 3.0 – 5.0

Purity Hydrolysis products—Perform the preliminary neu-

tralization with 0.1 mol/L sodium hydroxide VS in the Assay: not more than 0.7 mL of 0.1 mol/L sodium hydroxide VS is required for each 200 mg of Suxamethonium Chloride (C₁₄H₃₀Cl₂N₂O₄) taken.

Extractable volume <6.05> It meets the requirement.

Assay Transfer to a separator an accurately measured volume of Suxamethonium Chloride Injection, equivalent to about 0.2 g of suxamethonium chloride (C₁₄H₃₀Cl₂N₂O₄), add 30 mL of freshly boiled and cooled water, and wash the solution with five 20-mL portions of diethyl ether. Combine the diethyl ether washings, and extract the combined diethyl ether layer with two 10-mL portions of freshly boiled and cooled water. Wash the combined water extracts with two 10-mL portions of diethyl ether. Combine the solution and the water extracts, add 2 drops of bromothymol blue TS, and neutralize with 0.1 mol/L sodium hydroxide VS. Add accurately measured 25 mL of 0.1 mol/L sodium hydroxide VS, and boil for 40 minutes under a reflux condenser, and cool. Titrate <2.50> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS. Transfer 50 mL of the freshly boiled and cooled water to a flask, add 2 drops of bromothymol blue TS, neutralize the solution with 0.1 mol/L sodium hydroxide VS, and perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.07 mg of C₁₄H₃₀Cl₂N₂O₄

Containers and storage Containers—Hermetic containers.

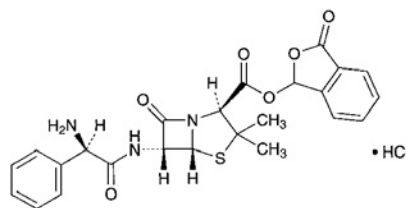
Storage—Not exceeding 5°C, and avoid freezing.

Expiration date 12 months after preparation.

Talampicillin Hydrochloride

Ampicillinphthalidyl Hydrochloride

タランピシリン塩酸塩



C₂₄H₂₃N₃O₆S.HCl: 517.98

3-Oxo-1,3-dihydroisobenzofuran-1-yl (2*S*,5*R*,6*R*)-6-[(2*R*)-2-amino-2-phenylacetyl-amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate monohydrochloride [47747-56-8]

Talampicillin Hydrochloride is the hydrochloride of ampicillin phthalidyl ester.

It contains not less than 600 μg (potency) and not more than 700 μg (potency) per mg, calculated on the anhydrous basis. The potency of Talampicillin Hydrochloride is expressed as mass (potency) of ampicillin (C₁₆H₁₉N₃O₄S: 349.40).

Description Talampicillin Hydrochloride occurs as a white to light yellowish white powder.

It is very soluble in methanol, and freely soluble in water

and in ethanol (99.5).

Identification (1) To 1 mL of a solution of Talampicillin Hydrochloride (1 in 30) add 1 mL of sodium hydroxide TS, mix, allow to stand for 5 minutes, and add 2 mL of dilute sulfuric acid and 2 to 3 drops of 2,4-dinitrophenylhydrazine TS: an orange-yellow precipitate is formed.

(2) Determine the infrared absorption spectrum of Talampicillin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Talampicillin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 10 mL of a solution of Talampicillin Hydrochloride (1 in 300) add 1 mL of dilute nitric acid, and add silver nitrate TS: a white precipitate is formed.

Optical rotation <2.49> $[\alpha]_D^{20}$: +151 – +171° (0.2 g calculated on the anhydrous basis, ethanol (99.5), 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Talampicillin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Talampicillin Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL, 2 mL and 3 mL of the sample solution, add ethanol (99.5) to each to make exactly 100 mL, and use these solutions as the standard solution (1), the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of tetrahydrofuran, ethyl acetate, water and ethanol (95) (4:4:2:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (99.5) (1 in 500) on the plate, and heat at 110°C for 5 minutes: the spots other than the principal spot from the sample solution is not more intense than the spot from the standard solution (3), and the total of the amount of each spot other than the principal spot from the sample solution, which is calculated by the comparison with the spots obtained from the standard solutions (1), (2) and (3), is not more than 5%.

(4) 2-Formylbenzoic acid—Dissolve 50 mg of Talampicillin Hydrochloride in ethanol (99.5) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 10 mg of 2-formylbenzoic acid in ethanol (99.5) to make exactly 100 mL. Pipet 5 mL of this solution, add ethanol (99.5) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of chloroform and acetic acid (100) (4:1) to a distance of about 13 cm, and air-dry the plate. Spray evenly a solution of 2,4-dinitrophenylhydrazine in diluted sulfuric acid (6 in 25) (1 in 500): the spot of 2-formylbenzoic

acid obtained from the sample solution is not more intense than that obtained from the standard solution.

Water <2.48> Not more than 3.0% (0.5 g, volumetric titration, direct titration).

Assay Weigh accurately an amount of Talampicillin Hydrochloride and Talampicillin Hydrochloride Reference Standard, equivalent to about 20 mg (potency), dissolve in water to make exactly 20 mL each, and use these solutions as the sample solution and the standard solution. The standard solution should be prepared before use. Pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add 2.0 mL of sodium hydroxide TS, and allow them to stand for exactly 15 minutes. Add 2.0 mL of diluted hydrochloric acid (1 in 10) and exactly 10 mL of 0.005 mol/L iodine VS, allow them to stand for exactly 15 minutes, and titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared. If necessary, add 0.2 to 0.5 mL of starch TS. Separately, pipet 2 mL each of the sample solution and the standard solution in separate 100-mL glass-stoppered flasks, add exactly 10 mL of 0.005 mol/L iodine VS, titrate <2.50> with 0.01 mol/L sodium thiosulfate VS until the color of the solution is disappeared, and make any necessary correction. For this titration, add 0.2 to 0.5 mL of starch TS, if necessary. Calculate the amount (mL) of 0.005 mol/L iodine VS, V_T and V_S , consumed by the sample solution and the standard solution, respectively.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of ampicillin (C}_{16}\text{H}_{19}\text{N}_3\text{O}_4\text{S)} \\ = W_S \times (V_T/V_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Talampicillin Hydrochloride Reference Standard

Containers and storage Containers—Tight containers.

Talc

タルク

Talc is a native, hydrous magnesium silicate, sometimes containing a small portion of aluminum silicate.

Description Talc occurs as a white to grayish white, fine, crystalline powder. It is odorless and tasteless.

It is unctuous, and adheres readily to the skin.

It is practically insoluble in water, in ethanol (95) and in diethyl ether.

Identification (1) Mix 0.2 g of Talc with 0.9 g of anhydrous sodium carbonate and 1.3 g of potassium carbonate, and heat the mixture in a platinum or nickel crucible until fusion is complete. Cool, and transfer the fused mixture to a beaker with the aid of 50 mL of hot water. Add hydrochloric acid until it ceases to cause effervescence, add 10 mL of hydrochloric acid, and evaporate the mixture on a water bath to dryness. Cool, add 20 mL of water, boil, and filter. Add 10 mL of a solution of methylene blue trihydrate (1 in 10,000) to the residue, and wash with water: the precipitate is blue in color.

(2) Dissolve 2 g of ammonium chloride and 5 mL of ammonia TS in the filtrate obtained in (1), filter if necessary, and

add disodium hydrogenphosphate TS: a white, crystalline precipitate is produced.

Purity (1) Acid-soluble substances—Weigh accurately about 1 g of Talc, heat with 20 mL of dilute hydrochloric acid at 50°C for 15 minutes with stirring. Cool, add water to make exactly 50 mL, and filter. Centrifuge, if necessary, until the filtrate becomes clear. To 25 mL of this filtrate add 1 mL of dilute sulfuric acid, evaporate to dryness, and ignite to constant mass at 800 ± 25°C: the amount of the residue is not more than 2.0%.

(2) Acid or alkali, and water-soluble substances—To 10.0 g of Talc, add 50 mL of water, weigh, and boil for 30 minutes, supplying water lost by evaporation. Cool, add water to restore the original mass, and filter. Centrifuge, if necessary, until the filtrate becomes clear: the filtrate is neutral. Evaporate 20 mL of the filtrate to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 4.0 mg.

(3) Water-soluble iron—Make 10 mL of the filtrate obtained in (2) weakly acidic with hydrochloric acid, and add dropwise potassium hexacyanoferrate (II) TS: the liquid does not acquire a blue color.

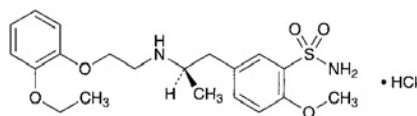
(4) Arsenic <1.11>—To 0.5 g of Talc add 5 mL of dilute sulfuric acid, and heat gently to boiling with shaking. Cool immediately, filter, and wash the residue with 5 mL of dilute sulfuric acid, then with 10 mL of water. Combine the filtrate and the washings, evaporate to 5 mL on a water bath, and perform the test with this solution as the test solution (not more than 4 ppm).

Loss on ignition <2.43> Not more than 5.0% (1 g, 450–550°C, 3 hours).

Containers and storage Containers—Well-closed containers.

Tamsulosin Hydrochloride

タムスロシン塩酸塩



$C_{20}H_{28}N_2O_5S \cdot HCl$: 444.97

5-[(2*R*)-2-[2-(2-Ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzenesulfonamide monohydrochloride [106463-17-6]

Tamsulosin Hydrochloride, when dried, contains not less than 98.5% and not more than 101.0% of $C_{20}H_{28}N_2O_5S \cdot HCl$.

Description Tamsulosin Hydrochloride occurs as white crystals.

It is freely soluble in formic acid, sparingly soluble in water, slightly soluble in acetic acid (100), and very slightly soluble in ethanol (99.5).

Melting point: about 230°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Tamsulosin Hydrochloride (3 in 160,000) as

directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tamsulosin Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of an ice cooled solution of Tamsulosin Hydrochloride (3 in 400) add 3 mL of dilute nitric acid, shake well, allow to stand at room temperature for 30 minutes, and filter: the filtrate responds to the Qualitative Tests <1.09> for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: -17.5 – -20.5° (after drying, 0.15 g, water, warming, after cooling, 20 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tamsulosin Hydrochloride according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—

(i) Dissolve 50 mg of Tamsulosin Hydrochloride in 10 mL of the mobile phase, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 2.5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than the peak area of tamsulosin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 225 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To 700 mL of this solution add 300 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 6 minutes.

Time span of measurement: Until tamsulosin is eluted, beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μL of this solution is equivalent to 1.4 to 2.6% of that from 10 μL of the standard solution.

System performance: Dissolve 5 mg of Tamsulosin Hydrochloride and 10 mg of propyl parahydroxybenzoate in 20 mL of the mobile phase. To 2 mL of this solution add the

mobile phase to make 20 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, tamsulosin and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

(ii) Perform the test with 10 μ L each of the sample solution and standard solution which are obtained in above (i) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tamsulosin obtained from the sample solution is not larger than the peak area of tamsulosin from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Purity (2) (i).

Mobile phase: Dissolve 4.4 mL of perchloric acid and 1.5 g of sodium hydroxide in 950 mL of water, adjust the pH to 2.0 with sodium hydroxide TS, and add water to make 1000 mL. To this solution add 1000 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of tamsulosin is about 2.5 minutes.

Time span of measurement: About 5 times as long as the retention time of tamsulosin, beginning after the peak of tamsulosin.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add the mobile phase used in the Purity (2) (i) to make exactly 50 mL. Confirm that the peak area of tamsulosin obtained from 10 μ L of this solution is equivalent to 1.4 to 2.6% of that from 10 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Purity (2) (i).

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tamsulosin is not more than 4.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Tamsulosin Hydrochloride, previously dried, dissolve in 5 mL of formic acid, add 75 mL of a mixture of acetic acid (100) and acetic anhydride (3:2), and immediately titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 44.50 mg of C₂₀H₂₈N₂O₅S.HCl

Containers and storage Containers—Well-closed containers.

Tannic Acid

タンニン酸

Tannic Acid is the tannin usually obtained from nutgalls or rhusgalls.

Description Tannic Acid occurs as a yellowish white to light brown, amorphous powder, glistening leaflets, or spongy masses. It is odorless or has a faint, characteristic odor, and has a strongly astringent taste.

It is very soluble in water and in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) To 5 mL of a solution of Tannic Acid (1 in 400) add 2 drops of iron (III) chloride TS: a blue-black color develops. Allow the solution to stand: a blue-black precipitate is produced.

(2) To 5 mL of a solution of Tannic Acid (1 in 20) add 1 drop each of albumin TS, gelatin TS, or 1 mL of starch TS: a precipitate is produced in each solution.

Purity (1) Gum, dextrin and sucrose—Dissolve 3.0 g of Tannic Acid in 15 mL of boiling water: the solution is clear or slightly turbid. Cool, and filter the solution. To 5 mL of the filtrate add 5 mL of ethanol (95): no turbidity is produced. Add further 3 mL of diethyl ether to this solution: no turbidity is produced.

(2) Resinous substances—To 5 mL of the filtrate obtained in (1) add 10 mL of water: no turbidity is produced.

Loss on drying <2.41> Not more than 12.0% (1 g, 105°C, 2 hours).

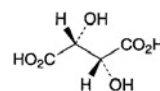
Residue on ignition <2.44> Not more than 1.0% (0.5 g).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tartaric Acid

酒石酸



C₄H₆O₆: 150.09
(2*R*,3*R*)-2,3-Dihydroxybutanedioic acid [87-69-4]

Tartaric Acid, when dried, contains not less than 99.7% of C₄H₆O₆.

Description Tartaric Acid occurs as colorless crystals or a white, crystalline powder. It is odorless, and has a strong acid taste.

It is very soluble in water, freely soluble in ethanol (95), and slightly soluble in diethyl ether.

A solution of Tartaric Acid (1 in 10) is dextrorotatory.

Identification (1) Ignite Tartaric Acid gradually: it decomposes and an odor of burning sugar is perceptible.

(2) A solution of Tartaric Acid (1 in 10) changes blue lit-

mus paper to red, and responds to the Qualitative Tests <1.09> for tartrate.

Purity (1) Sulfate <1.14>—Perform the test with 0.5 g of Tartaric Acid. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.048%).

(2) Oxalate—Dissolve 1.0 g of Tartaric Acid in 10 mL of water, and add 2 mL of calcium chloride TS: no turbidity is produced.

(3) Heavy metals <1.07>—Proceed with 2.0 g of Tartaric Acid according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Calcium—Neutralize a solution of 1.0 g of Tartaric Acid in 10 mL of water with ammonia TS, and add 1 mL of ammonium oxalate TS: no turbidity is produced.

(5) Arsenic <1.11>—Prepare the test solution with 2.0 g of Tartaric Acid according to Method 1, and perform the test (not more than 1 ppm).

Loss on drying <2.41> Not more than 0.5% (3 g, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.05% (1 g).

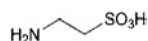
Assay Weigh accurately about 1.5 g of Tartaric Acid, previously dried, dissolve in 40 mL of water, and titrate <2.50> with 1 mol/L sodium hydroxide VS (indicator: 2 drops of phenolphthalein TS).

$$\begin{aligned} \text{Each mL of 1 mol/L sodium hydroxide VS} \\ = 75.04 \text{ mg of } \text{C}_4\text{H}_6\text{O}_6 \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Taurine

タウリン



$\text{C}_2\text{H}_7\text{NO}_3\text{S}$: 125.15
2-Aminoethanesulfonic acid
[107-35-7]

Taurine, when dried, contains not less than 99.0% and not more than 101.0% of $\text{C}_2\text{H}_7\text{NO}_3\text{S}$.

Description Taurine occurs as colorless or white crystals, or a white crystalline powder.

It is soluble in water, and practically insoluble in ethanol (99.5).

The pH of a solution prepared by dissolving 1.0 g of Taurine in 20 mL of freshly boiled and cooled water is between 4.1 and 5.6.

Identification Determine the infrared absorption spectrum of Taurine as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Clarity and color of solution—A solution obtained by dissolving 1.0 g of Taurine in 20 mL of water is clear and colorless.

(2) Chloride <1.03>—Perform the test with 1.0 g of Taurine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <1.14>—Perform the test with 2.0 g of Taurine. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of Taurine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 2.0 g of Taurine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Iron <1.10>—Prepare the test solution with 2.0 g of Taurine according to Method 1, and perform the test according to Method A. Prepare the control solution with 2.0 mL of Standard Iron Solution (not more than 10 ppm).

(7) Related substances—Dissolve 1.0 g of Taurine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water, ethanol (99.5), 1-butanol and acetic acid (100) (150:150:100:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin-butanol TS on the plate, and heat at 105°C for 5 minutes: the spot other than the principle spot with the sample solution is not more than one spot, and it is not more intense than the spot with the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Taurine, previously dried, dissolve in 50 mL of water, add 5 mL of formaldehyde solution, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L sodium hydroxide VS} \\ = 12.52 \text{ mg of } \text{C}_2\text{H}_7\text{NO}_3\text{S} \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Teceleukin (Genetical Recombination)

テセロイキン(遺伝子組換え)

Met-Ala-Pro-Thr-Ser-Ser-Ser-Thr-Lys-Lys-Thr-Gln-Leu-Gln-Leu-Glu-His-Leu-Leu-Leu-
 Asp-Leu-Gln-Met-Ile-Leu-Asn-Gly-Ile-Asn-Asn-Tyr-Lys-Asn-Pro-Lys-Leu-Thr-Arg-Met-
 Leu-Thr-Phe-Lys-Phe-Tyr-Met-Pro-Lys-Lys-Ala-Thr-Glu-Leu-Lys-His-Leu-Gln-Cys-Leu-
 Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu-Ala-Gln-Ser-Lys-Asn-Phe-His-
 Leu-Arg-Pro-Arg-Asp-Leu-Ile-Ser-Asn-Ile-Asn-Val-Ile-Val-Leu-Glu-Leu-Lys-Gly-Ser-
 Glu-Thr-Thr-Phe-Met-Cys-Glu-Tyr-Ala-Asp-Glu-Thr-Ala-Thr-Ile-Val-Glu-Phe-Leu-Asn-
 Arg-Trp-Ile-Thr-Phe-Cys-Gln-Ser-Ile-Ile-Ser-Thr-Leu-Thr

C₆₉₈H₁₁₂₇N₁₇₉O₂₀₄S₈: 15547.01
 [136279-32-8]

The desired product of Teceleukin (Genetical Recombination) is a protein consisting of 134 amino acid residues manufactured by *E. coli* through expression of human interleukin-2 cDNA. It is a solution and possesses a T-lymphocyte activating effect.

It contains potency between 7.7×10^6 and 1.54×10^7 units/mL, and not less than 7.7×10^6 units per mg of protein.

Description Teceleukin (Genetical Recombination) occurs as a clear and colorless liquid.

Identification (1) Measure accurately an appropriate amount of Teceleukin (Genetical Recombination), add accurately to a concentration of 200 units per mL of culture medium for assay of teceleukin, and use this solution as the sample stock solution. Dilute reference anti-interleukin-2 antibody for teceleukin with culture medium for assay of teceleukin to a concentration of approximately 200 neutral units/mL and use this solution as the interleukin-2 neutral antibody solution. Accurately add an equivalent volume of the interleukin-2 neutral antibody solution to the sample stock solution, shake, and then leave for 1 hour in a 37°C incubator in air containing 5% carbon dioxide. This solution is the sample solution. Prepare a standard solution by accurately adding an equivalent volume of culture medium for assay of teceleukin to the sample stock solution, mixing, and then processing in the same way. Process the sample and standard solutions according to the assay method, determine their respective dilution coefficients, D_N and D_T , and then determine the neutralization rate, which should be at least 90%, using the following formula.

$$\text{Neutralization rate (\%)} = \{(D_T - D_N) / D_T\} \times 100$$

However, please note if the mean values of the absorbance of the maximum uptake control solution and absorbance of the minimum uptake control solution do not fit the standard curve, the neutralization coefficient is to be determined within the following range.

$$\text{Neutralization coefficient (\%)} > \{(D_T - 2) / D_T\} \times 100$$

(2) Place a volume of Teceleukin (Genetical Recombination) corresponding to approximately 50 µg of protein into 2 test tubes for hydrolysis, evaporate to dryness under vacuum,

and use one as the sample (1). To the other, add 50 µL of a mixture of formic acid and hydrogen peroxide (30) (9:1) that has been left at room temperature for one hour, cool for 4 hours in ice, add 0.5 mL of water, and then evaporate to dryness under vacuum to give the sample (2). To 1.3 mL of methanesulfonic acid add 3.7 mL of water, mix well, add and dissolve 10 mg of 3-(2-aminoethyl)indole, to make a 4 mol/L methanesulfonic acid solution. Dissolve 39.2 g of trisodium citrate dihydrate, 33 mL of hydrochloric acid, 40 mL of thiodiglycol, and 4 mL of laurmacrogol solution (1 in 4) in 700 mL of water, adjust the pH to 2.2, add water to 1000 mL, add 100 µL of capric acid, and mix to make a sodium citrate solution for dilution. Add 50 µL of freshly prepared 4 mol/L methanesulfonic acid to the sample (1) and sample (2), cool to -70°C, and then deaerate under vacuum. Heat to 115°C ± 2°C for 24 hours after sealing these test tubes under reduced pressure. After cooling, unseal, add 50 µL of 4 mol/L sodium hydroxide TS followed by 0.4 mL of sodium citrate solution for dilution to make the sample solution (1) and sample solution (2). Separately, accurately measure 0.25 mmol amounts of L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-lysine hydrochloride, ammonium chloride, L-histidine hydrochloride monohydrate, and L-arginine hydrochloride as well as 0.125 mmol of L-cysteine and then dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. This is the amino acid standard stock solution. Accurately measure 1 mL of this solution, and add sodium citrate solution for dilution to make exactly 25 mL. This is solution A. Accurately weigh approximately 20 mg of L-tryptophan and dissolve in water to make exactly 1000 mL. This is solution B. Accurately measure 10 mL of both solution A and solution B, combine together, and add sodium citrate solution for dilution to make exactly 50 mL. This is the amino acid standard solution. Separately, accurately weigh approximately 17 mg of L-cysteic acid and dissolve in sodium citrate solution for dilution to make exactly 50 mL. Accurately measure 1 mL of this solution and add sodium citrate solution for dilution to make exactly 100 mL. This is the cysteic acid standard solution. Accurately measure 0.25 mL of the sample solution (1), the sample solution (2), amino acid standard solution, and the cysteic acid standard solution. When the test is conducted by Liquid Chromatography <2.01> under the following conditions, peaks for the 18 amino acids are observed in the chromatogram obtained from the sample solution (1). Also, measure the peak area of each amino acid in the sample solution (1) and the amino acid standard solution, and taking the molar number of alanine in the sample solution (1) as 5.0, determine the concentrations of aspartic acid, glutamic acid, proline, glycine, methionine, leucine, tyrosine, phenylalanine, lysine, histidine, tryptophan, and arginine and then calculate the molar ratio for each amino acid. Also, measure the cysteic acid peak areas of the sample solution (2) and the cysteic acid standard solution, determine the concentration of cysteine, and, taking the molar number of alanine in the sample solution (2) as 5.0, calculate the molar ratio of cysteine. When determining the molar ratios of the respective amino acids, aspartic acid is 11.4 to 12.6, glutamic acid 17.1 to 18.9, proline 4.5 to 5.5, glycine 1.8 to 2.2, cysteine 2.7 to 3.3, methionine 4.5 to 5.5, leucine 20.9 to 23.1, tyrosine 2.7 to 3.3, phenylalanine 5.4 to 6.6, lysine 10.5 to 11.6, histidine 2.7 to 3.3, tryptophan 0.7 to 1.2, and arginine 3.6 to 4.4.

Operating conditions—

Detector: Visible absorption photometer [wavelengths: 440 nm (proline) and 570 nm (amino acids other than proline)]

Column: A stainless steel column with an inside diameter of 4 mm and length of 25 cm packed with a strongly acidic ion exchange resin for liquid chromatography consisting of polystyrene to which sulphonate group binds.

Column temperature: A constant temperature of about 50°C when the sample is injected. After a certain time, increase the temperature to a constant temperature of about 62°C

Reaction temperature: A constant temperature of about 98°C.

Time for color formation: Approximately 2 minutes

Mobile phase: After preparing mobile phases A, B, and C according to the following table, add 0.1 mL of capric acid to each.

	Mobile phase A	Mobile phase B	Mobile phase C
Citric acid monohydrate	18.70 g	10.50 g	7.10 g
Trisodium citrate dihydrate	7.74 g	14.71 g	26.67 g
Sodium chloride	7.07 g	2.92 g	54.35 g
Ethanol (99.5)	60 mL	—	—
Benzyl alcohol	—	—	10 mL
Thiodiglycol	5 mL	5 mL	—
Lauromacrogol solution (1 in 4)	4 mL	4 mL	4 mL
Water	Appropriate amount	Appropriate amount	Appropriate amount
pH	3.2	4.3	4.7
Total volume	1000 mL	1000 mL	1000 mL

Changing mobile phases and column temperature: When operating under the above conditions using 0.25 mL of amino acid standard solution, the amino acids will elute in the following order; aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, ammonia, histidine, tryptophan, and arginine. Switchover to mobile phase A, mobile phase B, and mobile phase C, in sequence so that the resolution between the peaks of cystine and valine is 2.0 or more and that between ammonia and histidine is 1.5 or more. Also, increase the temperature after a constant length of time so that the resolution between the peaks of glutamic acid and proline is at least 2.0.

Reaction reagents: Dissolve 408 g of lithium acetate dihydrate in water, and add 100 mL of acetic acid (100) and water to make 1000 mL. To this solution add 1200 mL of dimethylsulfoxide and 800 mL of 2-methoxyethanol. This is solution (I). Separately, mix together 600 mL of dimethylsulfoxide and 400 mL of 2-methoxyethanol and then add 80 g of ninhydrin and 0.15 g of sodium borohydride. This is solution (II). After gassing 3000 mL of the solution (I) for 20 minutes with nitrogen, rapidly add 1000 mL of the solution (II) and then mix by gassing for 10 minutes with nitrogen.

Mobile phase flow rate: About 0.275 mL every minute

Reaction reagent flow rate: About 0.3 mL every minute

System suitability—

System performance: When 0.25 mL of the amino acid standard solution is run under the above conditions, the resolution between the peaks of threonine and serine is at least 1.5.

(3) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate, and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. After adjusting the pH to 8.0 using 1 mol/L hydrochloric acid TS, add water to make 100 mL. This is the molecular weight determination buffer solution. Accurately measure 20 μ L of Teceleukin (Genetical Recombination), add exactly 20 μ L of the molecular weight determination buffer solution and 2 μ L of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μ L of bromophenol blue solution (1 in 2000) and then shake. This is the sample solution. Separately, accurately measure 5 μ L of molecular weight marker for teceleukin, and add exactly 50 μ L of water, 55 μ L of the molecular weight determination buffer solution, and 5 μ L of 2-mercaptoethanol, and then heat for 5 minutes on a 90 to 100°C water bath without allowing any water evaporation from the mixture. After cooling, add exactly 1 μ L of bromophenol blue solution (1 in 2000), and shake well. This is the molecular weight standard solution. When conducting a test using SDS-polyacrylamide gel electrophoresis with 1 μ L each of the sample solution and the molecular weight standard solution, the molecular weight of the main band is between the range of 14,000 and 16,000.

Operating conditions—

Equipment: Horizontal electrophoresis vessel equipped with a cooling unit, a device that accumulates load voltage over time, and a direct current power source device that controls the amperage, voltage, wattage.

Spotting of solutions: Solutions are spotted on concentrating gel of polyacrylamide gel sheets.

Electrophoresis conditions

Polyacrylamide gel sheet: Polyester sheet to which a polyacrylamide gel (width, about 43 mm, length, about 50 mm, and thickness, about 0.5 mm) is closely adhered. The polyacrylamide gel consists of a concentrating gel with a gel support concentration of 7.5% and a 3% degree of crosslinking and a separating gel with corresponding values of 20% and 2%. The gel contains pH 6.5 Tris-acetate buffer.

Buffer solution for electrode: Prepared by dissolving 35.83 g of tricine, 24.23 g of 2-amino-2-hydroxymethyl-1,3-propanediol, and 5.5 g of sodium lauryl sulfate in water to make 1000 mL.

Cooling temperature of gel support plate: 15°C

Running conditions

Pre-electrophoresis and electrophoresis: The voltage, amperage, and wattage should not exceed 250 V, 10 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Immediately after adding sample: The voltage, amperage, and wattage should not exceed 250 V, 1 mA, and 3 W, respectively. The amperage and wattage should be proportional to the number of polyacrylamide sheets.

Electrophoresis time

Before adding sample: Until value of load voltage integrated with respect to time reaches 60 V·h.

Immediately after adding sample: Until value of load voltage integrated with respect to time reaches 1 V·h.

Main electrophoresis: Until value of load voltage integrated with respect to time reaches 140 V·h.

Fixation and staining

Dissolve 25 g of anhydrous sodium carbonate anhydride and 0.8 mL of formaldehyde solution in water to make 1000 mL. This is the developing solution. After immersing the polyacrylamide gel sheet in a mixture of ethanol (99.5), water and acetic acid (100) (5:4:1) for 2 minutes, immerse for 2 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (17:2:1). Change the mixture, immerse for another 4 minutes, immerse in water for 2 minutes to rinse the polyacrylamide gel sheet, and change the water to immerse for 2 minutes. This procedure is carried out with heating to 50°C. Next, while heating at 40°C, immerse for 10 to 15 minutes in diluted silver nitrate TS (1 in 7), warm to 30°C, and gently rinse the polyacrylamide gel sheet with water. While warming at 30°C, immerse the polyacrylamide gel sheet in freshly prepared developing solution. After obtaining adequate color formation, immerse the polyacrylamide gel sheet in diluted acetic acid (100) (1 in 20) to terminate the color formation.

Estimation of molecular weight

Plot graphs for each band obtained from the molecular weight standard solution, distance from the border of the concentrating gel and separating gel, and the logarithm of the molecular weight of proteins in each band. Determine the molecular mass by reading the corresponding position of the major band obtained from the sample solution on the graph.

(4) The isoelectric point determined from the electrophoresis position is 7.4 to 7.9 when 3 μ L of Teceleukin (Genetical Recombination) and 8 μ L of isoelectric marker for teceleukin are tested by the polyacrylamide gel isoelectric method.

Operating conditions—

Equipment: Horizontal electrophoretic vessel with cooling unit and direct current power source that can perform constant wattage control.

Preparation of polyacrylamide gel: Dissolve 1.62 g of polyacrylamide and 50 mg of *N,N'*-methylenebisacrylamide in water to make 25 mL. Accurately measure 7.5 mL of this solution, 2 mL of a 10 mL solution prepared by adding water to 5 g of glycerin, and 0.64 mL of a pH 3 to pH 10 amphoteric electrolyte solution, and degas under reduced pressure while stirring thoroughly. Next, accurately measure 74 μ L of freshly prepared ammonium peroxodisulfate solution (1 in 50), 3 μ L of *N,N,N',N'*-tetramethylethylenediamine, and 50 μ L of freshly prepared riboflavin sodium phosphate solution (1 in 1000), stir well, immediately pour on a gel preparation plate (10 cm wide, 11 cm long, and 0.8 mm thick), and then expose to a fluorescent light source for 60 minutes to gelate.

Spotting

Add Teceleukin (Genetical Recombination) or isoelectric marker for teceleukin 30 minutes after starting electrophoresis to wells in gel plates to which plastic tape (3.5 mm wide, 3.5 mm long, 0.4 mm thick) has been applied in advance and that have undergone gelation.

Electrophoresis conditions

Cathode solution: Sodium hydroxide TS

Anode solution: DL-aspartic acid solution (133 in 25,000)

Cooling temperature of gel support plate: $2 \pm 1^\circ\text{C}$

Running conditions: After starting the electrophoresis, a constant wattage of 10 W for 20 minutes and 20 W thereafter. However, the voltage should be 3000 V or less.

Running time: 120 to 140 minutes while blowing Nitrogen into the electrophoresis vessel.

Fixation and washing

Dissolve 28.75 g of trichloroacetic acid and 8.65 g of 5-sulfosalicylic acid dihydrate in 75 mL of methanol and 175 mL of water. Immerse the gel in this solution for 60 minutes to fix the protein to the gel. After fixation, immerse for 10 minutes in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Staining and decolorization

Dissolve 0.11 g of Coomassie brilliant blue G-250 in 25 mL of ethanol (99.5), and add 8 mL of acetic acid (100) and water to make 100 mL. This is the staining solution. Immerse the gel for 10 minutes while heating at 60°C in freshly filtered staining solution. After staining, decolorize by immersing in a mixture of water, ethanol (99.5) and acetic acid (100) (67:25:8).

Determination of isoelectric point

Plot the protein isoelectric points and the distance from the cathode of each band obtained from the isoelectric markers for teceleukin. Determine the isoelectric point from the corresponding position of the major bands obtained from the sample solution.

pH <2.54> 2.7 to 3.5

Purity (1) Host cell-derived protein—Take an appropriate amount of Teceleukin (Genetical Recombination) and add an exact amount of diluted acetic acid (100) (1 in 350) to make a solution containing between 0.68 and 0.72 mg of protein in one mL. This is the sample stock solution. Dissolve 1.52 g of 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride and 10.94 g of 2-amino-2-hydroxymethyl-1,3-propanediol in water to make 200 mL. Dissolve 0.5 g of bovine serum albumin in 25 mL of this solution. This is 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution. Accurately measure 0.5 mL of the sample stock solution, add exactly 30 μ L of sodium carbonate TS, stir, and immediately add exactly 0.47 mL of the 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make the sample solution. Accurately measure 10 mL of diluted acetic acid (100) (1 in 350), add 0.6 mL of sodium carbonate TS, and then add 2 w/v% bovine serum albumin-Tris-hydrochloride buffer solution to make exactly 20 mL. This is the dilution solution. Add the *E. coli* protein stock solution to this dilution solution to make a solution containing 0.015 μ g of *E. coli* protein in one mL. This is standard solution (1). Accurately dilute this solution serially two-fold with the dilution solution to make standard solutions (2) to (8) having different concentrations of *E. coli* protein. Dissolve 0.5 g of bovine serum albumin in 100 mL of 0.01 mol/L phosphate buffer-sodium chloride TS, pH 7.4. This is the wash solution. Accurately measure 0.1 mL of the sample solution, standard solutions (1) to (8), and dilution solution as a blank standard solution and place each in 3 wells in solid phase plates (place dilution solution in 6 wells), cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 5 to 16 hours. Next, remove the solution from each well by aspiration, add 0.25 mL of the wash solution, mix again by shaking in a horizontal direction, and then remove by aspiration. Repeat this procedure 2 more times by adding 0.25 mL of the wash solution to each well. Freshly dilute peroxidase marker antibody stock solution with 1 w/v% bovine serum albumin-phosphate buffer-sodium chloride TS, add exactly 0.1 mL to each well, cover with plastic wrap, mix by shaking in a horizontal direction, and then leave standing at a constant temperature of about 25°C for 16 to 24 hours.

Next, remove the solution in the wells by aspiration, add 0.25 mL of the wash solution, mix by shaking in a horizontal direction, and then remove the solution by aspiration. Using 0.25 mL of the wash solution, repeat this procedure 2 more times for each well. To each well, accurately add 0.1 mL of teceleukin chromophore solution, stir gently, and then shield from light and leave standing for 30 minutes at a constant temperature of about 25°C. Add exactly 0.1 mL of diluted sulfuric acid (3 in 50) to each well and then mix by gently shaking horizontally. Perform the test with these solutions as directed under Ultraviolet-visible spectrophotometry <2.24>, and measure the absorbances A_{T2} and A_{S2} at 450 nm and A_{T1} and A_{S1} at 510 nm. Prepare a standard curve by plotting the values obtained from each standard solution ($A_{S2} - A_{S1}$) on a graph having the concentration of *E. coli* protein (ng/mL) in logarithmic scale on the horizontal axis and the absorbance values on the vertical axis. Match the values obtained from the sample solution ($A_{T2} - A_{T1}$) to the standard curve, determine the concentration A of *E. coli* protein in the sample solution, and take the mean. The amount of *E. coli* protein is not more than 5 ng when the amount of *E. coli* protein per mg of protein is determined using the following formula.

Amount (ng) of *E. coli*-derived protein per mg protein
= A/C

C : Protein concentration (mg/mL) in sample solution

The test is valid if the *E. coli* protein concentration is 0.3 ng/mL or less when the concentration is obtained by fitting the absorbance value at detection limit, calculated from the following formula using absorbance value of the dilution solution, to the standard curve.

Absorbance at detection limit

$$= \bar{X} + 3.3 \times \sqrt{\left[\frac{\sum_{i=1}^6 (X_i - \bar{X})^2}{6 - 1} \right]}$$

X_i : Individual absorbance values obtained from the dilution solution

\bar{X} : The mean of absorbance values obtained from the dilution solution

6: The number of wells in the microplate containing dilution solution

(2) Tetracycline hydrochloride—Serially subculture through 2 passages at 35 to 37°C the test bacteria *Micrococcus luteus* A_TCC9341 in a slant culture of test bacteria inoculation media for teceleukin and then dilute this 100-fold by adding sterilized purified water. This is the test bacteria solution. Store the test bacteria solution at 5°C or less and use the solution within 5 days. Dilute the test bacteria solution serially by adding sterilized purified water, add an appropriate amount to 100 mL of normal agar medium for teceleukin, conduct a preliminary test, and determine the amount of tetracycline hydrochloride that shows an inhibition zone corresponding to standard solution containing 0.5 µg (potency) in 1 mL. Add this amount to 100 mL of normal agar medium for teceleukin dissolved and then cooled to 45 to 50°C and mix. Pipet 25 mL of this solution into square Petri dishes (135 × 95 mm) and spread horizontally to solidify. Prepare plates for testing by making an appropriate number of wells in this agar medium. The volume of the test bacteria solution to which 100 mL of normal agar medium for teceleukin has been added is 0.25 to 1.0 mL. Accurately measure an ap-

propriate amount of Tetracycline Hydrochloride Reference Standard and dilute accurately with water to make a clear solution with a concentration of 1 mg (potency)/mL. Accurately measure an appropriate amount of this solution and dilute precisely with water to make standard solutions with concentrations of 4, 2, 1 and 0.5 µg (potency)/mL. Separately, dilute Teceleukin (Genetical Recombination) with diluted acetic acid (100) (3 in 1000) if needed, or alternatively concentrate under reduced pressure, to make a sample solution with a protein concentration of 0.8 to 1.2 mg/mL. Accurately measure 25 µL of the sample solution and each standard solution, and add each to the wells in the same test plate. Repeat the same procedure for at least 3 more test plates. Leave the test plates at room temperature for 30 to 60 minutes and then incubate for 16 to 18 hours at 35 to 37°C. Measure the inhibition zones to a diameter of 0.25 mm. Determine the mean among the test plates for each of the solutions.

Prepare a standard curve by plotting a graph with the concentration of each standard solution in logarithmic scale on the horizontal axis and the diameter of the inhibition zone on the vertical axis. Match the diameter of the inhibition zone of teceleukin from the standard curve and determine A , the concentration of tetracycline hydrochloride. When the amount of tetracycline hydrochloride per mg of protein is determined by the following formula, the amount is not more than 0.7 µg. However, if an inhibition zone is not seen, or is seen but the diameter is smaller than 0.5 µg/mL on the standard curve, A is taken as being 0.5 µg/mL or less.

Amount [µg (potency)] of tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$) per mg of protein = A/P

P : The protein concentration (mg/mL) of the sample solution.

(3) Desmethionyl form—Add water to an appropriate amount of teceleukin to make a sample solution with a protein concentration of about 0.17 mg/mL. Perform the test with 1.2 mL of this solution as directed under Liquid Chromatography <2.01> under the following conditions. Determine using automatic integration the peak area, A_2 , of teceleukin and the peak area of the desmethionyl form with a relative retention time of about 0.8 relative to teceleukin, A_1 . The content of the desmethionyl form is not more than 1.0% when determined using the following formula.

Amount (%) of desmethionyl form = $\{A_1/(A_1 + A_2)\} \times 100$

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 280 nm)

Columns: Two stainless steel columns with inside diameters of 7.5 mm and lengths of 7.5 cm connected in sequence and packed with 10 µm synthetic polymer bound to diethylaminoethyl base for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Mix 0.658 g of diethanolamine in 400 mL of water, adjust the pH to 9.0 by adding 1 mol/L hydrochloric acid TS, and then add water to make 500 mL.

Mobile phase B: Add 300 mL of water to 2.6 mL of a pH 6 to 9 amphoteric electrolyte solution and 0.5 mL of a pH 8 to 10.5 amphoteric electrolyte solution, adjust to pH 7 with diluted hydrochloric acid (9 in 100), and then add water to make 400 mL.

Switching mobile phases and sample injection: Inject the sample solution while running the mobile phase A. Repeatedly inject 10 times a sample solution volume of 0.11 mL followed by a single injection of 100 μ L. After injecting the entire volume and running mobile phase A for 60 minutes, switch to mobile phase B. After measuring the sample solution and after running 1 mol/L sodium chloride TS for 10 minutes for posttreatment and cleaning of the columns, inject 100 μ L of sodium hydroxide TS while running the mobile phase A and then 55 minutes later start injection of the next sample solution.

Flow: Adjust the flow of the mobile phase B so that the retention time for teceleukin is 45 to 65 minutes. Measure the retention time from the point at which the mobile phase is switched to the mobile phase B.

System suitability—

System performance: Dissolve in water a mixture of two kinds of equine heart-derived myoglobin whose isoelectric points are 6.76 and 7.16 to make a concentration of approximately 0.5 mg/mL. Mix together 50 μ L of this solution, 50 μ L of Teceleukin (Genetical Recombination), and 1.47 mL of water. When 1.2 mL of this solution is run under the above conditions, myoglobin and teceleukin are eluted in this order, and their respective peaks are completely separated.

(4) Dimer—Prepare a sample solution by adding 20 μ L of 0.2% sodium laurylsulfate TS to 20 μ L of Teceleukin (Genetical Recombination). Perform the test as directed under Liquid Chromatography <2.01> using 20 μ L of this solution under the following conditions. Determine using automated integration the teceleukin peak area, A_2 , and the peak area, A_1 , of the dimer with a relative retention time of 0.8 to 0.9 in relation to teceleukin. The amount of the dimer is not more than 1.0% by the following formula.

$$\text{Amount (\%)} \text{ of dimer} = [A_1 / (A_1 + A_2)] \times 100$$

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column with an inside diameter of 7.5 mm and 60 cm in length, packed with glycol etherified silica gel for liquid chromatography (particle diameter: 10 μ m)

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.0 g of sodium lauryl sulfate in 0.1 mol/L sodium phosphate buffer, pH 7.0, to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of teceleukin is between 30 and 40 minutes.

System suitability—

System performance: Add 20 μ L of 0.2% sodium lauryl sulfate TS to 20 μ L of a solution consisting of 5 mg of carbonic anhydrase and 5 mg of α -lactoalbumin dissolved in 100 mL of water. When 20 μ L of this solution is tested under the above conditions, carbonic anhydrase and α -lactoalbumin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: Measure exactly 1.0 mL of the sample solution, add the mobile phase to exactly 20 mL. To exactly 1 mL of this solution add the mobile phase to make exactly 10 mL. When the test is repeated 3 times with 20 μ L of this solution under the above conditions, the relative standard deviation of the teceleukin peak area is not more than 7%.

(5) Other related proteins—Perform the test on 5 μ L of Teceleukin (Genetical Recombination) as directed under Liquid Chromatography <2.01> under the following conditions, and measure the area of each peak using automatic integration. When the amounts are determined by the area percent method, the total amount of peaks other than the teceleukin and solvent peaks is not more than 1.0%.

Operating conditions—

Detector: Ultraviolet absorption photometer (wavelength: 220 nm)

Column: A stainless steel column with an inside diameter of 4.6 mm and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter)

Column temperature: A constant temperature of about 30°C.

Mobile phase A: A solution of trifluoroacetic acid in a mixture of water and acetonitrile (19:1) (1 in 1000)

Mobile phase B: A solution of trifluoroacetic acid in acetonitrile (7 in 10,000)

Mobile phase flow: Control the concentration gradient by changing the mobile phase A and mobile phase B as shown in the table below.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 ~ 12	60 → 50	40 → 50
12 ~ 25	50	50
25 ~ 45	50 → 0	50 → 100
45 ~ 50	0	100

Flow rate: 1.0 mL/min

Time span of measurement: a range that is approximately 1.2-fold the retention time of teceleukin.

System suitability—

System performance: Add 3.8 μ L of water and 16.6 μ L of polysorbet 80 solution (1 in 100) to 83.6 μ L of Teceleukin (Genetical Recombination) and let stand for at least one hour. When 5 μ L of this solution is tested by running under the above conditions, there is complete separation between the teceleukin peak and the peak with a relative retention time of about 0.98 in relation to the teceleukin peak.

(6) Acetic acid—Measure exactly 0.25 mL of Teceleukin (Genetical Recombination) and add exactly 0.25 mL of the internal standard solution to make the sample solution. Separately, measure exactly 3 mL of acetic acid (100) and add water to make exactly 100 mL. Take exactly 10 mL of this solution and add water to make exactly 100 mL. Measure exactly 2 mL of this solution and add exactly 2 mL of the internal standard solution to make the standard solution. Perform the test with 1 μ L each of the sample solution and the standard solution by Gas Chromatography <2.02> under the following conditions. Determine the ratios of the peak area of acetic acid to that of the internal standard, Q_T and Q_S , and the amount of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination) determined by the following formula is between 2.85 and 3.15 mg.

Amount (mg) of acetic acid ($C_2H_4O_2$) in 1 mL of Teceleukin (Genetical Recombination)

$$= (Q_T / Q_S) \times 1.5 \times 1.049 \times 2$$

1.5: Concentration ($\mu\text{L}/\text{mL}$) of acetic acid (100) in the standard solution

1.049: Density ($\text{mg}/\mu\text{L}$) of acetic acid (100) at 25°C

2: Dilution coefficient

Internal standard solution—Diluted propionic acid (1 in 500)
Operating conditions—

Detector: Hydrogen flame ionization detector

Column: A glass column with an inside diameter of 1.2 mm and 40 m in length, whose inside is covered with chemically-bound polyethylene glycol for gas chromatography $1.0\ \mu\text{m}$ in thickness.

Column temperature: A constant temperature of about 110°C .

Carrier gas: Helium

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 8 minutes.

System suitability—

System performance: When $1\ \mu\text{L}$ of the standard solution is run under the above conditions, acetic acid and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.0.

System repeatability: When the test is repeatedly run 6 times under the above conditions using $1\ \mu\text{L}$ of standard solution, the relative standard deviation of the ratio of the acetic acid peak area to the internal standard peak area is not more than 5%.

Bacterial endotoxins <4.01> Less than 5EU per mg of protein

Specific activity Accurately measure an appropriate amount of Teceleukin (Genetical Recombination), and add water accurately so that 1 mL contains about 0.1 mg. This is the sample solution. Separately, measure precisely about 25 mg of human serum albumin for assay, dissolve in water, and add water to make 50 mL. Measure exactly an appropriate amount of this solution, and accurately dilute with water to make standard solutions with concentrations of 0.05, 0.10, and 0.15 mg/mL. Accurately measure 1 mL each of the sample solution, the standard solutions, and water, add 2.5 mL of alkaline copper solution, mix, leave for at least 10 minutes to dissolve, add exactly 2.5 mL of water and 0.5 mL of diluted Folin reagent (1 in 2), immediately shake vigorously, and then leave for 30 minutes at 37°C . Perform the test on these solutions, with water as a control, as directed under Ultraviolet-visible Spectrophotometry <2.24>, and measure the absorbance at 750 nm. With the concentration of the standard solution as the x -axis and the absorbance as the y -axis, perform linear regression using their respective reciprocals, and determine the protein content.

Determine the ratio of the potency determined by Assay and the protein content.

Assay Accurately measure an appropriate amount of Teceleukin (Genetical Recombination) and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL (estimated value). This is the sample solution. Separately, dissolve Interleukin-2 Reference Substance in 1 mL of sterilized purified water, and, depending on the cell sensitivity, dilute precisely by adding culture medium for assay of teceleukin to a constant concentration of 10 to 50 units/mL. This is the standard solution. Add exactly $50\ \mu\text{L}$

of culture medium for assay of teceleukin to all but 8 wells in a microtest plate. Add $50\ \mu\text{L}$ of the sample solution and the standard solution to 2 wells each containing culture medium for assay of teceleukin. From these 4 wells, remove exactly $50\ \mu\text{L}$ and add to 4 other wells containing culture medium for assay of teceleukin. From these 4 wells, remove exactly $50\ \mu\text{L}$ and add to 4 other wells containing culture medium for assay of teceleukin and repeat this procedure to prepare 2 wells that contain each of 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 dilutions of the sample and standard solutions. Add $50\ \mu\text{L}$ of the standard solution to each of the 8 empty wells to make maximum uptake controls. Eight wells containing only culture medium for assay of teceleukin serve as the minimum uptake controls. After adding exactly $50\ \mu\text{L}$ of cell suspension solution for teceleukin to each well in a microtest plate, leave for 15 to 17 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. After adding $25\ \mu\text{L}$ of MTT TS to each of the wells in the plate, leave for 4 hours in an incubator at 37°C filled with air containing 5% carbon dioxide. Transfer the culture medium in all of the wells to empty wells in another microtest plate. To each of the empty wells from which the culture medium was removed, add $100\ \mu\text{L}$ of hydrochloric acid-2-propanol TS, and then shake the plates horizontally for 5 minutes to elute the pigment. After returning the transferred culture medium to each original well, perform the test with the solution in each well, determine the difference in absorption at wavelengths of 560 nm and 690 nm, and calculate the mean values of the identical respective solutions in the two wells (dilution solutions of the sample solution and standard solutions) as well as the 8 wells containing the maximum or minimum uptake controls. Prepare standard curves by plotting the values obtained from each dilution solution of the sample solution, with the dilution coefficient of the sample solution on the microtest plates in logarithmic scale on the horizontal axis and the absorbance on the vertical axis. Determine the mean absorbance values of the maximum and minimum uptake controls, find the values on the standard curve, and then determine the dilution coefficient, D_T . Perform the same plot for the dilution solution of the standard solution, determine the dilution coefficient, D_S , and then determine the potency in 1 mL by the following formula.

Teceleukin potency (units) in 1 mL of Teceleukin (Genetical Recombination)

$$= S \times (D_T/D_S) \times d$$

S : Concentration of standard solution (units/mL)

d : Dilution coefficient when sample solution prepared

Containers and storage Containers—Tight containers

Storage—Store at -70°C or below.

Teceleukin for Injection (Genetical Recombination)

注射用テセロイキン(遺伝子組換え)

Teceleukin for Injection (Genetical Recombination) is a preparation for injection which is dissolved before use.

It contains not less than 70.0% and not more than

150.0% of the labeled amount of teceleukin (genetical recombination) ($C_{698}H_{1127}N_{179}O_{204}S_8$; 15547.01).

Method of preparation Prepare as directed under Injection, with Teceleukin (Genetical Recombination).

Description Teceleukin for Injection (Genetical Recombination) occurs as a white, light mass or powder.

Identification (1) Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample stock solution containing about 200 units/mL (estimate). Proceed as directed in the Identification (1) and (3) under Teceleukin (Genetical Recombination).

(2) Dissolve 0.242 g of 2-amino-2-hydroxymethyl-1,3-propanediol, 5.0 g of sodium lauryl sulfate and 74 mg of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 60 mL of water. Adjust to pH 8.0 with 1 mol/L hydrochloric acid TS, add water to make 100 mL, and use this solution as the buffer solution for molecular mass determination. Separately, dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of water. To exactly 100 μ L of this solution add exactly 100 μ L of the buffer solution for molecular mass determination and 10 μ L of 2-mercaptoethanol, and heat on a water bath for 5 minutes without allowing any water evaporation from the mixture. After cooling, add exactly 1 μ L of bromophenol blue solution (1 in 2000), mix, and use this solution as the sample solution. Proceed as directed in Identification (3) under Teceleukin (Genetical Recombination); a band appears in the range of molecular mass between 14,000 and 16,000.

pH <2.54> Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water; the pH of the solution is between 7.0 and 7.7.

Purity Clarity and color of solution—Dissolve the content of one vial of Teceleukin for Injection (Genetical Recombination) in 1 mL of water; the solution is clear and colorless.

Loss on drying <2.41> Transfer the content of the vial of Teceleukin for Injection (Genetical Recombination) to a weighing bottle under the atmosphere not exceeding 10% relative humidity, and perform the test as directed in the Water content determination described in the Minimum Requirements for Biological Products: not more than 3%.

Bacterial endotoxins <4.01> Less than 5 EU/350,000 units.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test. Calculate as $|M - A| = 0$.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Dissolve the content of 1 vial of Teceleukin for Injection (Genetical Recombination) in exactly 1 mL of sterilized purified water, dilute exactly with culture medium for assay of teceleukin to make the sample solution containing a definite concentration of 10 to 50 units/mL (estimate). Proceed as directed in the Assay under Teceleukin (Genetical

Recombination), and calculate the amount (unit) of teceleukin in 1 vial by the following formula.

Amount (unit) of teceleukin in 1 vial = $S \times (D_T/D_S) \times d \times l$

S: Concentration (unit/mL) of the standard solution

d: Rate of dilution to prepare the sample solution

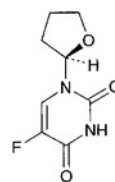
l: Volume (mL) of the sample solution

Containers and storage Containers—Hermetic containers.

Storage—Light-resistant, not exceeding 10°C, avoiding freezing.

Tegafur

テガフル



and enantiomer

$C_8H_9FN_2O_3$; 200.17

5-Fluoro-1-[(2*RS*)-tetrahydrofuran-2-yl]uracil
[17902-23-7]

Tegafur, when dried, contains not less than 98.0% of $C_8H_9FN_2O_3$.

Description Tegafur occurs as a white, crystalline powder.

It is soluble in methanol and in acetone, and sparingly soluble in water and in ethanol (95).

It dissolves in dilute sodium hydroxide TS.

A solution of Tegafur in methanol (1 in 50) shows no optical rotation.

Identification (1) Prepare the test solution with 0.01 g of Tegafur as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as an absorbing liquid; the test solution responds to the Qualitative Tests <1.09> (2) for fluoride.

(2) Determine the absorption spectrum of a solution of Tegafur in 0.01 mol/L sodium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tegafur, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.5 g of Tegafur in 50 mL of water; the pH of this solution is between 4.2 and 5.2.

Melting point <2.60> 166 – 171°C

Purity (1) Clarity and color of solution—Dissolve 0.2 g of Tegafur in 10 mL of dilute sodium hydroxide TS; the solution is clear and colorless.

(2) Chloride <1.03>—Dissolve 0.8 g of Tegafur in 40 mL

of water by warming, cool, filter if necessary, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Tegafur in 40 mL of water by warming, cool, filter if necessary, and add 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution in a platinum crucible with 1.0 g of Tegafur according to Method 4, incinerating by ignition between 750°C and 850°C, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.10 g of Tegafur in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and ethanol (95) (5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

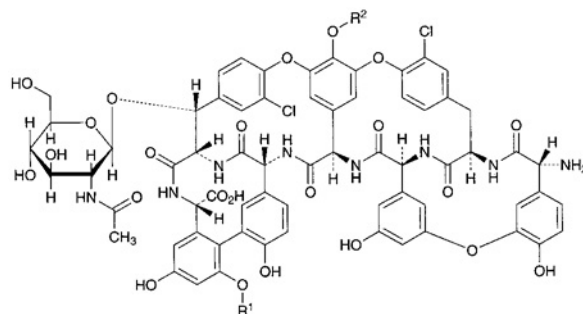
Assay Weigh accurately about 0.15 g of Tegafur, previously dried, place in an iodine bottle, dissolve in 75 mL of water, and add exactly 25 mL of 1/60 mol/L potassium bromate VS. Add rapidly 1.0 g of potassium bromide and 12 mL of hydrochloric acid, stopper the bottle tightly at once, and allow to stand for 30 minutes with occasional shaking. To this solution add 1.6 g of potassium iodide, shake gently, allow to stand for exactly 5 minutes, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 2 mL of starch TS). Perform a blank determination.

Each mL of 1/60 mol/L potassium bromate VS
= 10.01 mg of $C_{88}H_{97}Cl_2N_9O_{33}$

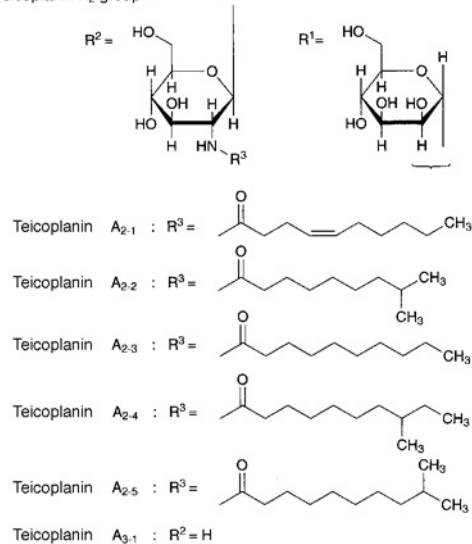
Containers and storage Containers—Tight containers.

Teicoplanin

テイコプラニン



Teicoplanin A_2 group :



Teicoplanin $A_{2.1}$

$C_{88}H_{95}Cl_2N_9O_{33}$: 1877.64

(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-(4*Z*)-dec-4-enoylamino-2-deoxy- β -D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α -D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [91032-34-7]

Teicoplanin $A_{2.2}$

$C_{88}H_{97}Cl_2N_9O_{33}$: 1879.66

(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy- β -D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methylnonanoylamino)- β -D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α -D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-

bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [91032-26-7]

Teicoplanin A_{2,3}

C₈₈H₉₇Cl₂N₉O₃₃: 1879.66
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-(2-decanoylamino-2-deoxy-β-D-glucopyranosyloxy)-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [91032-36-9]

Teicoplanin A_{2,4}

C₈₉H₉₉Cl₂N₉O₃₃: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(8-methyldecanoylamino)-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [91032-37-0]

Teicoplanin A_{2,5}

C₈₉H₉₉Cl₂N₉O₃₃: 1893.68
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-56-[2-deoxy-2-(9-methyldecanoylamino)-β-D-glucopyranosyloxy]-6,11,40,44-tetrahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [91032-38-1]

Teicoplanin A_{3,1}

C₇₂H₆₆Cl₂N₈O₂₈: 1564.25
(3*S*,15*R*,18*R*,34*R*,35*S*,38*S*,48*R*,50*aR*)-34-(2-Acetylamino-2-deoxy-β-D-glucopyranosyloxy)-15-amino-22,31-dichloro-6,11,40,44,56-pentahydroxy-42-(α-D-mannopyranosyloxy)-2,16,36,50,51,59-hexaoxo-2,3,16,17,18,19,35,36,37,38,48,49,50,50a-tetradecahydro-1*H*,15*H*,34*H*-20,23:30,33-dietheno-3,18:35,48-bis(iminomethano)-4,8:10,14:25,28:43,47-tetrametheno-28*H*-[1,14,6,22]dioxadiazacyclooctacosino[4,5-*m*][10,2,16]benzoxadiazacyclotetracosine-38-carboxylic acid [93616-27-4]

[61036-62-2, Teicoplanin]

Teicoplanin is a mixture of glycopeptide substances having antibacterial activity produced by the growth of *Actinoplanes teichomyceticus*.

It contains not less than 900 μg (potency) per mg, calculated on the anhydrous, de-sodium chloride and de-residual solvents basis. The potency of Teicoplanin is expressed as mass (potency) of teicoplanin (C₇₂₋₈₉H₆₈₋₉₉Cl₂N₈₋₉O₂₈₋₃₃).

Description Teicoplanin occurs as a white to light yellowish white powder.

It is freely soluble in water, sparingly soluble in *N,N*-dimethylformamide, and practically insoluble in acetonitrile, in methanol, in ethanol (95), in acetone, in acetic acid (100) and in diethyl ether.

Identification (1) To 1 mL of a solution of Teicoplanin (1 in 100) add 2 mL of ninhydrin TS, and warm for 5 minutes: a blue-purple color develops.

(2) To 1 mL of a solution of Teicoplanin (3 in 100) add slowly 2 mL of anthrone TS, and shake gently: a dark brown color develops.

(3) Determine the infrared absorption spectra of Teicoplanin and Teicoplanin Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the spectrum of Teicoplanin Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 0.5 g of Teicoplanin in 10 mL of water: the pH of the solution is between 6.3 and 7.7.

Content ratio of the active principle Dissolve about 20 mg of Teicoplanin in water to make 10 mL, and use this solution as the sample solution. Perform the test with 20 μL of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the sum of peak areas of teicoplanin A₂ group, S_a, the sum of peak areas of teicoplanin A₃ group, S_b, and the sum of peak areas of other contents, S_c from the sample solution by the automatic integration method. Calculate the content ratio of them by the formula given below: teicoplanin A₂ group, teicoplanin A₃ group, and the other are not less than 80.0%, not more than 15.0% and not more than 5.0%, respectively.

The elution order of each content and the relative retention time of each content to the retention time of teicoplanin A_{2,2} are shown in the following table.

Name of content	Elution order	Relative retention time
teicoplanin A ₃ group		≤0.42
teicoplanin A _{3,1}	1	0.29
teicoplanin A ₂ group		0.42 <, ≤ 1.25
teicoplanin A _{2,1}	2	0.91
teicoplanin A _{2,2}	3	1.00
teicoplanin A _{2,3}	4	1.04
teicoplanin A _{2,4}	5	1.17
teicoplanin A _{2,5}	6	1.20
others		1.25 <

Content ratio (%) of teicoplanin A₂ group
 $= \{S_a / (S_a + 0.83S_b + S_c)\} \times 100$

Content ratio (%) of teicoplanin A₃ group
 $= \{0.83S_b / (S_a + 0.83S_b + S_c)\} \times 100$

Content ratio (%) of others
 $= \{S_c / (S_a + 0.83S_b + S_c)\} \times 100$

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 1650 mL of water, add 300 mL of acetonitrile, adjust pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Mobile phase B: Dissolve 7.80 g of sodium dihydrogen phosphate dihydrate in 550 mL of water, add 1400 mL of acetonitrile, adjust the pH to 6.0 with sodium hydroxide TS, and add water to make 2000 mL.

Flowing of the mobile phase: Flow mobile phase A for 10 minutes before injection. After injection, control the gradient by mixing the mobile A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 32	100 → 70	0 → 30
32 – 40	70 → 50	30 → 50
40 – 42	50 → 100	50 → 0

Flow rate: 1.8 mL per minute.

Time span of measurement: About 1.7 times as long as the retention time of teicoplanin A_{2,2}, beginning after the solvent peak.

System suitability—

Test for required detection: Confirm that peak height of teicoplanin A_{2,2} obtained from the sample solution is equivalent to 90% of the full scale.

System performance: When the procedure is run with 20 μL of the sample solution under the above operating conditions, the symmetry factor of the peak of teicoplanin A_{3,1} is not more than 2.2.

System repeatability: When the test is repeated 3 times with 20 μL of the sample solution under the above operating conditions, the relative standard deviation of the peak area of teicoplanin A_{2,2} is not more than 2.0%.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Sodium chloride—Weigh accurately about 0.5 g of Teicoplanin, dissolve in 50 mL of water, titrate <2.50> with 0.1 mol/L silver nitrate VS (indicator: 1 mL of potassium chromate TS), and calculate an amount of sodium chloride: not more than 5.0%.

Each mL of 0.1 mol/L silver nitrate VS
 $= 5.844$ mg of NaCl

(3) Heavy metals <1.07>—Being specified separately.

(4) Arsenic <1.11>—Being specified separately.

(5) Residual solvents <2.46>—Weigh accurately about 0.1 g of Teicoplanin, dissolve in *N,N*-dimethylformamide to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 1 g each of methanol and acetone, and add *N,N*-dimethylformamide to make exactly 100 mL. Pipet 1 mL of this solution, add *N,N*-dimethylformamide to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 4 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following condition. Calculate the peak area of methanol, A₁, and the peak area of acetone, A₂, obtained from the sample solution, and the peak area of methanol, A_{S1}, and the peak area of acetone, A_{S2}, obtained from the standard solution by the automatic integration method, and calculate the amounts of methanol and acetone by the following formula: not more than 0.5% and not more than 1.0%, respectively.

Amount (%) of methanol
 $= W_{S1} \times (A_1 / A_{S1}) \times 0.001 \times (1 / W_{T1}) \times 100$

W_{S1}: Amount (g) of methanol
 W_{T1}: Amount (g) of Teicoplanin

Amount (%) of acetone
 $= W_{S2} \times (A_2 / A_{S2}) \times 0.001 \times (1 / W_{T2}) \times 100$

W_{S2}: Amount (g) of acetone
 W_{T2}: Amount (g) of Teicoplanin

Operating conditions—

Detector: Hydrogen flame-ionization detector.

Column: A glass column 2 mm in inside diameter and 3 m in length, packed with graphite carbon for gas chromatography, 150 to 180 μm in particle diameter, coated with 0.1% of polyethylene glycol esterified.

Column temperature: Inject the sample at a constant temperature of about 70°C, maintain the temperature for 4 minutes, then program to increase the temperature at the rate of 8°C per minute to 210°C.

Detector temperature: A constant temperature of about 240°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention times of methanol and acetone are about 2 minutes and 5 minutes, respectively.

System suitability—

Test for required detection: Confirm that the peak height of acetone obtained from 4 μL of the standard solution is equivalent to about the full scale.

System performance: When the procedure is run with 4 μL of the standard solution under the above operating conditions, methanol and acetone are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 3 times with 4 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetone is not more than 3%.

Water <2.48> Not more than 15.0% (0.2 g, volumetric titration, direct titration).

Bacterial endotoxins <4.01> Less than 0.75 EU/mg (potency).

Blood pressure depressant Being specified separately.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

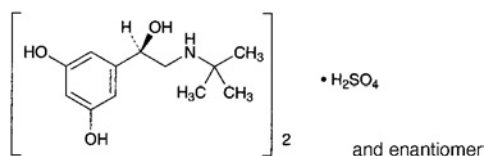
- (i) Test organism—*Bacillus subtilis* ATCC 6633
- (ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.
- (iii) Standard solutions—Weigh accurately an amount of Teicoplanin Reference Standard equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL, and use this solution as the standard stock solution. Keep the standard stock solution at not exceeding 5°C and use within 14 days. Take exactly a suitable amount of this solution before use, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 µg (potency) and 40 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.
- (iv) Sample solutions—Weigh accurately an amount of Teicoplanin equivalent to about 50 mg (potency), dissolve in phosphate buffer solution, pH 6.0 to make exactly 50 mL. Take exactly a suitable amount of this solution, add phosphate buffer solution, pH 6.0 to make solutions so that each mL contains 160 µg (potency) and 40 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 5°C.

Terbutaline Sulfate

テルブタリン硫酸塩



(C₁₂H₁₉NO₃)₂·H₂SO₄: 548.65

5-[(1*R,S*)-2-(1,1-Dimethylethylamino)-1-hydroxyethyl]benzene-1,3-diol hemisulfate [23031-32-5]

Terbutaline Sulfate contains not less than 98.5% of (C₁₂H₁₉NO₃)₂·H₂SO₄, calculated on the anhydrous basis.

Description Terbutaline Sulfate is white to slightly brownish white crystals or crystalline powder. It is odorless or has a faint odor of acetic acid.

It is freely soluble in water, and practically insoluble in acetonitrile, in ethanol (95), in acetic acid (100), in chloroform, and in diethyl ether.

It is gradually colored by light and by air.

Melting point: about 255°C (with decomposition).

Identification (1) Dissolve 1 mg of Terbutaline Sulfate in 1 mL of water, and add 5 mL of Tris buffer solution, pH 9.5, 0.5 mL of 4-aminoantipyrine solution (1 in 50) and 2 drops of potassium hexacyanoferrate (III) solution (2 in 25): a reddish purple color is produced.

(2) Determine the absorption spectrum of a solution of Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS (1 in

10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths. This maximum can be biphasic.

(3) A solution of Terbutaline Sulfate (1 in 50) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve Terbutaline Sulfate in 10 mL of water: the pH of this solution is between 4.0 and 4.8.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Terbutaline Sulfate in 10 mL of water: the solution is clear and colorless or slightly yellow.

(2) Chloride <1.03>—Perform the test with 2.0 g of Terbutaline Sulfate. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.004%).

(3) Acetic acid—Dissolve 0.50 g of Terbutaline Sulfate in a solution of phosphoric acid (59 in 1000) to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 1.50 g of acetic acid (100) in a solution of phosphoric acid (59 in 1000) to make exactly 100 mL. Dilute 2 mL of this solution, accurately measured, with a solution of phosphoric acid (59 in 1000) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 2 µL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following operating conditions. Measure the peak areas, A_T and A_S, of acetic acid for the two solutions: A_T is not larger than A_S.

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column 3 mm in inside diameter and 1 m in length, packed with 10% of macrogol 6000 on 180- to 250-µm terephthalic acid for gas chromatography.

Column temperature: A constant temperature at about 120°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of acetic acid is about 5 minutes.

System suitability—

System performance: Mix 0.05 g each of acetic acid (100) and propionic acid in 100 mL of diluted phosphoric acid (59 in 1000). When the procedure is run with 2 µL of this solution under the above conditions, acetic acid and propionic acid are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 2 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of acetic acid is not more than 3.0%.

(4) 3,5-Dihydroxy-*ω*-tert-butylaminoacetophenone sulfate—Dissolve 0.50 g of Terbutaline Sulfate in 0.01 mol/L hydrochloric acid TS to make exactly 25 mL, and perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance at a wavelength of 330 nm does not exceed 0.47.

(5) Heavy metals <1.07>—Proceed with 2.0 g of Terbutaline Sulfate as directed under Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Terbutaline Sulfate according to method 3, and perform the test (not more than 2 ppm).

Water <2.48> Not more than 0.5% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

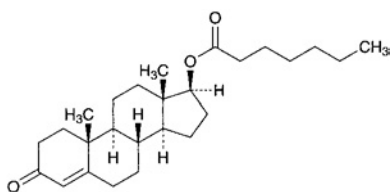
Assay Weigh accurately about 0.5 g of Terbutaline Sulfate, dissolve in 50 mL of a mixture of acetonitrile and acetic acid (100) (1:1) by stirring and warming. Allow to cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration, substituting a saturated solution of potassium chloride in methanol for the internal fluid).

Each mL of 0.1 mol/L perchloric acid VS
= 54.87 mg of $(C_{12}H_{19}NO_3)_2 \cdot H_2SO_4$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Testosterone Enanthate

テストステロンエナント酸エステル



$C_{26}H_{40}O_3$: 400.59

3-Oxoandrost-4-en-17 β -yl heptanoate [315-37-7]

Testosterone Enanthate, when dried, contains not less than 95.0% and not more than 105.0% of $C_{26}H_{40}O_3$.

Description Testosterone Enanthate occurs as white to pale yellow crystals, crystalline powder or a pale yellow-brown, viscous liquid. It is odorless or has a slight, characteristic odor.

It is very soluble in ethanol (95), in 1,4-dioxane and in diethyl ether, and practically insoluble in water.

Melting point: about 36°C

Identification Heat 25 mg of Testosterone Enanthate with 2 mL of a solution of potassium hydroxide in methanol (1 in 100) under a reflux condenser on a water bath for 1 hour, cool, and add 10 mL of water. Collect the produced precipitate by suction, wash with water until the last washing is neutral, and dry the precipitate in a desiccator (in vacuum, phosphorus (V) oxide) for 4 hours: the precipitate melts between 151°C and 157°C.

Optical rotation <2.49> $[\alpha]_D^{20}$: +77 – +88° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity Acidity—Dissolve 0.5 g of Testosterone Enanthate in 10 mL of ethanol (95) which has previously been rendered neutral to bromothymol blue TS, and add 2 drops of bromothymol blue TS and 0.50 mL of 0.01 mol/L sodium hydroxide VS: the color of the solution is light blue.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately about 0.1 g of Testosterone Enanthate, previously dried, and dissolve in ethanol (95) to make

exactly 100 mL. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Measure exactly 10 mL of this solution, and dilute with ethanol (95) to make exactly 100 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24> with this solution. Read the absorbance A of this solution at the wavelength of maximum absorption at about 241 nm.

Amount (mg) of $C_{26}H_{40}O_3$
= $(A/426) \times 100,000$

Containers and storage Containers—Tight containers.
Storage—Light-resistant, and not exceeding 30°C.

Testosterone Enanthate Injection

テストステロンエナント酸エステル注射液

Testosterone Enanthate Injection is an oily solution for injection.

It contains not less than 90% and not more than 110% of the labeled amount of testosterone enanthate ($C_{26}H_{40}O_3$: 400.59).

Method of preparation Prepare as directed under Injections, with Testosterone Enanthate.

Description Testosterone Enanthate Injection is a clear, colorless or pale yellow oily liquid.

Identification Measure a volume of Testosterone Enanthate Injection, equivalent to 0.05 g of Testosterone Enanthate according to the labeled amount, add 8 mL of petroleum ether, and extract with three 10-mL portions of diluted acetic acid (31) (7 in 10). Combine the extracts, wash with 10 mL of petroleum ether, add 0.5 mL of diluted sulfuric acid (7 in 10) to 0.1 mL of the extract, and heat on a water bath for 5 minutes. Cool, and add 0.5 mL of iron (III) chloride-acetic acid TS: the color of the solution is blue.

Extractable volume <6.05> It meets the requirement.

Assay Measure accurately a volume of Testosterone Enanthate Injection, equivalent to about 25 mg of testosterone enanthate ($C_{26}H_{40}O_3$), and dissolve in chloroform to make exactly 25 mL. Pipet 3 mL of this solution, add chloroform to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Testosterone Propionate Reference Standard, proceed in the same manner as for the sample solution, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add exactly 10 mL of isoniazid TS, add methanol to make exactly 20 mL, and allow to stand for 45 minutes. Determine the absorbances, A_T and A_S , of these solutions at 380 nm, respectively, as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution obtained by proceeding with 5 mL of chloroform as the blank.

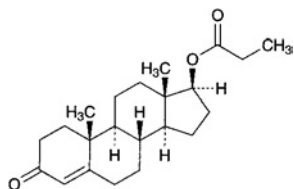
Amount (mg) of testosterone enanthate ($C_{26}H_{40}O_3$)
= $W_S \times (A_T/A_S) \times 1.1629$

W_S : Amount (mg) of Testosterone Propionate Reference Standard

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Testosterone Propionate

テストステロンプロピオン酸エステル



$C_{22}H_{32}O_3$: 344.49
3-Oxoandrost-4-en-17 β -yl propanoate [57-85-2]

Testosterone Propionate, when dried, contains not less than 97.0% and not more than 103.0% of $C_{22}H_{32}O_3$.

Description Testosterone Propionate occurs as white to pale yellow crystals or crystalline powder.

It is freely soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) Determine the absorption spectrum of a solution of Testosterone Propionate in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Testosterone Propionate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Testosterone Propionate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Testosterone Propionate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: +83 – +90° (after drying, 0.1 g, ethanol (95), 10 mL, 100 mm).

Melting point <2.60> 118 – 123°C

Purity Related substances—Dissolve 40 mg of Testosterone Propionate in 2 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of this solution, add ethanol (95) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, phosphorus (V) oxide, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (0.5 g).

Assay Weigh accurately each about 10 mg of Testosterone

Propionate and Testosterone Propionate Reference Standard, previously dried, and dissolve in methanol to make exactly 100 mL. To exactly 5 mL of these solutions add exactly 5 mL of the internal standard solution and methanol to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of testosterone propionate to that of the internal standard.

$$\begin{aligned} \text{Amount (mg) of } C_{22}H_{32}O_3 \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Testosterone Propionate Reference Standard

Internal standard solution—A solution of Progesterone in methanol (9 in 100,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 241 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of acetonitrile and water (7:3).

Flow rate: Adjust the flow rate so that the retention time of testosterone propionate is about 10 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, the internal standard and testosterone propionate are eluted in this order with the resolution between these peaks being not less than 9.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of testosterone propionate to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Testosterone Propionate Injection

テストステロンプロピオン酸エステル注射液

Testosterone Propionate Injection is an oily solution for injection.

It contains not less than 92.5% and not more than 107.5% of the labeled amount of testosterone propionate ($C_{22}H_{32}O_3$: 344.49).

Method of preparation Prepare as directed under Injections, with Testosterone Propionate.

Description Testosterone Propionate Injection is a clear, colorless or pale yellow oily liquid.

Identification Dissolve the residue obtained as directed in the procedure in the Assay in exactly 20 mL of methanol, and

use this solution as the sample solution. Separately, dissolve 1 mg of Testosterone Propionate Reference Standard in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and diethylamine (19:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the R_f values of the principal spot with the sample solution and of the spot with the standard solution are not different each other.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay (i) Chromatographic tube A glass tube about 1 cm in inside diameter and about 18 cm in length, with a glass filter (G3) at the lower end.

(ii) Chromatographic column To about 2 g of silica gel for liquid chromatography add 5 mL of dichloromethane, and mix gently. Transfer and wash into the chromatographic tube with the aid of dichloromethane, allow to elute the dichloromethane through the column, and put a filter paper on the upper end of the silica gel.

(iii) Standard solution Weigh accurately about 10 mg of Testosterone Propionate Reference Standard, previously dried at 105°C for 4 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make exactly 20 mL.

(iv) Sample stock solution To exactly a volume of Testosterone Propionate Injection, equivalent to about 20 mg of testosterone propionate ($C_{22}H_{32}O_3$), add dichloromethane to make exactly 20 mL.

(v) Procedure Transfer exactly 2 mL of the sample stock solution into the chromatographic column, and elute to the upper surface of the silica gel. Wash the inner surface of the chromatographic tube with 15 mL of dichloromethane, elute to the upper surface of the silica gel, and discard the effluent. Elute 15 mL of a mixture of dichloromethane and methanol (39:1), discard the first 5 mL of the effluent, and collect the subsequent effluent. Wash the lower part of the column with a few amount of dichloromethane, combine the washings and the effluent, and evaporate the solvent under reduced pressure. Dissolve the residue so obtained with methanol to make exactly 20 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and methanol to make exactly 20 mL, and use this solution as the sample solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed in the Assay under Testosterone Propionate.

$$\begin{aligned} & \text{Amount (mg) of testosterone propionate } (C_{22}H_{32}O_3) \\ & = W_S \times (Q_T / Q_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of Testosterone Propionate Reference Standard

Internal standard solution—A solution of Progesterone in

methanol (9 in 100,000).

Containers and storage Containers—Hermetic containers.

Freeze-dried Tetanus Antitoxin, Equine

乾燥破傷風ウマ抗毒素

Freeze-dried Tetanus Antitoxin, Equine, is a preparation for injection which is dissolved before use.

It contains tetanus antitoxin in immunoglobulin of horse origin.

It conforms to the requirements of Freeze-dried Tetanus Antitoxin, Equine, in the Minimum Requirements for Biological Products.

Description Freeze-dried Tetanus Antitoxin, Equine, becomes a clear, colorless to light yellow-brown liquid or slightly white-turbid liquid on addition of solvent.

Adsorbed Tetanus Toxoid

沈降破傷風トキソイド

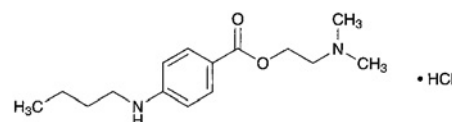
Adsorbed Tetanus Toxoid is a liquid for injection containing tetanus toxoid prepared by treating tetanus toxin with formaldehyde by a method involving no appreciable loss of the immunogenicity and rendered insoluble by the addition of aluminum salt.

It conforms to the requirements of Adsorbed Tetanus Toxoid in the Minimum Requirements for Biological Products.

Description Adsorbed Tetanus Toxoid becomes a uniform white-turbid liquid on shaking.

Tetracaine Hydrochloride

テトラカイン塩酸塩



$C_{15}H_{24}N_2O_2 \cdot HCl$: 300.82

2-(Dimethylamino)ethyl 4-(butylamino)benzoate monohydrochloride [136-47-0]

Tetracaine Hydrochloride, when dried, contains not less than 98.5% of $C_{15}H_{25}N_2O_2 \cdot HCl$.

Description Tetracaine Hydrochloride occurs as white crystals or crystalline powder. It is odorless, and has a slightly bitter taste followed by a sense of numbness on the tongue.

It is very soluble in formic acid, freely soluble in water, soluble in ethanol (95), sparingly soluble in ethanol (99.5), slightly soluble in acetic anhydride, and practically insoluble

in diethyl ether.

A solution of Tetracaine Hydrochloride (1 in 10) is neutral.
Melting point: about 148°C

Identification (1) Dissolve 0.5 g of Tetracaine Hydrochloride in 50 mL of water, add 5 mL of ammonia TS, shake, and allow to stand in a cold place. Collect the precipitate, wash with water until the washings is neutral, and dry in a desiccator (silica gel) for 24 hours: it melts <2.60> between 42°C and 44°C.

(2) Dissolve 0.1 g of Tetracaine Hydrochloride in 8 mL of water, and add 3 mL of ammonium thiocyanate TS: a crystalline precipitate is produced. Collect the precipitate, recrystallize from water, and dry at 80°C for 2 hours: it melts <2.60> between 130°C and 132°C.

(3) Determine the absorption spectrum of a solution of Tetracaine Hydrochloride in ethanol (99.5) (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tetracaine Hydrochloride (1 in 10) responds to the Qualitative Tests <1.09> for chloride.

Purity Heavy metals <1.07>—Proceed with 1.0 g of Tetracaine Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

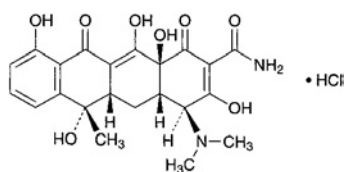
Assay Weigh accurately about 0.5 g of Tetracaine Hydrochloride, previously dried, dissolve in 2 mL of formic acid, add 80 mL of acetic anhydride, allow to stand at 30°C on a water bath for 15 minutes, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 30.08 mg of C₁₅H₂₄N₂O₂·HCl

Containers and storage Containers—Tight containers.

Tetracycline Hydrochloride

テトラサイクリン塩酸塩



C₂₂H₂₄N₂O₈·HCl: 480.90
(4*S*,4*aS*,5*aS*,6*S*,12*aS*)-4-Dimethylamino-3,6,10,12,12*a*-pentahydroxy-6-methyl-1,11-dioxo-1,4,4*a*,5,5*a*,6,11,12*a*-octahydrotetracene-2-carboxamide monohydrochloride [64-75-5]

Tetracycline Hydrochloride is the hydrochloride of a tetracycline substance having antibacterial activity pro-

duced by the growth of *Streptomyces aureofaciens*.

It contains not less than 950 μg (potency) and not more than 1010 μg (potency) per mg, calculated on the dried basis. The potency of Tetracycline Hydrochloride is expressed as mass (potency) of tetracycline hydrochloride (C₂₂H₂₄N₂O₈·HCl).

Description Tetracycline Hydrochloride occurs as a yellow to pale brownish yellow crystalline powder.

It is freely soluble in water, and sparingly soluble in ethanol (95).

Identification (1) Determine the absorption spectrum of a solution of Tetracycline Hydrochloride (1 in 62,500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tetracycline Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tetracycline Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tetracycline Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tetracycline Hydrochloride (1 in 100) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Tetracycline Hydrochloride in 100 mL of water: the pH of the solution is between 1.8 and 2.8.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tetracycline Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 1 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tetracycline Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 25 mg of Tetracycline Hydrochloride in 50 mL of 0.01 mol/L hydrochloric acid TS, and use this solution as the sample solution. Pipet 3 mL of the sample solution, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the areas of each peak by the automatic integration method: the peak areas other than tetracycline from the sample solution is not more than the peak area of tetracycline from the standard solution, and the total of the peak areas other than tetracycline from the sample solution is not more than 3 times of the peak area of tetracycline from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 7 times as long as the retention time of tetracycline beginning after the solvent peak.

System suitability—

Test for required detection: Pipet 3 mL of the standard so-

lution, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and confirm that the peak area of tetracycline obtained from 20 μ L of this solution is equivalent to 1 to 5% of that of tetracycline obtained from 20 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (1.0 g).

Assay Weigh accurately an amount of Tetracycline Hydrochloride and Tetracycline Hydrochloride Reference Standard, equivalent to about 25 mg (potency), and dissolve each in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the peak area, A_T and A_S , of tetracycline of each solution.

$$\begin{aligned} \text{Amount } [\mu\text{g (potency)}] \text{ of } \text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8\cdot\text{HCl} \\ = W_S \times (A_T/A_S) \times 1000 \end{aligned}$$

W_S : Amount [mg (potency)] of Tetracycline Hydrochloride Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with styrene-divinylbenzene copolymer for liquid chromatography (0.01 μ m in pore diameter).

Column temperature: A constant temperature of about 60°C.

Mobile phase: Dissolve 3.5 g of dipotassium hydrogenphosphate, 2.0 g of tetrabutylammonium hydrogensulfate and 0.4 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 300 mL of water, adjust to pH 9.0 with sodium hydroxide TS, add 90.0 g of *t*-butanol, and add water to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of tetracycline is about 5 minutes.

System suitability—

System performance: Dissolve 0.05 g of Tetracycline Hydrochloride Reference Standard in water to make 25 mL. Heat 5 mL of this solution on a water bath for 60 minutes, then add water to make 25 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, the retention time of 4-epitetracycline is about 3 minutes, and 4-epitetracycline and tetracycline are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tetracycline is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thallium (²⁰¹Tl) Chloride Injection

塩化タリウム (²⁰¹Tl) 注射液

Thallium (²⁰¹Tl) Chloride Injection is an aqueous solution for injection

It contains thallium-201 (²⁰¹Tl) in the form of thal-
lous chloride.

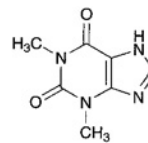
It conforms to the requirements of Thallium (²⁰¹Tl) Chloride Injection in the Minimum Requirements for Radiopharmaceuticals.

Test for Extractable Volume of Parenteral Preparations and Insoluble Particulate Matter Test for Injections are not applied to this injection.

Description Thallium (²⁰¹Tl) Chloride Injection is a clear, colorless liquid.

Theophylline

テオフィリン



$\text{C}_7\text{H}_8\text{N}_4\text{O}_2$: 180.16

1,3-Dimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione [58-55-9]

Theophylline, when dried, contains not less than 99.0% of $\text{C}_7\text{H}_8\text{N}_4\text{O}_2$.

Description Theophylline occurs as white crystals or crystalline powder.

It is soluble in *N,N*-dimethylformamide, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the absorption spectrum of a solution of Theophylline in 0.1 mol/L hydrochloric acid TS (1 in 200,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Theophylline, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 271 – 275°C

Purity (1) Acidity—To 0.5 g of Theophylline add 75 mL of water, 2.0 mL of 0.01 mol/L sodium hydroxide VS and 1 drop of methyl red TS: a yellow color develops.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Theophylline according to Method 4, and perform the test. Pre-

pare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Theophylline according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.10 g of Theophylline in 3 mL of *N,N*-dimethylformamide, add 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of acetone, chloroform, methanol, 1-butanol and ammonia solution (28) (3:3:2:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Theophylline, previously dried, and dissolve in 100 mL of water, add exactly 20 mL of 0.1 mol/L silver nitrate VS, shake the mixture, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 18.02 mg of $C_7H_8N_4O_2$

Containers and storage Containers—Well-closed containers.

Thiamazole

チアマゾール



$C_4H_6N_2S$: 114.17

1-Methyl-1*H*-imidazole-2-thiol [60-56-0]

Thiamazole, when dried, contains not less than 98.0% of $C_4H_6N_2S$.

Description Thiamazole occurs as white to pale yellowish white crystals or crystalline powder. It has a faint, characteristic odor, and has a bitter taste.

It is freely soluble in water and in ethanol (95), and slightly soluble in diethyl ether.

The pH of the solution (1 in 50) is between 5.0 and 7.0.

Identification (1) Dissolve 5 mg of Thiamazole in 1 mL of water, shake with 1 mL of sodium hydroxide TS, and add 3 drops of sodium pentacyanonitrosylferrate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it

changes to blue.

(2) To 2 mL of a solution of Thiamazole (1 in 200) add 1 mL of sodium carbonate TS and 1 mL of diluted Folin's TS (1 in 5): a deep blue color develops.

Melting point <2.60> 144 – 147°C

Purity (1) Selenium—Proceed with 0.10 g of Thiamazole as directed under Oxygen Flask Combustion Method <1.06>, using 25 mL of diluted nitric acid (1 in 30) as the absorbing liquid, and prepare the test solution. Apply a small amount of water to the upper part of apparatus A, pull out C carefully, and transfer the test solution to a beaker. Wash C, B and the inner side of A with 25 mL of water, and combine the washings with the test solution. Boil gently for 10 minutes, cool to room temperature, add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh exactly 40 mg of selenium, dissolve in 100 mL of diluted nitric acid (1 in 2), heat to dissolve on a water bath if necessary, and add water to make exactly 1000 mL. Pipet 5 mL of this solution, and add water to make exactly 200 mL. To 2 mL of this solution, exactly measured, add diluted nitric acid (1 in 60) to make exactly 50 mL, and use this solution as the standard solution. Pipet 40 mL each of the sample solution and standard solution into separate beakers, and adjust each solution with ammonia solution (28) to a pH of 1.8 to 2.2. To each solution add 0.2 g of hydroxylammonium chloride, shake gently to dissolve, then add 5 mL of 2,3-diaminonaphthalene TS, shake, and allow to stand for 100 minutes. Transfer these solutions to corresponding separators, rinse the beakers with 10 mL of water, combine the rinsings in the respective separators, shake well with 5.0 mL of cyclohexane for 2 minutes, and extract. Centrifuge the cyclohexane extracts to remove any water remaining in these solutions. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution prepared with 40 mL of diluted nitric acid (1 in 60) in the same manner as the blank. The absorbance of the sample solution at the wavelength of maximum absorbance at about 378 nm does not exceed the absorbance of the standard solution.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Thiamazole according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Thiamazole according to Method 1, and perform the test (not more than 2 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.25 g of Thiamazole, previously dried, dissolve in 75 mL of water, add 15 mL of 0.1 mol/L sodium hydroxide VS from a burette, and add 30 mL of 0.1 mol/L silver nitrate VS with stirring. Add 1 mL of bromothymol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS, until a persistent blue-green color is produced. Determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.42 mg of $C_4H_6N_2S$

Containers and storage Containers—Well-closed contain-

ers.

Storage—Light-resistant.

Thiamazole Tablets

チアマゾール錠

Thiamazole Tablets contain not less than 94% and not more than 106% of the labeled amount of thiamazole ($C_4H_6N_2S$: 114.17).

Method of preparation Prepare as directed under Tablets, with Thiamazole.

Identification (1) To a quantity of powdered Thiamazole Tablets, equivalent to 0.05 g of Thiamazole according to the labeled amount, add 20 mL of hot ethanol (95), shake for 15 minutes, filter, and evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water, filter if necessary, and use this solution as the sample solution. To 1 mL of the sample solution add 1 mL of sodium hydroxide TS, shake, and add 3 drops of sodium pentacyanonitrosylferate (III) TS: a yellow color develops, and it gradually changes to yellow-green to green. To this solution add 1 mL of acetic acid (31): it changes to blue.

(2) With 2 mL of the sample solution obtained in (1), proceed as directed in the Identification (2) under Thiamazole.

Assay Weigh accurately and powder not less than 20 Thiamazole Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.15 g of thiamazole ($C_4H_6N_2S$), add 80 mL of water, shake for 15 minutes, add water to make exactly 100 mL, and centrifuge. Filter, discard the first 20 mL of the filtrate, pipet 50 mL of the subsequent filtrate, add 1 mL of bromothymol blue TS, and if a blue color develops, neutralize with 0.1 mol/L hydrochloric acid VS until the color of the solution changes to green. To this solution add 4.5 mL of 0.1 mol/L sodium hydroxide VS from a burette, add 15 mL of 0.1 mol/L silver nitrate VS while stirring, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS. Continue the titration until a persistent blue-green color is produced, and determine the total volume of 0.1 mol/L sodium hydroxide VS consumed.

Each mL of 0.1 mol/L sodium hydroxide VS
= 11.42 mg of $C_4H_6N_2S$

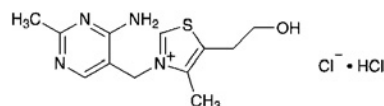
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Thiamine Chloride Hydrochloride

Vitamin B₁ Hydrochloride

チアミン塩化物塩酸塩



$C_{12}H_{17}ClN_4OS \cdot HCl$: 337.27

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium chloride monohydrochloride [67-03-8]

Thiamine Chloride Hydrochloride contains not less than 98.5% of $C_{12}H_{17}ClN_4OS \cdot HCl$, calculated on the anhydrous basis.

Description Thiamine Chloride Hydrochloride occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor.

It is freely soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 245°C (with decomposition).

Identification (1) To 5 mL of a solution of Thiamine Chloride Hydrochloride (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(2) Determine the absorption spectrum of a solution of Thiamine Chloride Hydrochloride (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Thiamine Chloride Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Thiamine Chloride Hydrochloride, previously dried at 105°C for 2 hours, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum, or the spectrum of Thiamine Chloride Hydrochloride Reference Standard previously dried at 105°C for 2 hours: both spectra exhibit similar intensities of absorption at the same wave numbers. In case when some differences are found between the spectra, repeat the test with residues obtained by dissolving these substances in water, evaporating to dryness, and drying at 105°C for 2 hours.

(4) A solution of Thiamine Chloride Hydrochloride (1 in 500) responds to the Qualitative Tests <1.09> for chloride.

pH <2.54> Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 100 mL of water: the pH of this solution is between 2.7 and 3.4.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Thiamine Chloride Hydrochloride in 10 mL of water: the solution is clear, and has no more color than the following control solution.

Control solution: To 1.5 mL of 1/60 mol/L potassium dichromate VS add water to make 1000 mL.

(2) Sulfate <1.14>—Weigh 1.5 g of Thiamine Chloride Hydrochloride, and perform the test. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.011%).

(3) Nitrate—Dissolve 0.5 g of Thiamine Chloride Hydrochloride in 25 mL of water. Add 2 mL of sulfuric acid to 2 mL of this solution, shake, cool, and superimpose iron (II) sulfate TS: no dark brown ring is produced at the junction of the two layers.

(4) Heavy metals <1.07>—Proceed with 1.0 g of Thiamine Chloride Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Related substances—Dissolve 0.10 g of Thiamine Chloride Hydrochloride in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution, as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the area of each peak by the automatic integration method: the total area of the peaks other than thiamine is not larger than the peak area of thiamine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of thiamine.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 50 mL. Confirm that the peak area of thiamine obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of thiamine obtained from 10 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiamine is not more than 1.0%.

Water <2.48> Not more than 5.0% (30 mg, coulometric titration).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Chloride Hydrochloride and Thiamine Chloride Hydrochloride Reference Standard (separately determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution

as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of thiamine to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of } C_{12}H_{17}ClN_4OS.HCl \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Thiamine Chloride Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution methyl benzoate in methanol (1 in 50).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.1 g of sodium 1-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamine Chloride Hydrochloride Injection

Vitamin B₁ Hydrochloride Injection

チアミン塩化物塩酸塩注射液

Thiamine Chloride Hydrochloride Injection is an aqueous solution for injection.

It contains not less than 95% and not more than 115% of the labeled amount of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$: 337.27).

Method of preparation Prepare as directed under Injections, with Thiamine Chloride Hydrochloride.

Description Thiamine Chloride Hydrochloride Injection is a clear, colorless liquid.

pH: 2.5 – 4.5

Identification To a volume of Thiamine Chloride

Hydrochloride Injection, equivalent to 0.05 g of Thiamine Chloride Hydrochloride according to the labeled amount, add water to make 25 mL. Proceed with 5 mL of this solution as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

Extractable volume <6.05> It meets the requirement.

Assay Dilute with 0.001 mol/L hydrochloric acid TS if necessary, then measure exactly a volume of Thiamine Chloride Hydrochloride Injection, equivalent to about 20 mg of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$), and add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride Reference Standard (separately determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 20 mL of methanol and 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add 0.001 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

$$\begin{aligned} & \text{Amount (mg) of thiamine chloride hydrochloride} \\ & (C_{12}H_{17}ClN_4OS.HCl) \\ & = W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Thiamine Chloride Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thiamine Chloride Hydrochloride Powder

Vitamin B₁ Hydrochloride Powder

チアミン塩化物塩酸塩散

Thiamine Chloride Hydrochloride Powder contains not less than 95% and not more than 115% of the labeled amount of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$; 337.27).

Method of preparation Prepare as directed under Powders, with Thiamine Chloride Hydrochloride.

Identification To a portion of Thiamine Chloride Hydrochloride Powder, equivalent to 0.02 g of Thiamine Chloride Hydrochloride according to the labeled amount, add 50 mL of water and 10 mL of dilute acetic acid, shake, and filter. Proceed with 5 mL of the filtrate as directed in the Identification (1) under Thiamine Chloride Hydrochloride.

Purity Rancidity—Thiamine Chloride Hydrochloride Powder has no unpleasant or rancid odor. It is tasteless.

Assay Weigh accurately a quantity of Thiamine Chloride Hydrochloride Powder, equivalent to about 20 mg of thiamine chloride hydrochloride ($C_{12}H_{17}ClN_4OS.HCl$), add 60 mL of 0.01 mol/L hydrochloric acid TS, and heat on a water bath for 30 minutes. Shake vigorously for 10 minutes, cool, add methanol to make exactly 100 mL, and centrifuge. Pipet 25 mL of the supernatant, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of Thiamine Chloride Hydrochloride Reference Standard (separately determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), and dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 50 mL. To 10 mL of this solution, exactly measured, add 50 mL of 0.01 mol/L hydrochloric acid TS, and add methanol to make exactly 100 mL. To 25 mL of this solution, exactly measured, add exactly 5 mL of the internal standard solution, add water to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Thiamine Chloride Hydrochloride.

$$\begin{aligned} & \text{Amount (mg) of thiamine chloride hydrochloride} \\ & (C_{12}H_{17}ClN_4OS.HCl) \\ & = W_S \times (Q_T/Q_S) \times (1/5) \end{aligned}$$

W_S : Amount (mg) of Thiamine Chloride Hydrochloride Reference Standard, calculated on the anhydrous basis

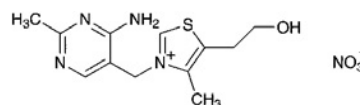
Internal standard solution—A solution of methyl benzoate in methanol (1 in 200).

Containers and storage Containers—Tight containers. Storage—Light-resistant.

Thiamine Nitrate

Vitamin B₁ Nitrate

チアミン硝化物



$C_{12}H_{17}N_5O_4S$: 327.36

3-(4-Amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium nitrate [532-43-4]

Thiamine Nitrate, when dried, contains not less than 98.0% and not more than 102.0% of $C_{12}H_{17}N_5O_4S$.

Description Thiamine Nitrate occurs as white crystals or crystalline powder. It is odorless or a slight, characteristic odor.

It is sparingly soluble in water, and very slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

Melting point: about 193°C (with decomposition).

Identification (1) Take 2-mL portions of a solution of Thiamine Nitrate (1 in 500), and add 2 to 3 drops of iodine

TS: a red-brown precipitate or turbidity is produced. Upon further addition of 1 mL of 2,4,6-trinitrophenol TS, a yellow precipitate or turbidity is produced.

(2) To 1 mL of a solution of Thiamine Nitrate (1 in 500) add 1 mL of lead (II) acetate TS and 1 mL of a solution of sodium hydroxide (1 in 10), and warm: the color of the solution changes through yellow to brown, and on standing, a black-brown precipitate is produced.

(3) To 5 mL of a solution of Thiamine Nitrate (1 in 500) add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and examine under ultraviolet light (main wavelength: 365 nm): the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline.

(4) A solution of Thiamine Nitrate (1 in 50) responds to the Qualitative Tests <1.09> (1) and (2) for nitrate.

pH <2.54> Dissolve 1.0 g of Thiamine Nitrate in 100 mL of water: the pH of this solution is between 6.5 and 8.0.

Purity (1) Chloride <1.03>—Perform the test with 0.20 g of Thiamine Nitrate. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.053%).

(2) Sulfate <1.14>—Dissolve 1.5 g of Thiamine Nitrate in 30 mL of water and 2 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS and 2 mL of dilute hydrochloric acid, and add water to make 50 mL (not more than 0.011%).

(3) Heavy metals <1.07>—Dissolve 1.0 g of Thiamine Nitrate in 30 mL of water by warming, cool, and add 12 mL of 6 mol/L acetic acid TS and water to make 50 mL. Perform the test with this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Loss on drying <2.41> Not more than 1.0% (0.5 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 0.1 g each of Thiamine Nitrate, previously dried, and Thiamine Chloride Hydrochloride Reference Standard (separately determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), and dissolve them in the mobile phase to make exactly 50 mL. To 10 mL each of the solutions, accurately measured, add exactly 5 mL each of the internal standard solution, add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions and calculate the ratios, Q_T and Q_S , of the peak area of thiamine to that of the internal standard.

Amount (mg) of $C_{12}H_{17}N_2NaO_2S = W_S \times (Q_T/Q_S) \times 0.9706$

W_S : Amount (mg) of Thiamine Chloride Hydrochloride Reference Standard, calculated on the anhydrous basis

Internal standard solution—A solution of methyl benzoate in methanol (1 in 50).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: Dissolve 1.1 g of sodium l-octanesulfonate in 1000 mL of diluted acetic acid (100) (1 in 100). To 600 mL of this solution add 400 mL of a mixture of methanol and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of thiamine is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, thiamine and the internal standard are eluted in this order with the resolution between these peaks being not less than 6.

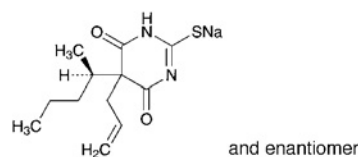
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of thiamine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamylal Sodium

チアミラルナトリウム



$C_{12}H_{17}N_2NaO_2S$: 276.33

Monosodium 5-allyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [337-47-3]

Thiamylal Sodium contains not less than 97.5% and not more than 101.0% of $C_{12}H_{17}N_2NaO_2S$, calculated on the dried basis.

Description Thiamylal Sodium occurs as light yellow crystals or powder.

It is very soluble in water, and freely soluble in ethanol (95).

The pH of a solution of Thiamylal Sodium (1 in 10) is between 10.0 and 11.0.

It is hygroscopic.

It is gradually decomposed by light.

Its solution in ethanol (95) (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Thiamylal Sodium in ethanol (95) (7 in 1,000,000)

as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Thiamylal Sodium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Thiamylal Sodium (1 in 10) responds to Qualitative Tests <1.09> for sodium salt.

Purity (1) Clarity and color of solution—To 1.0 g of Thiamylal Sodium in a 11- to 13-mL glass-stoppered test tube add 10 mL of freshly boiled and cooled water, stopper tightly, allow to stand, and dissolve by occasional gentle shaking: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Thiamylal Sodium according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Thiamylal Sodium in 10 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL and 3 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use these solutions as the standard solution (1) and the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography, develop with a mixture of toluene, methanol and ethyl acetate (40:7:3) to a distance of about 12 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for a night: the spot appeared around *R_f* 0.1 obtained with the sample solution is not more intense than the spot with the standard solution (2), and the spot other than the principal spot, the spot at origin and the spot mentioned above obtained with the sample solution is not more intense than the spot with the standard solution (1).

Loss on drying <2.41> Not more than 2.0% (1 g, 105°C, 1 hour).

Assay Weigh accurately about 0.25 g of Thiamylal Sodium, dissolve in 50 mL of methanol and 5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 10 mL of this solution, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Separately, weigh accurately about 23 mg of Thiamylal Reference Standard, previously dried at 105°C for 1 hour, dissolve in 50 mL of methanol and 0.5 mL of dilute hydrochloric acid, and add methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of thiamylal to that of the internal standard.

Amount (mg) of $C_{12}H_{17}N_2NaO_2S$

$$= W_s \times (Q_T/Q_S) \times 10 \times 1.0864$$

W_s : Amount (mg) of Thiamylal Reference Standard

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and 0.05 mol/L acetic acid-sodium acetate buffer solution, pH 4.6 (13:7).

Flow rate: Adjust the flow rate so that the retention time of thiamylal is about 6 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, thiamylal and the internal standard are eluted in this order with the resolution between these peaks being not less than 12.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of thiamylal to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiamylal Sodium for Injection

注射用チアミラルナトリウム

Thiamylal Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93.0% and not more than 107.0% of the labeled amount of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$; 276.33).

Method of preparation Prepare as directed under Injections, with 100 parts of Thiamylal Sodium and 7 parts of Dried Sodium Carbonate in mass.

Description Thiamylal Sodium for Injection occurs as light yellow crystals, powder or masses.

It is hygroscopic.

It is gradually decomposed by light.

Identification (1) To 1.0 g of Thiamylal Sodium for Injection add 20 mL of ethanol (95), shake vigorously, and filter. Dissolve the precipitate so obtained in 1 mL of water, and add 1 mL of barium chloride TS: a white precipitate is produced. Centrifuge this solution, take off the supernatant liquid, and to the precipitate add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) To 50 mg of Thiamylal Sodium for Injection add 100 mL of ethanol (95), shake vigorously, and filter. To 3 mL of the filtrate add ethanol (95) to make 200 mL. Determine the absorption spectrum of this solution as directed under

Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 236 nm and 240 nm, and between 287 nm and 291 nm.

pH <2.54> The pH of a solution obtained by dissolving 1.0 g of Thiamylal Sodium for Injection in 40 mL of water is between 10.5 and 11.5.

Purity Related substances—To 0.10 g of Thiamylal Sodium for Injection add 10 mL of ethanol (95), shake vigorously, filter, and use the filtrate as the sample solution. Proceed as directed in the Purity (3) under Thiamylal Sodium.

Bacterial endotoxins <4.01> Less than 1.0 EU/mg.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Open carefully 10 containers of Thiamylal Sodium for Injection, dissolve the contents with water, wash out the inside of each container with water, combine them, and add water to make exactly V mL so that each mL contains about 5 mg of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$). Pipet 5 mL of this solution, and add 0.5 mL of dilute hydrochloric acid and methanol to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution and the mobile phase to make 200 mL, and use this solution as the sample solution. Proceed the test with the sample solution as directed in the Assay under Thiamylal Sodium.

Amount (mg) of thiamylal sodium ($C_{12}H_{17}N_2NaO_2S$) in 1 container

$$= W_S \times (Q_T/Q_S) \times (V/50) \times 1.0864$$

W_S : Amount (mg) of Thiamylal Reference Standard

Internal standard solution—A solution of phenyl benzoate in methanol (3 in 500).

Containers and storage Containers—Hermetic containers. Storage—Light-resistant.

Thianthol

チアントール

Thianthol consists of dimethylthianthrene and ditoluene disulfide.

It contains not less than 23.5% and not more than 26.5% of sulfur (S: 32.07).

Description Thianthol is a yellowish, viscous liquid. It has a faint, agreeable odor.

It is freely soluble in diethyl ether, slightly soluble in ethanol (95), and practically insoluble in water.

It, when cold, may separate crystals, which melt on warming.

Specific gravity d_{20}^{20} : 1.19 – 1.23

Identification To 0.1 g of Thianthol add cautiously 5 mL of sulfuric acid: a blue-purple color develops. Add 5 to 6 drops of nitric acid to the solution: the color of the solution changes to yellow-red with evolution of gas.

Purity (1) Acidity or alkalinity—Shake 10 g of Thianthol with 20 mL of water, allow to stand, and separate the water layer. The solution is neutral.

(2) Sulfate—To 10 mL of the water layer obtained in (1) add 2 to 3 drops of barium chloride TS: no opalescence is produced.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 10 mg of Thianthol, and proceed as directed in the sulfur determination of Oxygen Flask Combustion Method <1.06>, using a mixture of 5 mL of diluted sodium hydroxide TS (1 in 10) and 1.0 mL of hydrogen peroxide TS as an absorbing liquid.

Containers and storage Containers—Tight containers.

Compound Thianthol and Salicylic Acid Solution

複方チアントール・サリチル酸液

Compound Thianthol and Salicylic Acid Solution contains not less than 1.8 w/v% and not more than 2.2 w/v% of salicylic acid ($C_7H_6O_3$: 138.12), and not less than 1.8 w/v% and not more than 2.2 w/v% of phenol (C_6H_6O : 94.11).

Method of preparation

Thianthol	200 mL
Salicylic Acid	20 g
Phenol	20 g
Olive Oil	50 mL
Ether	100 mL
Petroleum Benzin	a sufficient quantity
To make 1000 mL	

Dissolve Salicylic Acid and Phenol in Ether, add Thianthol, Olive Oil and Petroleum Benzin to this solution, mix and dissolve to make 1000 mL.

Description Compound Thianthol and Salicylic Acid Solution is a light yellow liquid, having a characteristic odor.

Identification (1) Place 1 mL of Compound Thianthol and Salicylic Acid Solution to a porcelain dish, and evaporate on a water bath to dryness. To the residue add cautiously 5 mL of sulfuric acid: the color of the solution changes to yellow-red with evolution of gas (thianthol).

(2) Shake 10 mL of Compound Thianthol and Salicylic Acid Solution with 10 mL of sodium hydrogen carbonate TS, and separate the water layer. To 0.5 mL of the water layer add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 50 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(3) Wash the upper phase obtained in (2) with 10 mL of sodium hydrogen carbonate TS, and extract with 10 mL of

dilute sodium hydroxide TS. Shake 1 mL of the extract with 1 mL of sodium nitrate TS and 1 mL of dilute hydrochloric acid, and add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(4) To 1 mL of Compound Thianthol and Salicylic Acid Solution add 10 mL of ethanol (95), mix, and use this solution as the sample solution. Dissolve 0.01 g each of salicylic acid, phenol and thianthol in 5 mL each of ethanol (95), and use each solution as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): three spots obtained from the sample solution and the corresponding spots of standard solutions (1), (2) and (3) show the same *R_f* value. Spray evenly iron (III) chloride TS on the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Assay Measure exactly 2 mL of Compound Thianthol and Salicylic Acid Solution, add exactly 10 mL of the internal standard solution, then add 70 mL of diluted methanol (1 in 2), mix well, and add diluted methanol (1 in 2) to make 100 mL. Filter, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Weigh accurately about 0.2 g of salicylic acid for assay, previously dried in a desiccator (silica gel) for 3 hours, and about 0.2 g of phenol for assay, dissolve in diluted methanol (1 in 2) to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution and diluted methanol (1 in 2) to make 100 mL, and use this solution as the standard solution. With 5 μ L each of the sample solution and standard solution, perform the test as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_{Ta} and Q_{Tb} , of the peak area of salicylic acid and phenol to that of the internal standard in the sample solution, and the ratios, Q_{Sa} and Q_{Sb} , of the peak area of salicylic acid and phenol to that of the internal standard in the standard solution.

$$\begin{aligned} \text{Amount (mg) of salicylic acid (C}_7\text{H}_6\text{O}_3) \\ = W_{Sa} \times (Q_{Ta}/Q_{Sa}) \times (1/5) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of phenol (C}_6\text{H}_6\text{O)} \\ = W_{Sb} \times (Q_{Tb}/Q_{Sb}) \times (1/5) \end{aligned}$$

W_{Sa} : Amount (mg) of salicylic acid for assay

W_{Sb} : Amount (mg) of phenol for assay

Internal standard solution—A solution of theophylline in methanol (1 in 1000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 270 nm).

Column: A stainless steel column about 4 mm in inside diameter and 25 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of 0.1 mol/L phosphate buffer solution, pH 7.0, and methanol (3:1).

Flow rate: Adjust the flow rate so that the retention time of salicylic acid is about 6 minutes.

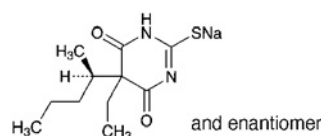
Selection of column: Dissolve 0.2 g of benzoic acid, 0.2 g of salicylic acid and 0.05 g of theophylline in 100 mL of diluted methanol (1 in 2). To 10 mL of this solution add 90 mL of diluted methanol (1 in 2). Proceed with 10 μ L of this solution under the above operating conditions. Use a column giving elution of benzoic acid, salicylic acid and theophylline in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding 25°C.

Thiopental Sodium

チオペンタールナトリウム



$C_{11}H_{17}N_2NaO_2S$: 264.32

Monosodium 5-ethyl-5-[(1*RS*)-1-methylbutyl]-4,6-dioxo-1,4,5,6-tetrahydropyrimidine-2-thiolate [71-73-8]

Thiopental Sodium, when dried, contains not less than 97.0% of $C_{11}H_{17}N_2NaO_2S$.

Description Thiopental Sodium occurs as a light yellow powder. It has a faint, characteristic odor.

It is very soluble in water, freely soluble in ethanol (95), and practically insoluble in diethyl ether.

A solution of Thiopental Sodium (1 in 10) is alkaline.

It is hygroscopic.

Its solution gradually decomposes on standing.

Identification (1) Dissolve 0.2 g of Thiopental Sodium in 5 mL of sodium hydroxide TS, and add 2 mL of lead (II) acetate TS: a white precipitate, which dissolves upon heating, is produced. Boil the solution thus obtained: a black precipitate forms gradually, and the precipitate responds to the Qualitative Tests <1.09> for sulfide.

(2) Dissolve 0.5 g of Thiopental Sodium in 15 mL of water, add 10 mL of dilute hydrochloric acid to produce white precipitate, and extract with four 25-mL portions of chloroform. Combine the chloroform extracts, evaporate on a water bath, and dry at 105°C for 2 hours: the residue melts <2.60> between 157°C and 162°C.

(3) A solution of Thiopental Sodium (1 in 10) responds to the Qualitative Tests <1.09> (1) and (2) for sodium salt.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Thiopental Sodium in 10 mL of freshly boiled and cooled water: the solution is clear and light yellow.

(2) Heavy metals <1.07>—Dissolve 2.0 g of Thiopental Sodium in 76 mL of water, add 4 mL of dilute hydrochloric acid, shake, and filter through a glass filter (G4). To 40 mL of the filtrate add 2 mL of ammonium acetate TS, dilute with water to 50 mL, and perform the test using this solution as the test solution. Prepare a control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid, 2 mL of ammonium acetate TS and water to make 50 mL

(not more than 20 ppm).

(3) Neutral and basic substances—Weigh accurately about 1 g of Thiopental Sodium, dissolve in 10 mL of water and 5 mL of sodium hydroxide TS, and shake vigorously with 40 mL of chloroform. Separate the chloroform layer, wash with two 5-mL portions of water, filter, and evaporate the filtrate on a water bath to dryness. Dry the residue at 105°C for 1 hour: the amount of the residue is not more than 0.50%.

(4) Related substances—Dissolve 50 mg of Thiopental Sodium in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Measure each peak area of each solution by the automatic integration method: the total area of peaks other than those of thiopental in the sample solution is not larger than the peak area of thiopental in the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1 g of potassium dihydrogen phosphate in 1000 mL of water, and adjust the pH to 3.0 with phosphoric acid. To 700 mL of this solution add 300 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of thiopental is about 15 minutes.

Time span of measurement: About 1.5 times as long as the retention time of thiopental.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 10 mL. Confirm that the peak area of thiopental obtained from 20 μ L of this solution is equivalent to 15 to 25% of that of thiopental obtained from 20 μ L of the standard solution.

System performance: Dissolve 5 mg each of isopropyl parahydroxybenzoate and propyl parahydroxybenzoate in 50 mL of acetonitrile, and add water to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, isopropyl parahydroxybenzoate and propyl parahydroxybenzoate are eluted in this order with the resolution between these peaks being not less than 1.9.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of thiopental is not more than 2.0%.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Assay Weigh accurately about 0.5 g of Thiopental Sodium, previously dried, transfer to a separator, dissolve in 20 mL of water, add 5 mL of ethanol (95) and 10 mL of dilute hydrochloric acid, and extract with 50 mL of chloroform, then with three 25-mL portions of chloroform. Combine the chloroform extracts, wash with two 5-mL portions of water,

and extract the washings with two 10-mL portions of chloroform. Filter the combined chloroform extracts into a conical flask, and wash the filter paper with three 5-mL portions of chloroform. Combine the filtrate and the washings, and add 10 mL of ethanol (95). Titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS until the color of the solution changes from yellow through light blue to purple (indicator: 2 mL of alizarin yellow GG-thymolphthalein TS). Perform a blank determination with a mixture of 160 mL of chloroform and 30 mL of ethanol (95), and make any necessary correction.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS = 26.43 mg of $C_{11}H_{17}N_2NaO_2S$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Thiopental Sodium for Injection

注射用チオペンタールナトリウム

Thiopental Sodium for Injection is a preparation for injection which is dissolved before use.

It contains not less than 93% and not more than 107% of the labeled amount of thiopental sodium ($C_{11}H_{17}N_2NaO_2S$: 264.32).

Method of preparation Prepare as directed under Injections, with 100 parts of Thiopental Sodium and 6 parts of Dried Sodium Carbonate in mass.

Description Thiopental Sodium for Injection is a light yellow powder or mass, and has a slight, characteristic odor.

It is very soluble in water, and practically insoluble in dehydrated diethyl ether.

It is hygroscopic.

Identification (1) Dissolve 0.1 g of Thiopental Sodium for Injection in 10 mL of water, and add 0.5 mL of barium chloride TS: a white precipitate is formed. Collect the precipitate, and add dilute hydrochloric acid dropwise: the precipitate dissolves with effervescence.

(2) Proceed as directed in the Identification under Thiopental Sodium.

pH <2.54> Dissolve 1 g of Thiopental Sodium for Injection in 40 mL of water: the pH of this solution is between 10.2 and 11.2.

Purity Proceed as directed in the Purity under Thiopental Sodium.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, 80°C, 4 hours).

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take 10 samples of Thiopental Sodium for Injection, and open each container carefully. Dissolve each content with water, wash each container with water, combine the washings with the former solution, and add water to make exactly 1000 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Measure exactly a volume (V mL) of this solution, equivalent to about 15 mg of thiopental sodi-

um ($C_{11}H_{17}N_2NaO_2S$), and add water to make exactly 1000 mL. Pipet 10 mL of this solution, add 15 mL of diluted dilute sodium hydroxide TS (1 in 100), add water to make exactly 30 mL, and use this solution as the sample solution. Separately, weigh accurately about 46 mg of thiopental for assay, previously dried at 105°C for 3 hours, dissolve in 50 mL of dilute sodium hydroxide TS, and add water to make exactly 200 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and determine the absorbances, A_T and A_S , at 304 nm.

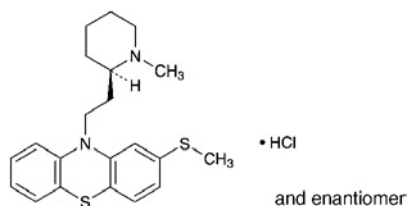
$$\begin{aligned} &\text{Amount (mg) of thiopental sodium (C}_{11}\text{H}_{17}\text{N}_2\text{NaO}_2\text{S)} \\ &\text{in each sample of Thiopental Sodium for Injection} \\ &= W_S \times (A_T/A_S) \times (300/V) \times 1.0907 \end{aligned}$$

W_S : Amount (mg) of thiopental sodium for assay

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant.

Thioridazine Hydrochloride

チオリダジン塩酸塩



$C_{21}H_{26}N_2S_2 \cdot HCl$: 407.04
10- {2-[(2RS)-1-Methylpiperidin-2-yl]ethyl} -2-methylsulfonyl-10H-phenothiazine monohydrochloride
[130-61-0]

Thioridazine Hydrochloride, when dried, contains not less than 99.0% of $C_{21}H_{26}N_2S_2 \cdot HCl$.

Description Thioridazine Hydrochloride occurs as a white to pale yellow, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in water, in methanol, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Thioridazine Hydrochloride (1 in 100) is between 4.2 and 5.2.

It is gradually colored by light.

Identification (1) Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of sulfuric acid: a deep blue color develops.

(2) Dissolve 0.01 g of Thioridazine Hydrochloride in 2 mL of water, and add 1 drop of cerium (IV) tetraammonium sulfate TS: a blue color develops, and the color disappears on the addition of excess of the reagent.

(3) Determine the infrared absorption spectrum of Thioridazine Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the

Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) To 5 mL of a solution of Thioridazine Hydrochloride (1 in 100) add 2 mL of ammonia TS, and heat on a water bath for 5 minutes. After cooling, filter, and acidify the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride.

Melting point <2.60> 159 – 164°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Thioridazine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Thioridazine Hydrochloride, according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Conduct this procedure under the protection from the sunlight. Dissolve 0.10 g of Thioridazine Hydrochloride in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, 2-propanol and ammonia solution (28) (74:25:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.35 g of Thioridazine Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} &\text{Each mL of 0.1 mol/L perchloric acid VS} \\ &= 40.70 \text{ mg of } C_{21}H_{26}N_2S_2 \cdot HCl \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Thiotepa

チオテパ



$C_6H_{12}N_3PS$: 189.22
Tris(aziridin-1-yl)phosphine sulfide [52-24-4]

Thiotepa, when dried, contains not less than 98.0%

of $C_6H_{12}N_3PS$.

Description Thiotepa occurs as colorless or white crystals, or white, crystalline powder. It is odorless.

It is freely soluble in water, in ethanol (95) and in diethyl ether.

A solution of Thiotepa (1 in 10) is neutral.

Identification (1) To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of hexaammonium heptamolybdate TS, and allow to stand: a dark blue color develops slowly when the solution is cold, or quickly when warm.

(2) To 5 mL of a solution of Thiotepa (1 in 100) add 1 mL of nitric acid: this solution responds to the Qualitative Tests <1.09> (2) for phosphate.

(3) Dissolve 0.1 g of Thiotepa in a mixture of 1 mL of lead (II) acetate TS and 10 mL of sodium hydroxide TS, and boil: the gas evolved changes moistened red litmus paper to blue, and the solution shows a grayish red color.

Melting point <2.60> 52 – 57°C

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Thiotepa in 20 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Thiotepa in a platinum crucible according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Dissolve 0.20 g of Thiotepa in 5 mL of water, and add 1 mL of nitric acid and 1 mL of sulfuric acid. Take this solution, prepare the test solution according to Method 2, and perform the test (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.20% (1 g, in vacuum, silica gel, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g, platinum crucible).

Assay Weigh accurately about 0.1 g of Thiotepa, previously dried, dissolve in 50 mL of a solution of potassium thiocyanate (3 in 20), add 25 mL of 0.05 mol/L sulfuric acid VS, exactly measured, and allow to stand for 20 minutes with occasional shaking. Titrate <2.50> the excess sulfuric acid with 0.1 mol/L sodium hydroxide VS until the color of the solution changes from red to light yellow (indicator: 3 drops of methyl red TS). Perform a blank determination.

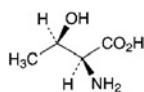
$$\begin{aligned} \text{Each mL of 0.05 mol/L sulfuric acid VS} \\ = 6.307 \text{ mg of } C_6H_{12}N_3PS \end{aligned}$$

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

L-Threonine

L-トレオニン



$C_4H_9NO_3$; 119.12

(2*S*,3*R*)-2-Amino-3-hydroxybutanoic acid [72-19-5]

L-Threonine, when dried, contains not less than

98.5% of $C_4H_9NO_3$.

Description L-Threonine occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor, and has a slightly sweet taste.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

Identification Determine the infrared absorption spectrum of L-Threonine, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: –26.0 – –29.0° (after drying, 1.5 g, water, 25 mL, 100 mm).

pH <2.54> Dissolve 0.20 g of L-Threonine in 20 mL of water: the pH of this solution is between 5.2 and 6.2.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of L-Threonine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Threonine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Threonine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Threonine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Threonine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of L-Threonine in 5 mL of dilute hydrochloric acid, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Threonine in 50 mL of water, and use this solution as the sample solution. Pipet 1 mL of this solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly the plate with a solution of ninhydrin in acetone (1 in 50), and heat the plate at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.20% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of L-Threonine, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS

= 11.91 mg of $C_4H_9NO_3$

Containers and storage Containers—Tight containers.

Thrombin

トロンビン

Thrombin is prepared from prothrombin obtained from blood of man or bull, through interaction with added thromboplastin in the presence of calcium ions, sterilized and lyophilized.

It contains not less than 80% and not more than 150% of the labeled Units of thrombin.

Each mg contains not less than 10 Units of thrombin.

Description Thrombin is a white to light yellow, amorphous substance.

Thrombin (500 Units) dissolves in 1.0 mL of isotonic sodium chloride solution clearly or with slight turbidity within 1 minute.

Loss on drying <2.41> Not more than 3% (50 mg, in vacuum, phosphorus (V) oxide, 4 hours).

Sterility <4.06> It meets the requirement.

Assay (i) Fibrinogen solution—Weigh accurately about 30 mg of fibrinogen, and dissolve in 3 mL of isotonic sodium chloride solution. Allow the solution to clot sufficiently with frequent shaking after the addition of about 3 Units of thrombin. Wash the precipitated clot thoroughly until the washings yield no turbidity on addition of silver nitrate TS, weigh the clot after drying at 105°C for 3 hours, and calculate the percentage of the clot in the fibrinogen. Dissolve the fibrinogen in isotonic sodium chloride solution so that the clot should be 0.20%, adjust the pH of the solution between 7.0 and 7.4 by addition of 0.05 mol/L dibasic sodium phosphate TS (or if necessary, use 0.5 mol/L disodium hydrogenphosphate TS), and dilute with isotonic sodium chloride solution to make a 0.10% solution.

(ii) Procedure—Dissolve Thrombin Reference Standard in isotonic sodium chloride solution, and prepare four kinds of standard solutions which contain 4.0, 5.0, 6.2, and 7.5 Units in 1 mL. Transfer accurately 0.10 mL each of the standard solutions maintained at a given degree $\pm 1^\circ\text{C}$ between 20°C and 30°C to a small test tube, 10 mm in inside diameter, 100 mm in length, blow out 0.90 mL of the fibrinogen solution at the same temperature into the test tube from a pipet, start a stop watch simultaneously, shake the tube constantly, and determine the time for the first appearance of clot. Calculate the average values of five determinations for the four kinds of standard solutions, respectively. If the deviation between the maximum and the minimum values of five determinations is more than 10% of the average value, reject the whole run, and try the experiment again. The concentration of the standard solution may be changed appropriately within the range between 14 and 60 seconds of the clotting time. The determination proceeds at the same temperature described above. Next, weigh accurately the whole contents of a single container of Thrombin, dissolve it in isotonic sodium chloride solution to provide a solution which is presumed

to contain about 5 Units in each mL, treat 0.10 mL of the solution with the same reagents in the same manner five times, determine the clotting times, and calculate the average value. Plot the average values of the clotting times of the four kinds of the standard solutions on a logarithmic graph, using Units as the abscissa and clotting times as the ordinate, and draw a calibration line which best fits the four plotted points. Using this line, read the Units U from the average value of the clotting times of the sample solution.

Units of a single container of Thrombin = $U \times 10 \times V$

V: The number of mL of the volume in which the contents of a single container of Thrombin has been dissolved.

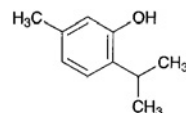
Calculate the units for 1 mg of the contents.

Containers and storage Containers—Hermetic containers. Storage—Not exceeding 10°C.

Expiration date Use within 36 months after the date of manufacture.

Thymol

チモール



$C_{10}H_{14}O$: 150.22

5-Methyl-2-(1-methylethyl)phenol [89-83-8]

Thymol contains not less than 98.0% of $C_{10}H_{14}O$.

Description Thymol occurs as colorless crystals or white, crystalline masses. It has an aromatic odor, and has a burning taste.

It is very soluble in acetic acid (100), freely soluble in ethanol (95) and in diethyl ether, and slightly soluble in water.

It sinks in water, but when warmed, it melts and rises to the surface of water.

Identification (1) To 1 mL of a solution of Thymol in acetic acid (100) (1 in 300) add 6 drops of sulfuric acid and 1 drop of nitric acid: a blue-green color develops by reflected light and a red-purple color develops by transmitted light.

(2) Dissolve 1 g of Thymol in 5 mL of a solution of sodium hydroxide (1 in 10) by heating in a water bath, and continue heating for several minutes: a light yellow-red color slowly develops. Allow this solution to stand at room temperature: the color changes to dark yellow-brown. Shake this solution with 2 to 3 drops of chloroform: a purple color gradually develops.

(3) Triturate Thymol with an equal mass of camphor or menthol: the mixture liquefies.

Melting point <2.60> 49 – 51°C

Purity (1) Non-volatile residue—Volatilize 2.0 g of Thymol by heating on a water bath, and dry the residue at 105°C for 2 hours: the mass is not more than 1.0 mg.

(2) Other phenols—Shake vigorously 1.0 g of Thymol with 20 mL of warm water for 1 minute, and filter. To 5 mL

of the filtrate add 1 drop of iron (III) chloride TS: a green color may develop, but no blue to purple color develops.

Assay Weigh accurately about 0.5 g of Thymol, dissolve in 10 mL of sodium hydroxide TS, and add water to make exactly 100 mL. Measure exactly 10 mL of the solution into an iodine flask, add 50 mL of water and 20 mL of dilute sulfuric acid, and cool in ice water for 30 minutes. Add exactly 20 mL of 0.05 mol/L bromine VS, stopper tightly immediately, allow to stand for 30 minutes in ice water with occasional shaking in a dark place, add 14 mL of potassium iodide TS and 5 mL of chloroform, stopper tightly, shake vigorously, and titrate <2.50> the liberated iodine with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Stopper tightly, shake vigorously near the end point, and continue the titration until the blue color in the chloroform layer disappears. Perform a blank determination.

Each mL of 0.05 mol/L bromine VS
= 3.755 mg of C₁₀H₁₄O

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Dried Thyroid

乾燥甲状腺

Dried Thyroid is the fresh thyroid gland, previously deprived of connective tissue and fat, minced, dried rapidly at a temperature not above 50°C, and powdered, or diluted with suitable diluents. It is obtained from domesticated animals that are used for food by man.

It contains not less than 0.30% and not more than 0.35% of iodine (I: 126.90) in the form of organic compounds peculiar to the thyroid gland.

Description Dried Thyroid occurs as a light yellow to grayish brown powder. It has a slight, characteristic, meat-like odor.

Identification Mount Dried Thyroid in diluted formaldehyde solution (1 in 10), stain in hematoxylin TS for 10 to 30 minutes, wash with water, soak in a mixture of 1 mL of hydrochloric acid and 99 mL of diluted ethanol (7 in 10) for 5 to 10 seconds, and again wash with water for about 1 hour. Stain in a solution of eosin Y (1 in 100) for 1 to 5 minutes, wash with water, dehydrate, and soak successively in diluted ethanol (7 in 10) for 5 to 10 seconds, in diluted ethanol (4 in 5) for 5 to 10 seconds, in diluted ethanol (9 in 10) for 1 to 2 minutes, in ethanol (95) for 1 to 5 minutes then in ethanol (99.5) for 1 to 5 minutes. Interpenetrate in xylene, seal with balsam, and examine under a microscope: epithelial nuclei forming follicles peculiar to the thyroid gland are observed.

Purity (1) Inorganic iodides—Mix 1.0 g of Dried Thyroid with 10 mL of a saturated solution of zinc sulfate heptahydrate, shake for 5 minutes, and filter. To 5 mL of the filtrate add 0.5 mL of starch TS, 4 drops of sodium nitrite TS and 4 drops of dilute sulfuric acid with thorough shaking: no blue color is produced.

(2) Fat—Extract 1.0 g of Dried Thyroid with diethyl ether for 2 hours using a Soxhlet extractor. Evaporate the

diethyl ether extract, and dry the residue at 105°C to constant mass: the mass of the residue is not more than 0.030 g.

Loss on drying <2.41> Not more than 6.0% (1 g, 105°C, constant mass).

Total ash <5.01> Not more than 5.0% (0.5 g).

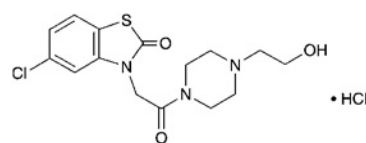
Assay Transfer about 1 g of Dried Thyroid, accurately weighed, to a crucible, add 7 g of potassium carbonate, mix carefully, and gently tap the crucible on the table to compact the mixture. Overlay with 10 g of potassium carbonate, and compact again thoroughly by tapping. Place the crucible in a muffle furnace preheated to a temperature between 600°C and 700°C, and ignite the mixture for 25 minutes. Cool, add 20 mL of water, heat gently to boiling, and filter into a flask. To the residue add 20 mL of water, boil, and filter into the same flask. Rinse the crucible and the char on the funnel with boiling water until the filtrate measures 200 mL. Add slowly 7 mL of freshly prepared bromine TS, 40 mL of diluted phosphoric acid (1 in 2), and boil until starch iodide paper is no longer colored blue by the evolved gas. Wash down inside of the flask with water, and continue boiling for 5 minutes. During the boiling add water from time to time to maintain a volume at not less than 200 mL. Cool, add 5 mL of a solution of phenol (1 in 20), again rinse inside of the flask with water, and allow to stand for 5 minutes. Add 2 mL of diluted phosphoric acid (1 in 2) and 5 mL of potassium iodide TS, and titrate <2.50> immediately the liberated iodine with 0.01 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.01 mol/L sodium thiosulfate VS
= 0.2115 mg of I

Containers and storage Containers—Tight containers.

Tiaramide Hydrochloride

チアラミド塩酸塩



C₁₅H₁₈ClN₃O₃.HCl: 392.30

5-Chloro-3-[2-[4-(2-hydroxyethyl)piperazin-1-yl]-2-oxoethyl]-1,3-benzothiazol-2(3H)-one monohydrochloride [35941-71-0]

Tiaramide Hydrochloride, when dried, contains not less than 98.5% of C₁₅H₁₈ClN₃O₃.HCl.

Description Tiaramide Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in water, slightly soluble in ethanol (95) and in acetic acid (100), and practically insoluble in acetic anhydride and in diethyl ether.

The pH of a solution of Tiaramide Hydrochloride (1 in 20) is between 3.0 and 4.5.

Melting point: about 265°C (with decomposition).

Identification (1) Dissolve 5 mg of Tiaramide Hydrochloride

ride in 5 mL of 0.1 mol/L hydrochloric acid TS, and add 3 drops of Dragendorff's TS: an orange precipitate is formed.

(2) Determine the infrared absorption spectrum of Tiaramide Hydrochloride, previously dried, as directed in the potassium chloride disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tiaramide Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of Tiaramide Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Tiaramide Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tiaramide Hydrochloride according to Method 1, and perform the test. In the procedure, add 20 mL of diluted hydrochloric acid (1 in 2) (not more than 2 ppm).

(4) Related substances—Dissolve 0.20 g of Tiaramide Hydrochloride in 10 mL of diluted ethanol (99.5) (7 in 10), and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add diluted ethanol (99.5) (7 in 10) to make exactly 100 mL. Pipet 2 mL of this solution, add diluted ethanol (99.5) (7 in 10) to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. After air-drying, immediately develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, air-dry the plate, and then dry at 100°C for 30 minutes. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution. Allow the plate to stand in iodine vapor for 30 minutes: the spots other than the principal spot and the spot of the starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tiaramide Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) by warming, cool, and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from red through purple to blue-purple (indicator: 3 drops of neutral red TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 39.23 mg of $C_{15}H_{18}ClN_3O_3S \cdot HCl$

Containers and storage Containers—Well-closed containers.

Tiaramide Hydrochloride Tablets

チアラミド塩酸塩錠

Tiaramide Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tiaramide ($C_{15}H_{18}ClN_3O_3S$; 355.84).

Method of preparation Prepare as directed under Tablets, with Tiaramide Hydrochloride.

Identification (1) Determine the absorption spectrum of the sample solution obtained in the Assay as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 285 nm and 289 nm, and between 292 nm and 296 nm.

(2) To a quantity of powdered Tiaramide Hydrochloride Tablets, equivalent to 0.1 g of tiaramide according to the labeled amount, add 10 mL of diluted ethanol (7 in 10), shake well, filter, and use the filtrate as the sample solution. Separately, dissolve 0.11 g of tiaramide hydrochloride for assay in 10 mL of diluted ethanol (7 in 10), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and dry the plate at 100°C for 30 minutes. Spray evenly Dragendorff's TS for spraying followed by diluted nitric acid (1 in 50) on the plate: the principal spot obtained with the sample solution and the spot with the standard solution are yellow-red in color and have the same R_f value.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Tiaramide Hydrochloride Tablets add a volume of 0.1 mol/L hydrochloric acid TS, equivalent to 3/5 volume of V mL which makes a solution so that each mL contains about 1 mg of tiaramide ($C_{15}H_{18}ClN_3O_3S$) according to the labeled amount, shake for 60 minutes, then add 0.1 mol/L hydrochloric acid TS to make exactly V mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 55 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} &\text{Amount (mg) of tiaramide } (C_{15}H_{18}ClN_3O_3S) \\ &= W_S \times (A_T/A_S) \times (V/50) \times 0.907 \end{aligned}$$

W_S : Amount (mg) of tiaramide hydrochloride for assay

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Tiaramide Hydrochloride

Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test for a 50-mg tablet or 30 minutes after starting the test for a 100-mg tablet, and filter through a membrane filter with pore size of not more than 0.5 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, add water to make exactly V' mL so that each mL contains about 56 μg of tiaramide ($\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 25 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , at 294 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rates for a 50-mg tablet in 15 minutes and a 100-mg tablet in 30 minutes are not less than 80%, respectively.

Dissolution rate (%) with respect to the labeled amount of tiaramide ($\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 360 \times 0.907$$

W_S : Amount (mg) of tiaramide hydrochloride for assay

C : Labeled amount (mg) of tiaramide ($\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}$) in 1 tablet

Assay Weigh accurately the mass of more than 20 Tiaramide Hydrochloride Tablets, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g of tiaramide ($\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S}$), add 60 mL of 0.1 mol/L hydrochloric acid TS, shake for 30 minutes, add 0.1 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet 5 mL of the subsequent filtrate, add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.11 g of tiaramide hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in 0.1 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 294 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

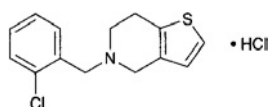
$$\begin{aligned} &\text{Amount (mg) of tiaramide (C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_3\text{S)} \\ &= W_S \times (A_T/A_S) \times 0.907 \end{aligned}$$

W_S : Amount (mg) of tiaramide hydrochloride for assay

Containers and storage Containers—Tight containers.

Ticlopidine Hydrochloride

チクロピジン塩酸塩



$\text{C}_{14}\text{H}_{14}\text{ClNS.HCl}$: 300.25
5-(2-Chlorobenzyl)-4,5,6,7-

tetrahydrothieno[3,2-c]pyridine monohydrochloride
[53885-35-1]

Ticlopidine Hydrochloride contains not less than 99.0% of $\text{C}_{14}\text{H}_{14}\text{ClNS.HCl}$, calculated on the anhydrous basis.

Description Ticlopidine Hydrochloride occurs as a white to pale yellowish white crystalline powder.

It is freely soluble in acetic acid (100), soluble in water and in methanol, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

Identification (1) Determine the infrared absorption spectrum of Ticlopidine Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) A solution of Ticlopidine Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> (2) for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Ticlopidine Hydrochloride according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ticlopidine Hydrochloride according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.5 g of Ticlopidine Hydrochloride in 20 mL of a solution of hydrochloric acid in methanol (1 in 20,000), and use this solution as the sample solution. To exactly 5 mL of the sample solution add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 200 mL, and use this solution as the standard solution (1). Separately, pipet 1 mL of the sample solution, add a solution of hydrochloric acid in methanol (1 in 20,000) to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution (1) on a plate of silica gel for thin-layer chromatography (Plate 1), and spot 10 μL each of the sample solution and standard solution (2) on another plate of silica gel for thin-layer chromatography (Plate 2). Develop the plates with an upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 15 cm, and air-dry the plates. Spray evenly a solution of ninhydrin in acetone (1 in 50) on Plate 1, and heat at 100°C for 20 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (1). Allow Plate 2 to stand in an iodine vapor for 30 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution (2).

(4) Formaldehyde—Dissolve 0.80 g of Ticlopidine Hydrochloride in 19.0 mL of water, add 1.0 mL of 4 mol/L sodium hydroxide TS, shake well, centrifuge, and filter the supernatant liquid. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, mix, and warm at 40°C for 40 minutes: the solution has no more color than the following control solution.

Control solution: Weigh accurately 0.54 g of formaldehyde solution, and add water to make exactly 1000 mL. To exactly 10 mL of this solution add water to make exactly 1000

mL. Prepare before use. To 8.0 mL of this solution add water to make 20.0 mL, and filter. To 5.0 mL of the filtrate add 5.0 mL of acetylacetone TS, and proceed in the same manner.

Water <2.48> Not more than 1.0% (0.3 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

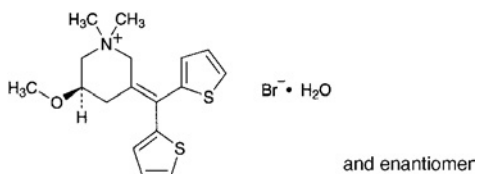
Assay Weigh accurately about 0.4 g of Ticlopidine Hydrochloride, dissolve in 20 mL of acetic acid (100), add 40 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 30.03 \text{ mg of } C_{14}H_{14}ClNS.HCl \end{aligned}$$

Containers and storage Containers—Well-closed containers.

Timepidium Bromide Hydrate

チメピジウム臭化物水和物



$C_{17}H_{22}BrNOS_2 \cdot H_2O$: 418.41
(*5RS*)-3-(Dithien-2-ylmethylene)-5-methoxy-1,1-dimethylpiperidinium bromide monohydrate
[35035-05-3, anhydride]

Timepidium Bromide Hydrate contains not less than 98.5% of timepidium bromide ($C_{17}H_{22}BrNOS_2$: 400.40), calculated on the anhydrous basis.

Description Timepidium Bromide Hydrate occurs as white crystals or crystalline powder.

It is very soluble in methanol and in acetic acid (100), freely soluble in ethanol (99.5), sparingly soluble in water and in acetic anhydride, and practically insoluble in diethyl ether.

The pH of a solution of Timepidium Bromide Hydrate in freshly boiled and cooled water (1 in 100) is between 5.3 and 6.3.

A solution of Timepidium Bromide Hydrate in methanol (1 in 20) shows no optical rotation.

Identification (1) To 1 mL of a solution of Timepidium Bromide Hydrate (1 in 100) add 1 mL of ninhydrin-sulfuric acid TS: a red purple color develops.

(2) Determine the absorption spectrum of a solution of Timepidium Bromide Hydrate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Timepidium Bromide Hydrate as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at

the same wave numbers.

(4) A solution of Timepidium Bromide Hydrate (1 in 100) responds to the Qualitative Tests <1.09> (1) for Bromide.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Timepidium Bromide Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Timepidium Bromide Hydrate in 10 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 100 mL. Pipet 1 mL of this solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, water, acetic acid (100) and ethyl acetate (5:4:1:1:1) to a distance of about 13 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Water <2.48> 3.5 – 5.0% (0.4 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

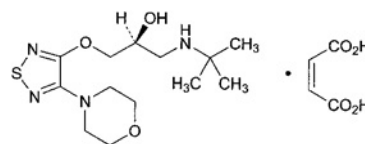
Assay Weigh accurately about 0.6 g of Timepidium Bromide Hydrate, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (2:1), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.1 mol/L perchloric acid VS} \\ = 40.04 \text{ mg of } C_{17}H_{22}BrNOS_2 \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Timolol Maleate

チモロールマレイン酸塩



$C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$: 432.49
(*2S*)-1-[(1,1-Dimethylethyl)amino]-3-(4-morpholin-4-yl-1,2,5-thiadiazol-3-yloxy)propan-2-ol monomaleate
[26921-17-5]

Timolol Maleate, when dried, contains not less than 98.0% and not more than 101.0% of $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$.

Description Timolol Maleate occurs as a white to pale yellow

lowish white crystalline powder.

It is freely soluble in acetic acid (100), and soluble in water and in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Melting point: about 197°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Timolol Maleate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Timolol Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) To 5 mL of a solution of Timolol Maleate (1 in 500) add 1 drop of potassium permanganate TS: the red color of the TS disappears immediately.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-5.7 - -6.2^\circ$ (after drying, 1.25 g, 1 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Timolol Maleate in 20 mL of water is between 3.8 and 4.3.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Timolol Maleate in 20 mL of water: the solution is clear, and its absorbance at 440 nm, determined as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.05.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Timolol Maleate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 30 mg of Timolol Maleate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 25 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than timolol and maleic acid is not larger than 1/5 times the peak area of timolol from the standard solution, and the total area of the peaks other than the peak of timolol and maleic acid is not larger than 1/2 times the peak area of timolol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 1.9 g of sodium 1-hexanesulfonate in 1800 mL of water, add 6.0 mL of triethylamine and 8.0 mL of formic acid, adjust to pH 3.0 with formic acid, and add water to make 2000 mL. To 1400 mL of this solution add 500 mL of methanol and 100 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of timolol is about 18 minutes.

Time span of measurement: About 2 times as long as the retention time of timolol beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mobile phase to make 10 mL. Confirm that the peak area of timolol obtained from 25 μ L of this solution is equivalent to 7 to 13% of that from 25 μ L of the standard solution.

System performance: When the procedure is run with 25 μ L of the sample solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of timolol are not less than 1500 and not more than 2.5, respectively.

System repeatability: When the test is repeated 6 times with 25 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of timolol is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, 100°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

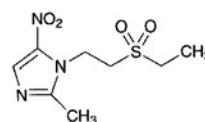
Assay Weigh accurately about 0.8 g of Timolol Maleate, previously dried, dissolve in 90 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 43.25 mg of $C_{13}H_{24}N_4O_3S \cdot C_4H_4O_4$

Containers and storage Containers—Tight containers.

Tinidazole

チニダゾール



$C_8H_{13}N_3O_4S$: 247.27

1-[2-(Ethylsulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole
[19387-91-8]

Tinidazole, when dried, contains not less than 98.5% and not more than 101.0% of $C_8H_{13}N_3O_4S$.

Description Tinidazole occurs as a light yellow, crystalline powder.

It is soluble in acetic anhydride and in acetone, sparingly soluble in methanol, slightly soluble in ethanol (99.5), and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Tinidazole in methanol (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of

Tinidazole as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 125 – 129°C

Purity (1) Sulfate <1.14>—To 2.0 g of Tinidazole add 100 mL of water, boil for 5 minutes, cool, add water to make 100 mL, and filter. Take 25 mL of the filtrate, and add 1 mL of dilute hydrochloric acid and water to make 50 mL. Use this solution as the test solution, and perform the test. Prepare the control solution with 0.45 mL of 0.005 mol/L sulfuric acid VS (not more than 0.043%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Tinidazole according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 2.0 g of Tinidazole according to Method 3, and perform the test (not more than 1 ppm).

(4) Related substances—Dissolve 50 mg of Tinidazole in 2 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and diethylamine (19:1) to a distance of about 10 cm, air-dry the plate, heat at 100°C for 5 minute, and cool. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

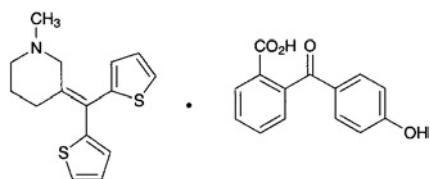
Assay Weigh accurately about 0.35 g of Tinidazole, previously dried, dissolve in 50 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 24.73 mg of C₈H₁₃N₃O₄S

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Tipepidine Hibenzate

チペピジンヒベンズ酸塩



C₁₅H₁₇NS₂·C₁₄H₁₀O₄; 517.66

3-(Dithien-2-ylmethylene)-1-methylpiperidine mono[2-(4-hydroxybenzoyl)benzoate] [31139-87-4]

Tipepidine Hibenzate, when dried, contains not less than 98.5% of C₁₅H₁₇NS₂·C₁₄H₁₀O₄.

Description Tipepidine Hibenzate occurs as a white to light yellow, crystalline powder. It is odorless and tasteless.

It is freely soluble in acetic acid (100), slightly soluble in methanol and in ethanol (95), very slightly soluble in water, and practically insoluble in diethyl ether.

Identification (1) Dissolve 0.01 g of Tipepidine Hibenzate in 5 mL of sulfuric acid: an orange-red color develops.

(2) Dissolve 0.3 g of Tipepidine Hibenzate in 10 mL of sodium hydroxide TS and 5 mL of water, and extract with two 20-mL portions of chloroform. Wash the chloroform extracts with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, and dissolve the residue in 0.5 mL of 1 mol/L hydrochloric acid TS and 5 mL of water. To 2 mL of this solution add 5 mL of Reinecke salt TS: a light red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Tipepidine Hibenzate in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum; both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectrum of Tipepidine Hibenzate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 189 – 193°C

Purity (1) Clarity of solution—Dissolve 1.0 g of Tipepidine Hibenzate in 10 mL of acetic acid (100): the solution is clear. Perform the test with this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: its absorbance at 400 nm is not more than 0.16.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Tipepidine Hibenzate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tipepidine Hibenzate according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—(i) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of all peaks other than the area of the hibenzic acid and tipepidine from the sample solution is not larger than the peak area of the tipepidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of a solution of ammonium acetate (1 in 100) and tetrahydrofuran (32:13).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 12 minutes.

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of tipepidine obtained from 20 μ L of the standard solution.

System performance: Dissolve 10 mg of Tipepidine Hibenzate and 3 mg of propyl parahydroxybenzoate in 100 mL of the mobile phase. When the procedure is run with 20 μ L of this solution under the above operating conditions, hibenzic acid, tipepidine and propyl parahydroxybenzoate are eluted in this order with the resolution between the peaks of tipepidine and propyl parahydroxybenzoate being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 1.5%.

(ii) Dissolve 10 mg of Tipepidine Hibenzate in 20 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the total area of all peaks other than the area of the hibenzic acid and tipepidine from the sample solution is not larger than 1/2 times the peak area of the tipepidine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of methanol and a solution of ammonium acetate (1 in 500) (13:7).

Flow rate: Adjust the flow rate so that the retention time of tipepidine is about 10 minutes.

Time span of measurement: As long as the retention time of tipepidine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of tipepidine obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of tipepidine obtained from 20 μ L of the standard solution.

System performance: Dissolve 12 mg of Tipepidine Hibenzate and 4 mg of xanthene in 50 mL of the mobile phase.

When the procedure is run with 10 μ L of this solution under the above operating conditions, hibenzic acid, tipepidine and xanthene are eluted in this order with the resolution between the peaks of tipepidine and xanthene being not less than 3.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tipepidine is not more than 3.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 60°C, in vacuum, phosphorus (V) oxide, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 1 g of Tipepidine Hibenzate, previously dried, dissolve in 40 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

$$\begin{aligned} \text{Each ml of 0.1 mol/L perchloric acid VS} \\ = 51.77 \text{ mg of } C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4 \end{aligned}$$

Containers and storage Containers— Well-closed containers.

Storage—Light-resistant.

Tipepidine Hibenzate Tablets

チペピジンヒベンズ酸塩錠

Tipepidine Hibenzate Tablets contain not less than 95% and not more than 105% of the labeled amount of tipepidine hibenzate ($C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$; 517.66).

Method of preparation Prepare as directed under Tablets, with Tipepidine Hibenzate.

Identification (1) To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 44 mg of Tipepidine Hibenzate according to the labeled amount, add 5 mL of water, shake for 1 minute, add 10 mL of sodium hydroxide TS, and extract with two 20-mL portions of chloroform. Combine the extracts, wash with 10 mL of water, and filter the chloroform layer. Evaporate the filtrate on a water bath to dryness, dissolve the residue in 0.2 mL of 1 mol/L hydrochloric acid TS and 2 mL of water, and add 5 mL of Reinecke salt TS: a light red precipitate is formed.

(2) To a quantity of powdered Tipepidine Hibenzate Tablets, equivalent to 11 mg of Tipepidine Hibenzate according to the labeled amount, add 30 mL of ethanol (99.5), and warm for 10 minutes with occasional shaking. After cooling, add ethanol (99.5) to make 50 mL, and filter. To 1 mL of the filtrate add ethanol (99.5) to make 20 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 282 nm and 286 nm.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Tipepidine Hibenzate Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium.

Use the dissolved solution 30 minutes after starting the test as the sample solution. Separately, weigh accurately about 0.11 g of tipegidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, and dissolve in 80 mL of diluted ethanol (99.5) (3 in 4) by warming occasionally. After cooling, add diluted ethanol (99.5) (3 in 4) to make exactly 100 mL, then pipet 20 mL of this solution, add water to make exactly 900 mL, and use this solution as the standard solution. Determine the absorbances, A_{T1} and A_{S1} , at 286 nm, and A_{T2} and A_{S2} , at 360 nm of the sample solution and standard solution as directed under Ultraviolet-visible Spectrophotometry <2.24>. The dissolution rate of Tipegidine Hibenzate Tablets in 30 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of tipegidine hibenzate ($C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$)

$$= W_S \times \{(A_T - A_{T2}/A_S - A_{S2}) \times (20/C)$$

W_S : Amount (mg) of tipegidine hibenzate for assay.

C : Labeled amount (mg) of tipegidine hibenzate ($C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$) in 1 tablet.

Assay Weigh accurately and powder not less than 20 Tipegidine Hibenzate Tablets. Weigh accurately a portion of the powder, equivalent to about 22 mg of tipegidine hibenzate ($C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$), add 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and warm for 10 minutes with occasional shaking. After cooling, add diluted methanol (1 in 2) to make exactly 50 mL, and filter. Discard the first 10 mL of the filtrate, pipet the subsequent 5 mL, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of tipegidine hibenzate for assay, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 60°C) for 3 hours, dissolve in 10 mL of diluted acetic acid (100) (1 in 2) and 30 mL of methanol, and add diluted methanol (1 in 2) to make exactly 50 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, then add diluted methanol (1 in 2) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tipegidine to that of the internal standard, respectively.

Amount (mg) of tipegidine hibenzate ($C_{15}H_{17}NS_2 \cdot C_{14}H_{10}O_4$)

$$= W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of tipegidine hibenzate for assay

Internal standard solution—A solution of dibucaine hydrochloride in methanol (1 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of sodium lauryl

sulfate in diluted phosphoric acid (1 in 1000) (1 in 500), acetonitrile and 2-propanol (3:2:1).

Flow rate: Adjust the flow rate so that the retention time of tipegidine is about 7 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, tipegidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tipegidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Titanium Oxide

酸化チタン

TiO₂: 79.87

Titanium Oxide, when dried, contains not less than 98.5% of TiO₂.

Description Titanium Oxide occurs as a white powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (99.5) and in diethyl ether.

It dissolves in hot sulfuric acid and in hydrofluoric acid, and does not dissolve in hydrochloric acid, in nitric acid and in dilute sulfuric acid.

When fused by heating with potassium hydrogen sulfate, with potassium hydroxide, or with potassium carbonate, it changes to soluble salts.

Shake 1 g of Titanium Oxide with 10 mL of water: the mixture is neutral.

Identification Heat 0.5 g of Titanium Oxide with 5 mL of sulfuric acid until white fumes are evolved, cool, add cautiously water to make 100 mL, and filter. To 5 mL of the filtrate add 2 to 3 drops of hydrogen peroxide TS: a yellow-red color develops.

Purity (1) Lead—Place 1.0 g of Titanium Oxide in a platinum crucible, add 10.0 g of potassium hydrogen sulfate, heat gently with caution at the beginning, then raise the temperature gradually, and heat strongly with occasional shaking until the contents fuse to yield a clear liquid. Cool, add 30 mL of a solution of diammonium hydrogen citrate (9 in 20) and 50 mL of water, dissolve by heating on a water bath, cool, add water to make 100 mL, and use this solution as the sample stock solution. Take 25 mL of the solution to a separator, add 10 mL of a solution of ammonium sulfate (2 in 5) and 5 drops of thymol blue TS, neutralize with ammonia TS, and add 2.5 mL of ammonia TS. To this solution add exactly 20 mL of a solution of dithizone in *n*-butyl acetate (1 in 500), shake for 10 minutes, and use this *n*-butyl acetate solution as the sample solution. Separately, place 6.0 mL of Standard Lead Solution in a platinum crucible, proceed as directed in

the sample solution, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution as directed under Atomic Absorption Spectrophotometry <2.23> according to the following conditions: the absorbance of the sample solution is smaller than that of the standard solution (not more than 60 ppm).

Gas: Combustible gas—Acetylene gas or hydrogen gas

Supporting gas—Air

Lamp: Lead hollow-cathode lamp

Wavelength: 283.3 nm

(2) Arsenic <1.11>—Perform the test with 20 mL of the sample stock solution obtained in (1) as the test solution: the stain is not deeper than the following standard stain.

Standard stain: Proceed in the same manner without Titanium Oxide, transfer 20 mL of the obtained solution to a generator bottle, add 2.0 mL of Standard Arsenic Solution, and proceed in the same manner as the test with the test solution (not more than 10 ppm).

(3) Water-soluble substances—Shake thoroughly 4.0 g of Titanium Oxide with 50 mL of water, and allow to stand overnight. Shake thoroughly with 2 mL of ammonium chloride TS, add further 2 mL of ammonium chloride TS if necessary, and allow titanium oxide to settle. Add water to make 200 mL, shake thoroughly, and filter through double filter paper. Discard the first 10 mL of the filtrate, evaporate 100 mL of the clear filtrate on a water bath, and heat strongly at 650°C to constant mass: the mass of the residue is not more than 5.0 mg.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

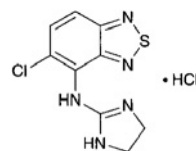
Assay Weigh accurately about 0.2 g of Titanium Oxide, previously dried, transfer to a crucible, and add 3 g of potassium disulfate. Cover, and heat gently at first, gradually raise the temperature, and then heat the fused contents for 30 minutes. Continue heating for 30 minutes at a higher temperature to make the fused mixture a deep yellow-red, almost clear liquid. Cool, transfer the contents of the crucible to a 250-mL beaker, wash the crucible with a mixture of 75 mL of water and 2.5 mL of sulfuric acid into the beaker, and heat on a water bath until the solution becomes almost clear. Dissolve 2 g of L-tartaric acid in the solution, add 2 to 3 drops of bromothymol blue TS, neutralize with ammonia TS, and acidify with 1 to 2 mL of diluted sulfuric acid (1 in 2). Pass hydrogen sulfide sufficiently through the solution, add 30 mL of ammonia TS, again saturate the solution with hydrogen sulfide, allow to stand for 10 minutes, and filter. Wash the precipitate on the filter paper with ten 25-mL portions of a mixture of ammonium L-tartrate solution (1 in 100) and ammonium sulfide TS (9:1). When the precipitate is filtered and washed, prevent iron (II) sulfide from oxidation by filling the solution on the filter paper. Combine the filtrate and the washings, add 40 mL of diluted sulfuric acid (1 in 2), and boil to expel hydrogen sulfide. Cool, and dilute with water to make 400 mL. Add gradually 40 mL of cupferron TS to the solution with stirring, and allow to stand. After sedimentation of a yellow precipitate, add again cupferron TS until a white precipitate is produced. Filter by slight suction using quantitative filter paper, wash with twenty portions of diluted hydrochloric acid (1 in 10), and remove water by stronger suction at the last washing. Dry the precipitate together with the filter paper at 70°C, transfer to a tared crucible, and heat very gently at first, and raise the temperature gradually after

smoke stops evolving. Heat strongly between 900°C and 950°C to constant mass, cool, and weigh as titanium oxide (TiO₂).

Containers and storage Containers—Well-closed containers.

Tizanidine Hydrochloride

チザニジン塩酸塩



C₉H₈ClN₅S.HCl: 290.17

5-Chloro-*N*-(4,5-dihydro-1*H*-imidazol-2-yl)-2,1,3-benzothiadiazole-4-amine monohydrochloride
[64461-82-1]

Tizanidine Hydrochloride, when dried, contains not less than 99.0% and not more than 101.0% of C₉H₈ClN₅S.HCl.

Description Tizanidine Hydrochloride occurs as a white to light yellowish white crystalline powder.

It is soluble in water, slightly soluble in ethanol (99.5), and practically insoluble in acetic anhydride and in acetic acid (100).

Melting point: about 290°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Tizanidine Hydrochloride in diluted 1 mol/L ammonia TS (1 in 10) (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tizanidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tizanidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tizanidine Hydrochloride according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 60 mg of Tizanidine Hydrochloride in 10 mL of a mixture of water and acetonitrile (17:3), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and acetonitrile (17:3) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than tizanidine is not larger than 1/5 times the peak area of tizanidine with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm for about 3 minutes after sample injection and 318 nm subsequently).

Column: A stainless steel column 4.6 mm in inside diameter and 12.5 cm in length, packed with octadecyl-silanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of water and formic acid (200:1), adjusted to pH 8.5 with ammonia water (28).

Mobile phase B: A mixture of acetonitrile and the mobile phase A (4:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	81 → 68	19 → 32
10 – 13	68	32
13 – 26	68 → 10	32 → 90
26 – 28	10	90

Flow rate: Adjust the flow rate so that the retention time of tizanidine is about 7 minute.

Time span of measurement: About 4 times as long as the retention time of tizanidine beginning after the solvent peak.

System suitability—

Test for required detectability: Measure exactly 2 mL of the standard solution, and add the mixture of water and acetonitrile (17:3) to make exactly 10 mL. Confirm that the peak area of tizanidine obtained with 10 μL of this solution is equivalent to 14 to 26% of that with 10 μL of the standard solution.

System performance: Dissolve 2 mg each of Tizanidine Hydrochloride and *p*-toluenesulfonic acid monohydrate in 100 mL of the mixture of water and acetonitrile (17:3). When the procedure is run with 10 μL of this solution under the above operating conditions, *p*-toluenesulfonic acid and tizanidine are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tizanidine is not more than 2.0%.

Loss on drying <2.41> Not more than 0.2% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Tizanidine Hydrochloride, previously dried, dissolve in 60 mL of a mixture of acetic anhydride and acetic acid (100) (7:3) with the aid of warming. After cooling, titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

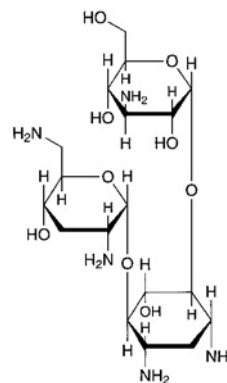
Each mL of 0.1 mol/L perchloric acid VS
= 29.02 mg of C₉H₈ClN₅S.HCl

Containers and storage Containers—Well-closed contain-

ers.

Tobramycin

トブラマイシン



C₁₈H₃₇N₅O₉; 467.51

3-Amino-3-deoxy-α-D-glucopyranosyl-(1→6)-
[2,6-diamino-2,3,6-trideoxy-α-D-ribo-hexopyranosyl-
(1→4)]-2-deoxy-D-streptamine [32986-56-4]

Tobramycin is an aminoglycoside substance having antibacterial activity produced by the growth of *Streptomyces tenebrarius*.

It contains not less than 900 μg (potency) and not more than 1060 μg (potency) per mg, calculated on the anhydrous basis. The potency of Tobramycin is expressed as mass (potency) of tobramycin (C₁₈H₃₇N₅O₉).

Description Tobramycin occurs as a white to pale yellowish white powder.

It is very soluble in water, freely soluble in formamide, slightly soluble in methanol, and very slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) Determine the spectrum of a solution of Tobramycin in heavy water for nuclear magnetic resonance spectroscopy (1 in 125) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H), using sodium 3-trimethylsilylpropanesulfonate for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits a double signal A at around δ5.1 ppm, a multiple signal B between δ2.6 ppm and δ4.0 ppm, and a multiple signal C between δ1.0 ppm and δ2.1 ppm. The ratio of the integrated intensity of these signals, A:B:C, is about 1:8:2.

(2) Dissolve 10 mg each of Tobramycin and Tobramycin Reference Standard in 1 mL of water, and use these solutions as the sample solution and the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 4 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia TS, 1-butanol and methanol (5:5:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 100°C for 5 minutes: the R_f values of the principal spots obtained from the sample solution and the standard solution are the same.

Optical rotation <2.49> [α]_D²⁰: +138 – +148° (1 g calculated

on the anhydrous basis, water, 25 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.10 g of Tobramycin in 10 mL of water is between 9.5 and 11.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tobramycin in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Tobramycin according to Method 2, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 30 ppm).

(3) Related substances—Dissolve 80 mg of Tobramycin in 10 mL of diluted ammonia solution (28) (1 in 250), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add diluted ammonia solution (28) (1 in 250) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ammonia solution (28), ethanol (95) and 2-butanone (1:1:1) to a distance of about 10 cm, air-dry the plate, then further dry at 110°C for 10 minutes. Immediately spray evenly a mixture of water and sodium hypochlorite TS (4:1) on the plate, air-dry the plate, then spray potassium iodide-starch TS on the plate: the spot other than the principal spot from the sample solution is not more intense than the spot from the standard solution.

Water <2.48> Not more than 11.0% (0.1 g, volumetric titration, direct titration). Use a mixture of formamide for water determination and methanol for water determination (3:1) instead of methanol for water determination.

Residue on ignition <2.44> Not more than 1.0% (0.5 g).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) under (1) Agar media for seed and base layer.

(iii) Standard solutions—Weigh accurately an amount of Tobramycin Reference Standard, equivalent to about 25 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution between 5°C and 15°C, and use within 30 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Tobramycin, equivalent to about 25 mg (potency), and dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 8.0 to make solutions so that each mL contains 8 μ g (potency) and 2 μ g (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

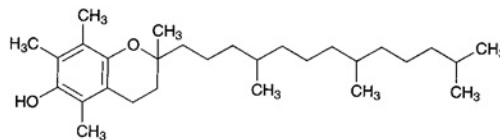
Containers and storage Containers—Tight containers.

Tocopherol

Vitamin E

dl- α -Tocopherol

トコフェロール



$C_{29}H_{50}O_2$: 430.71

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-ol [10191-41-0]

Tocopherol contains not less than 96.0% and not more than 102.0% of $C_{29}H_{50}O_2$.

Description Tocopherol is a clear, yellow to red-brown, viscous liquid. It is odorless.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether and with vegetable oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is oxidized by air and light, and acquires a dark red color.

Identification (1) Dissolve 0.01 g of Tocopherol in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Determine the infrared absorption spectrum of Tocopherol as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (292 nm): 71.0 – 76.0 (10 mg, ethanol (99.5), 200 mL).

Refractive index <2.45> n_D^{20} : 1.503 – 1.507

Specific gravity <2.56> d_{20}^{20} : 0.947 – 0.955

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol in 10 mL of ethanol (99.5): the solution is clear and has no more color than Matching Fluid C.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

Assay Dissolve about 50 mg each of Tocopherol and Tocopherol Reference Standard, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak heights, H_T and H_S , of tocopherol in the sample solution and standard solution.

$$\text{Amount (mg) of } C_{29}H_{50}O_2 = W_S \times (H_T/H_S)$$

W_S : Amount (mg) of Tocopherol Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 292 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ L in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol is about 10 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol and tocopherol acetate in 50 mL of ethanol (99.5). When the procedure is run with 20 μ L of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol is not more than 0.8%.

Containers and storage Containers—Tight containers.

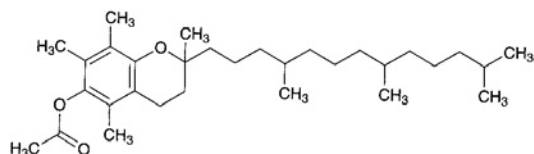
Storage—Light-resistant, and well-filled, or under nitrogen atmosphere.

Tocopherol Acetate

Vitamin E Acetate

dl- α -Tocopherol Acetate

トコフェロール酢酸エステル



$C_{31}H_{52}O_3$: 472.74

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl acetate [7695-91-2]

Tocopherol Acetate contains not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Description Tocopherol Acetate is a clear, colorless or yellow, viscous and odorless liquid.

It is miscible with ethanol (99.5), with acetone, with chloroform, with diethyl ether, with hexane and with fixed oils.

It is freely soluble in ethanol (95), and practically insoluble in water.

It is optically inactive.

It is affected by air and light.

Identification (1) Dissolve 0.05 g of Tocopherol Acetate in 10 mL of ethanol (99.5), add 2 mL of nitric acid, and heat at 75°C for 15 minutes: a red to orange color is produced.

(2) Determine the infrared absorption spectrum of

Tocopherol Acetate as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Acetate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (284 nm): 41.0 – 45.0 (10 mg, ethanol (99.5), 100 mL).

Refractive index <2.45> n_D^{20} : 1.494 – 1.499

Specific gravity <2.56> d_{20}^{20} : 0.952 – 0.966

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol Acetate in 10 mL of ethanol (99.5); the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Ferric Chloride Colorimetric Stock Solution add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) **Heavy metals** <1.07>—Carbonize 1.0 g of Tocopherol Acetate by gentle heating. Cool, add 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (1 in 10), and ignite the ethanol to burn. Cool, add 1 mL of sulfuric acid, proceed according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (20 ppm).

(3) **α -Tocopherol**—Dissolve 0.10 g of Tocopherol Acetate in exactly 10 mL of hexane, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol Reference Standard in hexane to make exactly 100 mL. Pipet 1 mL of this solution, add hexane to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α, α' -dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spot from the sample solution corresponding to that from the standard solution is not larger than and not more intense than the spot from the standard solution.

Assay Dissolve 50 mg each of Tocopherol Acetate and Tocopherol Acetate Reference Standard, accurately weighed, in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak heights, H_T and H_S , of tocopherol acetate in the sample solution and the standard solution, respectively.

$$\text{Amount (mg) of } C_{31}H_{52}O_3 = W_S \times (H_T/H_S)$$

W_S : Amount (mg) of Tocopherol Acetate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized

silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and water (49:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol acetate is about 12 minutes.

System suitability—

System performance: Dissolve 0.05 g each of Tocopherol Acetate and tocopherol in 50 mL of ethanol (99.5). When the procedure is run with 20 μL of this solution under the above operating conditions, tocopherol and tocopherol acetate are eluted in this order with the resolution between these peaks being not less than 2.6.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak heights of tocopherol acetate is not more than 0.8%.

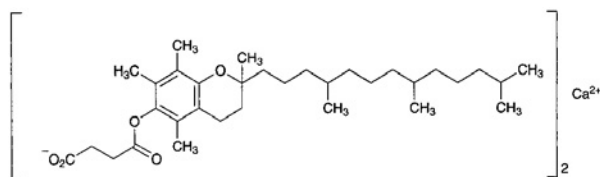
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tocopherol Calcium Succinate

Vitamin E Calcium Succinate

トコフェロールコハク酸エステルカルシウム



$\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$: 1099.62

Monocalcium bis {3-[2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yloxy-carbonyl]propanoate} [14638-18-7]

Tocopherol Calcium Succinate, when dried, contains not less than 96.0% and not more than 102.0% of $\text{C}_{66}\text{H}_{106}\text{CaO}_{10}$.

Description Tocopherol Calcium Succinate occurs as a white to yellowish white powder. It is odorless.

It is freely soluble in chloroform and in carbon tetrachloride, and practically insoluble in water, in ethanol (95) and in acetone.

Shake 1 g of Tocopherol Calcium Succinate with 7 mL of acetic acid (100); it dissolves, and produces a turbidity after being allowed to stand for a while.

It dissolves in acetic acid (100).

It is optically inactive.

Identification (1) Dissolve 0.05 g of Tocopherol Calcium Succinate in 1 mL of glacial acetic acid, add 9 mL of ethanol (99.5), and mix. To this solution add 2 mL of fuming nitric acid, and heat at 75°C for 15 minutes: a red to orange color develops.

(2) Dissolve 0.08 g of Tocopherol Calcium Succinate, previously dried, in 0.2 mL of carbon tetrachloride. Determine the infrared absorption spectrum of the solution as directed in the liquid film method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the

Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 5 g of Tocopherol Calcium Succinate in 30 mL of chloroform, add 10 mL of hydrochloric acid, shake for 10 minutes, then draw off the water layer, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (286 nm): 36.0 – 40.0 (10 mg, chloroform, 100 mL).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tocopherol Calcium Succinate in 10 mL of chloroform: the solution is clear, and has no more color than the following control solution.

Control solution: To 0.5 mL of Ferric Chloride Colorimetric Stock Solution add 0.5 mol/L hydrochloric acid TS to make 100 mL.

(2) Alkalinity—To 0.20 g of Tocopherol Calcium Succinate add 10 mL of diethyl ether, 2 mL of water, 1 drop of phenolphthalein TS and 0.10 mL of 0.1 mol/L hydrochloric acid VS, and shake: no red color develops in the water layer.

(3) Chloride <1.03>—Dissolve 0.10 g of Tocopherol Calcium Succinate in 4 mL of acetic acid (100), add 20 mL of water and 50 mL of diethyl ether, shake thoroughly, and collect the water layer. To the diethyl ether layer add 10 mL of water, shake, and collect the water layer. Combine the water layers, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution in the same manner using 0.60 mL of 0.01 mol/L hydrochloric acid VS in place of Tocopherol Calcium Succinate (not more than 0.212%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol Calcium Succinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tocopherol Calcium Succinate according to Method 3, and perform the test (not more than 2 ppm).

(6) α -Tocopherol—Dissolve 0.10 g of Tocopherol Calcium Succinate in exactly 10 mL of chloroform, and use this solution as the sample solution. Separately, dissolve 50 mg of Tocopherol Reference Standard in chloroform to make exactly 100 mL. Pipet 1 mL of this solution, add chloroform to make exactly 10 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of toluene and acetic acid (100) (19:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of iron (III) chloride hexahydrate in ethanol (99.5) (1 in 500) on the plate, then spray evenly a solution of α - α' -dipyridyl in ethanol (99.5) (1 in 200) on the same plate, and allow to stand for 2 to 3 minutes: the spots from the sample solution corresponding to the spots from the standard solution is not larger than and not more intense than the spots from the standard solution.

Loss on drying <2.41> Not more than 2.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Assay Weigh accurately about 50 mg each of Tocopherol Calcium Succinate and Tocopherol Succinate Reference Standard, previously dried, dissolve in a mixture of ethanol

(99.5) and diluted acetic acid (100) (1 in 5) (9:1) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Pipet exactly 20 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following operating conditions. Determine the peak heights, H_T and H_S , of tocopherol succinate in these solutions, respectively.

$$\begin{aligned} \text{Amount (mg) } C_{66}H_{106}CaO_{10} \\ = W_S \times (H_T/H_S) \times 1.0358 \end{aligned}$$

W_S : Amount (mg) of Tocopherol Succinate Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 284 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel (5 to 10 μ L in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of methanol, water and acetic acid (100) (97:2:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol succinate is about 8 minutes.

Selection of column: Dissolve 0.05 g each of tocopherol succinate and tocopherol in 50 mL of a mixture of ethanol (99.5) and diluted acetic acid (100) (1 in 5) (9:1). Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of tocopherol succinate and tocopherol in this order with the resolution between these peaks being not less than 2.0.

System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak height of tocopherol succinate is not more than 0.8%.

Containers and storage Containers—Tight containers.

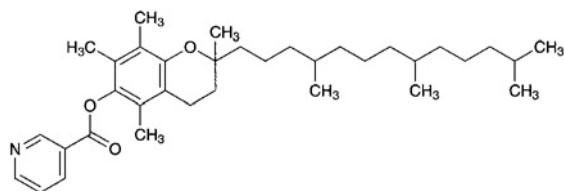
Storage—Light-resistant.

Tocopherol Nicotinate

Vitamin E Nicotinate

dl- α -Tocopherol Nicotinate

トコフェロールニコチン酸エステル



$C_{35}H_{53}NO_3$: 535.80

2,5,7,8-Tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl nicotinate [51898-34-1]

Tocopherol Nicotinate contains not less than 96.0% of nicotinic acid *dl*- α -tocopherol ($C_{35}H_{53}NO_3$).

Description Tocopherol Nicotinate occurs as a yellow to orange-yellow liquid or solid.

It is freely soluble in ethanol (99.5), and practically insoluble in water.

A solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 10) shows no optical rotation.

It is affected by light.

Identification (1) Determine the absorption spectrum of a solution of Tocopherol Nicotinate in ethanol (99.5) (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tocopherol Nicotinate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared spectrum of Tocopherol Nicotinate, if necessary melt by warming, as directed in the liquid film method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tocopherol Nicotinate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tocopherol Nicotinate according to Method 4, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tocopherol Nicotinate in 50 mL of ethanol (99.5), and use this solution as the sample solution. Pipet 7 mL of this solution, add ethanol (99.5) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than tocopherol nicotinate from the sample solution is not larger than the peak area of tocopherol nicotinate from the standard solution, and the area of a peak which has a retention time 0.8 to 0.9 times that of tocopherol nicotinate from the sample solution is not larger than 4/7 of the peak area of tocopherol nicotinate from the standard solution.

Operating conditions—

Detector, column, and column temperature: Proceed as directed in the operating conditions in the Assay.

Mobile phase: A mixture of methanol and water (19:1).

Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 20 minutes.

Time span of measurement: About 1.5 times as long as the retention time of tocopherol nicotinate beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the test solution for system suitability. Pipet 1 mL of the test solution for system suitability, add ethanol (99.5) to make exactly 10 mL. Confirm that the peak area of tocopherol nicotinate obtained from 10 μ L of this solution is equivalent to 7 to 13% of that of tocopherol nicotinate obtained from 10 μ L of the test solution for system suitability.

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol

(99.5). When the procedure is run with 10 μ L of this solution under the above operating conditions, tocopherol and tocopherol nicotinate are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 2.0%.

Assay Weigh accurately about 50 mg each of Tocopherol Nicotinate and Tocopherol Nicotinate Reference Standard, dissolve each in ethanol (99.5) to make exactly 50 mL, and use these solutions as the sample solution and standard solution, respectively. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine peak areas, A_T and A_S , of tocopherol nicotinate of these solutions.

$$\begin{aligned} \text{Amount (mg) of } C_{35}H_{53}NO_3 \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Tocopherol Nicotinate

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 264 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: Methanol

Flow rate: Adjust the flow rate so that the retention time of tocopherol nicotinate is about 10 minutes.

System suitability—

System performance: Dissolve 0.05 g of Tocopherol Nicotinate and 0.25 g of tocopherol in 100 mL of ethanol (99.5). When the procedure is run with 5 μ L of this solution under the above operating conditions, tocopherol and tocopherol nicotinate are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions: the relative standard deviation of the peak areas of tocopherol nicotinate is not more than 0.8%.

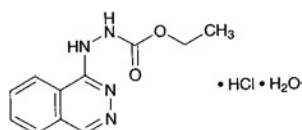
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Todalazine Hydrochloride Hydrate

Ecarazine Hydrochloride

トドラジン塩酸塩水和物



$C_{11}H_{12}N_4O_2 \cdot HCl \cdot H_2O$: 286.71

Ethyl 2-(phthalazin-1-yl)hydrazinecarboxylate

monohydrochloride monohydrate [3778-76-5, anhydride]

Todalazine Hydrochloride Hydrate contains not less than 98.5% of todalazine hydrochloride ($C_{11}H_{12}N_4O_2 \cdot HCl$: 268.70), calculated on the anhydrous basis.

Description Todalazine Hydrochloride Hydrate occurs as white crystals or crystalline powder. It has a slight, characteristic odor, and has a bitter taste.

It is very soluble in formic acid, freely soluble in methanol, soluble in water, sparingly soluble in ethanol (95), and practically insoluble in diethyl ether.

The pH of a solution of Todalazine Hydrochloride Hydrate (1 in 200) is between 3.0 and 4.0.

Identification (1) To 2 mL of a solution of Todalazine Hydrochloride Hydrate (1 in 200) add 5 mL of silver nitrate-ammonia TS: the solution becomes turbid, and a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Todalazine Hydrochloride Hydrate in 0.1 mol/L hydrochloric acid TS (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Todalazine Hydrochloride Hydrate as directed in the potassium chloride disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Todalazine Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (1) for chloride.

Purity (1) Clarity and color of solution—Dissolve 0.30 g of Todalazine Hydrochloride Hydrate in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Sulfate <1.14>—Proceed the test with 2.0 g of Todalazine Hydrochloride Hydrate. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.012%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Todalazine Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Todalazine Hydrochloride Hydrate according to Method 1, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 50 mg of Todalazine Hydrochloride Hydrate in 100 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of todalazine from the sample solution is not larger than the peak area of todalazine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 240 nm).

Column: A stainless steel column 3.9 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 1.10 g of sodium 1-heptane sulfonate in 1000 mL of diluted methanol (2 in 5). Adjust the pH of the solution to between 3.0 and 3.5 with acetic acid (100).

Flow rate: Adjust the flow rate so that the retention time of todralazine is about 8 minutes.

Time span of measurement: About twice as long as the retention time of todralazine beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 5 mL of the standard solution add the mobile phase to make exactly 25 mL. Confirm that the peak area of todralazine obtained from 10 μ L of this solution is equivalent to 15 to 25% of that of todralazine obtained from 10 μ L of the standard solution.

System performance: Dissolve 5 mg each of Todralazine Hydrochloride Hydrate and potassium biphthalate in 100 mL of the mobile phase. When the procedure is run with 10 μ L of this solution under the above operating conditions, phthalic acid and todralazine are eluted in this order with the resolution between these peaks being not less than 8.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of todralazine is not more than 2.0%.

Water <2.48> 6.0 – 7.5% (0.5 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

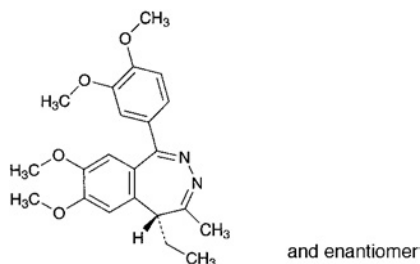
Assay Weigh accurately about 0.4 g of Todralazine Hydrochloride, dissolve in 5 mL of formic acid, add 70 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.87 mg of C₁₁H₁₂N₄O₂·HCl

Containers and storage Containers—Tight containers.

Tofisopam

トフィソパム



C₂₂H₂₆N₂O₄: 382.45
(5*RS*)-1-(3,4-Dimethoxyphenyl)-5-ethyl-7,8-dimethoxy-

4-methyl-5*H*-2,3-benzodiazepine [22345-47-7]

Tofisopam, when dried, contains not less than 98.0% of C₂₂H₂₆N₂O₄.

Description Tofisopam occurs as a pale yellowish white, crystalline powder.

It is freely soluble in acetic acid (100), soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

A solution of Tofisopam in ethanol (95) (1 in 100) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Tofisopam in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tofisopam, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 155 – 159°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tofisopam according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tofisopam according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.05 g of Tofisopam in 10 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 25 mL, pipet 1 mL of this solution, add acetone to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, methanol and formic acid (24:12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

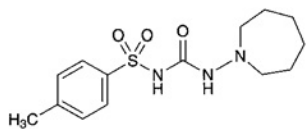
Assay Weigh accurately about 0.2 g of Tofisopam, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 38.25 mg of C₂₂H₂₆N₂O₄

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Tolazamide

トラザミド



$C_{14}H_{21}N_3O_3S$: 311.40

N-(Azepan-1-ylcarbonyl)-4-methylbenzenesulfonamide
[1156-19-0]

Tolazamide, when dried, contains not less than 97.5% and not more than 102.0% of $C_{14}H_{21}N_3O_3S$.

Description Tolazamide occurs as a white to pale yellow, crystalline powder. It is odorless.

It is freely soluble in chloroform, soluble in acetone, slightly soluble in ethanol (95) and in *n*-butylamine, and practically insoluble in water and in diethyl ether.

Melting point: about 168°C (with decomposition).

Identification (1) Dissolve 0.02 g of Tolazamide in 5 mL of water and 1 mL of *n*-butylamine, add 2 to 3 drops of copper (II) sulfate TS, and shake well. Shake well this solution with 5 mL of chloroform, and allow to stand: a green color develops in the chloroform layer.

(2) Determine the absorption spectrum of a solution of Tolazamide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolazamide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolazamide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolazamide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Tolazamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Tolazamide according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.20 g of Tolazamide in acetone to make exactly 10 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 200 mL, and use this solution as the standard solution (1). Separately, dissolve 20 mg of *p*-toluenesulfonamide in acetone to make exactly 200 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, cyclohexane and diluted ammonia

solution (28) (10 in 11) (200:100:60:23) to a distance of about 12 cm, and air-dry the plate. Heat the plate at 110°C for 10 minutes, and immediately expose to chlorine for 2 minutes. Expose the plate to cold wind until a very pale blue color develops when 1 drop of potassium iodide-starch TS is placed on a site below the starting line on the plate. Spray evenly potassium iodide-starch TS on the plate: the spot from the sample solution corresponding to the spot from the standard solution (2) is not more intense than the spot from the standard solution (2), and the spots other than the principal and above spots from the sample solution are not more intense than the spot from the standard solution (1).

(4) *N*-Aminohexamethyleneimine—To 0.50 g of Tolazamide add 2.0 mL of acetone, stopper the flask tightly, shake vigorously for 15 minutes. Add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, allow to stand for 15 minutes, and filter. To the filtrate add 1.0 mL of trisodium ferrous pentacyanoamine TS, and shake: the color developing within 30 minutes is not deeper than that of the following control solution.

Control solution: Dissolve 0.125 g of *N*-aminohexamethyleneimine in acetone to make exactly 100 mL. Pipet 1 mL of this solution, and add acetone to make exactly 100 mL. To 2.0 mL of this solution add 8.0 mL of disodium hydrogenphosphate-citric acid buffer solution, pH 5.4, shake, and proceed in the same manner.

Loss on drying <2.41> Not more 0.5% (1 g, in vacuum, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

Assay Weigh accurately about 30 mg each of Tolazamide and Tolazamide Reference Standard, previously dried, dissolve each in 10 mL of the internal standard solution, and use these solutions as the sample solution and standard solution, respectively. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tolazamide to that of the internal standard, respectively.

$$\begin{aligned} \text{Amount (mg) of } C_{14}H_{21}N_3O_3S \\ = W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Tolazamide Reference Standard

Internal standard solution—A solution of tolbutamide in ethanol-free chloroform (3 in 2000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of hexane, water-saturated hexane, tetrahydrofuran, ethanol (95) and acetic acid (100) (475:475:20:15:9).

Flow rate: Adjust the flow rate so that the retention time of tolazamide is about 12 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and tolazamide are eluted in this

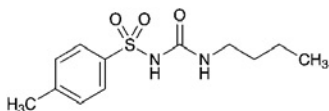
order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak area of tolbutamide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Tolbutamide

トルブタミド



$C_{12}H_{18}N_2O_3S$: 270.35

N-(Butylcarbamoyl)-4-methylbenzenesulfonamide
[64-77-7]

Tolbutamide, when dried, contains not less than 99.0% of $C_{12}H_{18}N_2O_3S$.

Description Tolbutamide occurs as white crystals or crystalline powder. It is odorless or has a slight, characteristic odor. It is tasteless.

It is soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

Identification (1) Boil 0.2 g of Tolbutamide with 8 mL of diluted sulfuric acid (1 in 3) under a reflux condenser for 30 minutes. Cool the solution in ice water, collect the precipitated crystals, recrystallize from water, and dry at 105°C for 3 hours: the crystals melt <2.60> between 135°C and 139°C.

(2) Render the filtrate obtained in (1) alkaline with about 20 mL of a solution of sodium hydroxide (1 in 5), and heat: an ammonia-like odor is perceptible.

Melting point <2.60> 126 – 132°C

Purity (1) Acidity—Warm 3.0 g of Tolbutamide with 150 mL of water at 70°C for 5 minutes, allow to stand for 1 hour in ice water, and filter. To 25 mL of the filtrate add 2 drops of methyl red TS and 0.20 mL of 0.1 mol/L sodium hydroxide VS: a yellow color develops.

(2) Chloride <1.03>—To 40 mL of the filtrate obtained in (1) add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.011%).

(3) Sulfate <1.14>—To 40 mL of the filtrate obtained in (1) add 1 mL of dilute hydrochloric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.021%).

(4) Heavy metals <1.07>—Proceed with 2.0 g of Tolbutamide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C,

3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tolbutamide, previously dried, and dissolve in 30 mL of neutralized ethanol. Add 20 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.04 mg of $C_{12}H_{18}N_2O_3S$

Containers and storage Containers—Well-closed containers.

Tolbutamide Tablets

トルブタミド錠

Tolbutamide Tablets contain not less than 95% and not more than 105% of the labeled amount of tolbutamide ($C_{12}H_{18}N_2O_3S$: 270.35).

Method of preparation Prepare as directed under Tablets, with Tolbutamide.

Identification Shake a quantity of powdered Tolbutamide Tablets, equivalent to 0.5 g of Tolbutamide according to the labeled amount, with 50 mL of chloroform, filter, and evaporate the filtrate to dryness. Proceed with the residue as directed in the Identification under Tolbutamide.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Take 1 tablet of Tolbutamide Tablets at 100 revolutions per minute according to the using 900 mL of phosphate buffer solution, pH 7.4, as the dissolution medium. Take 20 mL or more of the dissolved solution 30 minutes after the start of the test, and filter through a membrane filter (less than 0.8 μ m in pore size). Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL of a solution containing about 10 μ g of tolbutamide ($C_{12}H_{18}N_2O_3S$) per mL according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tolbutamide Reference Standard, previously dried at 105°C for 3 hours, dissolve in 10 mL of methanol, and add phosphate buffer solution, pH 7.4, to make exactly 100 mL. Pipet 2 mL of this solution, add water to make exactly 100 mL, and use this solution as standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 226 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively. The dissolution rate of Tolbutamide Tablets after 30 minutes should be not less than 80%.

Dissolution rate (%) to labeled amount
of tolbutamide ($C_{12}H_{18}N_2O_3S$)
= $W_S \times (A_T/A_S) \times (V'/V) \times (90/C) \times (1/5)$

W_S : Amount (mg) of Tolbutamide Reference Standard
 C : Labeled amount (mg) of tolbutamide ($C_{12}H_{18}N_2O_3S$) per tablet

Assay Weigh accurately and powder not less than 20 Tolbutamide Tablets. Weigh accurately a portion of the powder,

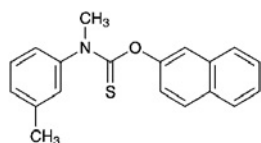
equivalent to about 0.5 g of tolbutamide ($C_{12}H_{18}N_2O_3S$), dissolve in 50 mL of neutralized ethanol, add 25 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 3 drops of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 27.04 mg of $C_{12}H_{18}N_2O_3S$

Containers and storage Containers—Well-closed containers.

Tolnaftate

トルナフタート



$C_{19}H_{17}NOS$: 307.41

O-Naphthalen-2-yl *N*-methyl-*N*-(3-methylphenyl)thiocarbamate [2398-96-1]

Tolnaftate, when dried, contains not less than 98.0% of $C_{19}H_{17}NOS$.

Description Tolnaftate occurs as a white powder. It is odorless.

It is freely soluble in chloroform, sparingly soluble in diethyl ether, slightly soluble in methanol and in ethanol (95), and practically insoluble in water.

Identification (1) To 0.2 g of Tolnaftate add 20 mL of potassium hydroxide-ethanol TS and 5 mL of water, and heat under a reflux condenser for 3 hours. After cooling, to 10 mL of this solution add 2 mL of acetic acid (100), and shake with 1 mL of lead (II) acetate TS: a black precipitate is formed.

(2) Determine the absorption spectrum of a solution of Tolnaftate in methanol (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tolnaftate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Tolnaftate, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Tolnaftate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 111 – 114°C (after drying).

Purity (1) Heavy metals <1.07>—Carbonize 1.0 g of Tolnaftate by gentle heating. After cooling, add 5 mL of nitric acid and 1 mL of sulfuric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid, and heat until white fumes are evolved. After cooling, add 2 mL of nitric acid and 0.5 mL of perchloric acid, and heat gradually until white fumes are evolved. Repeat this procedure twice, and heat until white fumes are no longer evolved. Incinerate the

residue by igniting between 500°C and 600°C for 1 hour. Proceed according to Method 2, and perform the test with 50 mL of the test solution so obtained. Prepare the control solution as follows: to 11 mL of nitric acid add 1 mL of sulfuric acid, 1 mL of perchloric acid and 2 mL of hydrochloric acid, proceed in the same manner as the test solution, and add 2.0 mL of Standard Lead Solution and water to make 50 mL (not more than 20 ppm).

(2) Related substances—Dissolve 0.50 g of Tolnaftate in 10 mL of chloroform, and use this solution as the sample solution. Pipet 2 mL of the sample solution, and add chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 5 minutes, and examine under ultraviolet light (wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum at a pressure not exceeding 0.67 kPa, 65°C, 3 hours).

Residue on ignition <2.44> Weigh accurately about 2.0 g of Tolnaftate, and carbonize by gradual heating. Moisten the substance with 1 mL of sulfuric acid, heat gradually until white fumes are no longer evolved, and ignite between 450°C and 550°C for about 2 hours to constant mass: the residue is not more than 0.1%.

Assay Weigh accurately about 50 mg of Tolnaftate and Tolnaftate Reference Standard, previously dried, dissolve each in 200 mL of methanol by warming in a water bath, cool, and add methanol to make exactly 250 mL. Pipet 5 mL each of the solutions, to each add methanol to make exactly 100 mL, and use these solutions as the sample solution and standard solution, respectively. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 257 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{19}H_{17}NOS \\ = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Tolnaftate Reference Standard

Containers and storage Containers—Tight containers.

Tolnaftate Solution

トルナフタート液

Tolnaftate Solution contains not less than 90% and not more than 110% of the labeled amount of tol-naftate ($C_{19}H_{17}NOS$: 307.41).

Method of preparation Prepare as directed under Liquids and Solutions, with Tolnaftate.

Identification (1) Spot 1 drop of Tolnaftate Solution on filter paper. Spray hydrogen hexachloroplatinate (IV)-potas-

sium iodide TS on the paper: a light yellow color develops in the spot.

(2) To a volume of Tolnaftate Solution, equivalent to 0.02 g of Tolnaftate according to the labeled amount, add chloroform to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.02 g of Tolnaftate Reference Standard in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with toluene to a distance of about 12 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot from the sample solution and that from the standard solution show the same R_f value.

Assay Pipet a volume of Tolnaftate Solution, equivalent to about 20 mg of tolinaftate ($C_{19}H_{17}NOS$), add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.4 g of Tolnaftate Reference Standard, previously dried in vacuum at a pressure not exceeding 0.67 kPa at 65°C for 3 hours, and dissolve in chloroform to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 4 mL of the internal standard solution, then add chloroform to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of tolinaftate to that of the internal standard, respectively.

$$\begin{aligned} & \text{Amount (mg) of tolinaftate (C}_{19}\text{H}_{17}\text{NOS)} \\ & = W_S \times (Q_T/Q_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Tolnaftate Reference Standard

Internal standard solution—A solution of diphenyl phthalate in chloroform (3 in 200).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of methanol and water (7:3).

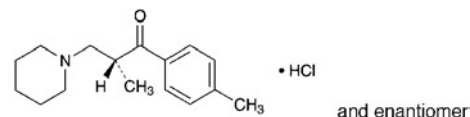
Flow rate: Adjust the flow rate so that the retention time of tolinaftate is about 14 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and tolinaftate in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Tolperisone Hydrochloride

トルペリゾン塩酸塩



$C_{16}H_{23}NO.HCl$: 281.82

(2*RS*)-2-Methyl-1-(4-methylphenyl)-3-piperidin-1-ylpropan-1-one monohydrochloride [3644-61-9]

Tolperisone Hydrochloride, when dried, contains not less than 98.5% of $C_{16}H_{23}NO.HCl$.

Description Tolperisone Hydrochloride occurs as a white, crystalline powder. It has a slight, characteristic odor.

It is very soluble in acetic acid (100), freely soluble in water and in ethanol (95), soluble in acetic anhydride, slightly soluble in acetone, and practically insoluble in diethyl ether.

It is hygroscopic.

The pH of a solution of Tolperisone Hydrochloride (1 in 20) is between 4.5 and 5.5.

Melting point: 167 – 174°C

Identification (1) Dissolve 0.2 g of Tolperisone Hydrochloride in 2 mL of ethanol (95), add 2 mL of 1,3-dinitrobenzene TS and 2 mL of sodium hydroxide TS, and heat: a red color develops.

(2) To 5 mL of a solution of Tolperisone Hydrochloride (1 in 20) add 2 to 3 drops of iodine TS: a red-brown precipitate is produced.

(3) Dissolve 0.5 g of Tolperisone Hydrochloride in 5 mL of water, add 2 mL of ammonia TS, and filter. Acidify 5 mL of the filtrate with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> for chloride.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (257 nm): 555 – 585 (after drying, 5 mg, ethanol (95), 500 mL).

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tolperisone Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test using 4.0 g of Tolperisone Hydrochloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Tolperisone Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Piperidine hydrochloride—Dissolve 0.20 g of Tolperisone Hydrochloride in water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve 1000 mg of piperidine hydrochloride in water to make exactly 1000 mL, and use this solution as the standard solution. Transfer 5.0 mL each of the sample solution and standard solution to different separators, add 0.1 mL each of a solution of copper (II) sulfate pentahydrate (1 in 20), then add 0.1 mL each of ammonia solution (28) and exactly 10 mL each of a mixture of isooctane and carbon disulfide (3:1), and shake vigorously for 30 minutes. Immediately after allowing to stand, separate

the isooctane-carbon disulfide mixture layer, and dehydrate with anhydrous sodium sulfate. Perform the test with these solutions as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of the sample solution at 438 nm is not more than that of the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

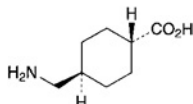
Assay Weigh accurately about 0.5 g of Tolperisone Hydrochloride, previously dried, dissolve in 70 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 28.18 mg C₁₆H₂₃NO.HCl

Containers and storage Containers—Well-closed containers.

Tranexamic Acid

トラネキサム酸



C₈H₁₅NO₂: 157.21

trans-4-(Aminomethyl)cyclohexanecarboxylic acid
[1197-18-8]

Tranexamic Acid, when dried, contains not less than 98.0% and not more than 101.0% of C₈H₁₅NO₂.

Description Tranexamic Acid occurs as white crystals or crystalline powder.

It is freely soluble in water, and practically insoluble in ethanol (99.5).

Identification Determine the infrared absorption spectrum of Tranexamic Acid as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Tranexamic Acid Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> The pH of a solution prepared by dissolving 1.0 g of Tranexamic Acid in 20 mL of water is between 7.0 and 8.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tranexamic Acid in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 1.0 g of Tranexamic Acid. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.014%).

(3) Heavy metals—Dissolve 2.0 g of Tranexamic Acid in water to make 20 mL, and use this solution as the sample stock solution. To 12 mL of the sample stock solution add 2 mL of hydrochloric acid-ammonium acetate buffer solu-

tion, pH 3.5, mix, add 1.2 mL of thioacetamide TS, mix immediately, and use this solution as the sample solution. Separately, proceed in the same manner as above with a mixture of 1 mL of Standard Lead Solution, 2 mL of the sample stock solution and 9 mL of water, and use the solution so obtained as the standard solution. Separately, proceed in the same manner with a mixture of 10 mL of water and 2 mL of the sample stock solution, and use the solution so obtained as the control solution. Conform that the color of the standard solution is slightly darker than that of the control solution. Compare the sample solution and the standard solution 2 minutes after they are prepared: the color of the sample solution is not more intense than that of the standard solution (not more than 10 ppm).

(4) Arsenic <1.11>—Prepare the test solution by dissolving 1.0 g of Tranexamic Acid in 10 mL of water, and perform the test (not more than 2 ppm).

(5) Related substances—Dissolve 0.20 g of Tranexamic Acid in water to make exactly 20 mL, and use this solution as the sample solution. Pipet 5 mL of the sample solution, and add water to make exactly 100 mL. Pipet 1 mL of this solution, add water to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area multiplied by relative response factor 1.2 of the peak, having the relative retention time of about 1.5 with respect to tranexamic acid, is not more than 2/5 of the peak area of tranexamic acid from the standard solution, and the area of the peak, having the relative retention time of about 2.1 with respect to tranexamic acid, is not more than 1/5 of the peak area of tranexamic acid from the standard solution. The area of each peak other than tranexamic acid and other than the peaks mentioned above is not more than 1/5 of the peak area of tranexamic acid from the standard solution. For this comparison, use the area of the peaks, having the relative retention time of about 1.1 and about 1.3, after multiplying by their relative response factors 0.005 and 0.006, respectively. The total area of the peaks other than tranexamic acid is not more than the peak area of tranexamic acid from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 3 times as long as the retention time of tranexamic acid beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 5 mL of the standard solution add water to make exactly 25 mL. Confirm that the peak area of tranexamic acid obtained from 20 μL of this solution is equivalent to 14 to 26% of that from 20 μL of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 7%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C,

2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Tranexamic Acid and Tranexamic Acid Reference Standard, previously dried, dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of tranexamic acid.

$$\text{Amount (mg) of } C_8H_{15}NO_2 = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Tranexamic Acid Reference Standard
Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 6.0 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 11.0 g of sodium dihydrogen phosphate in 500 mL of water, and add 5 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid or diluted phosphoric acid (1 in 10), add water to make 600 mL, and add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 20 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 0.6%.

Containers and storage Containers—Well-closed containers.

Tranexamic Acid Capsules

トラネキサム酸カプセル

Tranexamic Acid Capsules contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Capsules, with Tranexamic Acid.

Identification Take an amount of powdered contents of Tranexamic Acid Capsules, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and heat for 3 minutes: a dark purple color de-

velops.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 capsule of Tranexamic Acid Capsules at 50 revolutions per minute according to the Paddle method using a sinker and using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolved solution 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL, add water to make exactly V' mL so that each mL contains about 0.28 mg of tranexamic acid ($C_8H_{15}NO_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 28 mg of Tranexamic Acid Reference Standard, previously dried at 105°C for 2 hours, dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of tranexamic acid, A_T and A_S . The dissolution rate in 15 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of tranexamic acid ($C_8H_{15}NO_2$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 900$$

W_S : Amount (mg) of Tranexamic Acid Reference Standard

C : Labeled amount (mg) of tranexamic acid ($C_8H_{15}NO_2$) in 1 capsule

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 220 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 11.0 g of anhydrous sodium dihydrogen phosphate in 500 mL of water, and add 10 mL of triethylamine and 1.4 g of sodium lauryl sulfate. Adjust the pH to 2.5 with phosphoric acid, add water to make 600 mL, and add 400 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of tranexamic acid are not less than 4000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 2.0%.

Assay Weigh accurately the mass of the contents of not less than 20 Tranexamic Acid Capsules, and powder. Weigh accurately an amount of the powder, equivalent to about 0.1 g

of tranexamic acid ($C_8H_{15}NO_2$), add 30 mL of water, shake well, and add water to make exactly 50 mL. Centrifuge, filter the supernatant liquid through a membrane filter with pore size of not more than $0.45 \mu m$, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid Reference Standard, previously dried at $105^\circ C$ for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly $30 \mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of tranexamic acid.

$$\begin{aligned} &\text{Amount (mg) of tranexamic acid (C}_8\text{H}_{15}\text{NO}_2\text{)} \\ &= W_S \times (A_T/A_S) \times 2 \end{aligned}$$

W_S : Amount (mg) of Tranexamic Acid Reference Standard

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

Column temperature: A constant temperature of about $35^\circ C$.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with $30 \mu L$ of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with $30 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Tranexamic Acid Injection

トラネキサム酸注射液

Tranexamic Acid Injection is an aqueous injection.

It contains not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Injections, with Tranexamic Acid.

Description Tranexamic Acid Injection is a clear and colorless liquid.

Identification To a volume of Tranexamic Acid Injection, equivalent to 50 mg of Tranexamic Acid according to the labeled amount, add water to make 5 mL, add 1 mL of ninhydrin TS, and heat: a dark purple color develops.

pH <2.54> 7.0 – 8.0

Bacterial endotoxins <4.01> Not more than 0.12 EU/mg.

Extractable volume <6.05> It meets the requirement.

Foreign insoluble matter <6.06> Perform the test according to Method 1: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take accurately a volume of Tranexamic Acid Injection, equivalent to about 0.1 g of tranexamic acid ($C_8H_{15}NO_2$), add water to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid Reference Standard, previously dried at $105^\circ C$ for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly $30 \mu L$ each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of tranexamic acid.

$$\begin{aligned} &\text{Amount (mg) of tranexamic acid (C}_8\text{H}_{15}\text{NO}_2\text{)} \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Tranexamic Acid Reference Standard

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

Column temperature: A constant temperature of about $35^\circ C$.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with $30 \mu L$ of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with $30 \mu L$ of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Hermetic containers.

Tranexamic Acid Tablets

トラネキサム酸錠

Tranexamic Acid Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of tranexamic acid ($C_8H_{15}NO_2$: 157.21).

Method of preparation Prepare as directed under Tablets, with Tranexamic Acid.

Identification To an amount of powdered Tranexamic Acid Tablets, equivalent to 0.5 g of Tranexamic Acid according to the labeled amount, add 50 mL of water, shake well, and filter. To 5 mL of the filtrate add 1 mL of ninhydrin TS, and

heat for 3 minutes: a dark purple color develops.

Uniformity of dosage units <6.02> It meets the requirement of the Mass variation test.

Dissolution Being specified separately.

Assay Weigh accurately the mass of not less than 20 Tranexamic Acid Tablets, and powder. Weigh accurately a quantity of the powder, equivalent to about 5 g of tranexamic acid (C₈H₁₅NO₂), add 150 mL of water, disintegrate the tablets completely with the aid of ultrasonic waves, and add water to make exactly 200 mL. Centrifuge, pipet 4 mL of the supernatant liquid, and add water to make exactly 50 mL. Filter through a membrane filter with pore size of not more than 0.45 μm, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 50 mg of Tranexamic Acid Reference Standard, previously dried at 105°C for 2 hours, dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 30 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of tranexamic acid.

$$\begin{aligned} &\text{Amount (mg) of tranexamic acid (C}_8\text{H}_{15}\text{NO}_2) \\ &= W_S \times (A_T/A_S) \times 100 \end{aligned}$$

W_S: Amount (mg) of Tranexamic Acid Reference Standard

Operating conditions—

Detector, column, and mobile phase: Proceed as directed in the operating conditions in the Assay under Tranexamic Acid.

Column temperature: A constant temperature of about 35°C.

Flow rate: Adjust the flow rate so that the retention time of tranexamic acid is about 16 minutes.

System suitability—

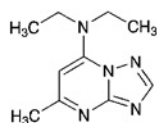
System performance: To 5 mL of the standard solution add 1 mL of a solution of 4-(aminomethyl)benzoic acid (1 in 10,000) and water to make 50 mL. When the procedure is run with 30 μL of this solution under the above operating conditions, tranexamic acid and 4-(aminomethyl)benzoic acid are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 30 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of tranexamic acid is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trapidil

トラピジル



C₁₀H₁₅N₅: 205.26

7-Diethylamino-5-methyl[1,2,4]triazolo[1,5-a]pyrimidine

[15421-84-8]

Trapidil, when dried, contains not less than 98.5% of C₁₀H₁₅N₅.

Description Trapidil occurs as a white to pale yellowish white, crystalline powder.

It is very soluble in water and in methanol, freely soluble in ethanol (95), in acetic anhydride and in acetic acid (100), and sparingly soluble in diethyl ether.

The pH of a solution of Trapidil (1 in 100) is between 6.5 and 7.5.

Identification (1) To 5 mL of a solution of Trapidil (1 in 50) add 3 drops of Dragendorff's TS: an orange color develops.

(2) Determine the absorption spectrum of a solution of Trapidil (1 in 125,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Absorbance <2.24> E_{1cm}^{1%} (307 nm): 860 – 892 (after drying, 20 mg, water, 2500 mL).

Melting point <2.60> 101 – 105°C

Purity (1) Clarity and color of solution—Dissolve 2.5 g of Trapidil in 10 mL of water: the solution is clear and colorless to pale yellow.

(2) Chloride <1.03>—Perform the test with 0.5 g of Trapidil. Prepare the control solution with 0.25 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.018%).

(3) Ammonium—Place 0.05 g of Trapidil in a glass-stoppered conical flask, thoroughly moisten with 10 drops of sodium hydroxide TS, and stopper the flask. Allow it to stand at 37°C for 15 minutes: the gas evolved does not change moistened red litmus paper to blue.

(4) Heavy metals <1.07>—Dissolve 1.0 g of Trapidil in 40 mL of water, and add 1.5 mL of dilute hydrochloric acid, 2 mL of dilute acetic acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 10 ppm).

(5) Arsenic <1.11>—Prepare the test solution with 1.0 g of Trapidil according to Method 1, and perform the test (not more than 2 ppm).

(6) Related substances—Dissolve 0.10 g of Trapidil in 4 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add methanol to make exactly 20 mL. Pipet 1 mL of this solution, add methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (85:13:2) to a distance of about 10 cm, and air-dry the plate. Allow the plate to stand in iodine vapor for 60 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, in vacuum, silica gel, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

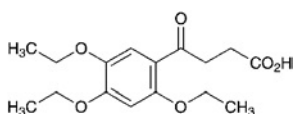
Assay Weigh accurately about 0.2 g of Trepidil, previously dried, dissolve in 20 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.53 mg of C₁₀H₁₅N₅

Containers and storage Containers—Tight containers.

Trepibutone

トレピブトン



C₁₆H₂₂O₆: 310.34
4-Oxo-4-(2,4,5-triethoxyphenyl)butanoic acid
[41826-92-0]

Trepibutone, when dried, contains not less than 98.5% of C₁₆H₂₂O₆.

Description Trepibutone occurs as white to yellowish white crystals or crystalline powder. It is odorless, and is tasteless or has a slight, characteristic aftertaste.

It is soluble in acetone, sparingly soluble in ethanol (95), slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification (1) Determine the absorption spectrum of a solution of Trepibutone in diluted dilute sodium hydroxide TS (1 in 10) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the spectrum of a solution of Trepibutone in deuterated chloroform for the nuclear magnetic resonance spectroscopy (1 in 10), using tetramethylsilane for the nuclear magnetic resonance spectroscopy as an internal reference compound, as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (¹H): it exhibits a sharp multiple signal A at around δ 1.5 ppm, a triplet signal B at around δ 2.7 ppm, a triplet signal C at around δ 3.3 ppm, a multiple signal D at around δ 4.2 ppm, a sharp single signal E at around δ 6.4 ppm, a sharp single signal F at around δ 7.4 ppm, and a single signal G at around δ 10.5 ppm. The ratio of integrated intensity of each signal, A:B:C:D:E:F:G, is about 9:2:2:6:1:1:1.

Melting point <2.60> 146 – 150°C

Purity (1) Chloride <1.03>—Dissolve 0.5 g of Trepibutone in 30 mL of acetone, and add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.30 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL

(not more than 0.021%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Trepibutone according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 0.10 g of Trepibutone in 10 mL of acetone, and use this solution as the sample solution. Pipet 2 mL of the sample solution, add acetone to make exactly 100 mL. To exactly 10 mL of this solution add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of isopropylether, acetone, water and formic acid (100:30:3:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm); the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

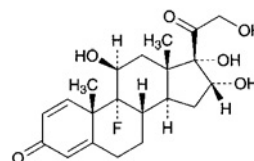
Assay Weigh accurately about 0.5 g of Trepibutone, previously dried, dissolve in 50 mL of ethanol (95), add 50 mL of water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 31.03 mg of C₁₆H₂₂O₆

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Triamcinolone

トリアムシノロン



C₂₁H₂₇FO₆: 394.43
9-Fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione [124-94-7]

Triamcinolone, when dried, contains not less than 97.0% and not more than 103.0% of C₂₁H₂₇FO₆.

Description Triamcinolone occurs as a white, crystalline powder. It is odorless.

It is freely soluble in *N,N*-dimethylformamide, slightly soluble in methanol, in ethanol (95) and in acetone, and practically insoluble in water, in 2-propanol and in diethyl ether.

Melting point: about 264°C (with decomposition).

Identification (1) Dissolve 1 mg of Triamcinolone in 6 mL of ethanol (95), add 5 mL of 2,6-di-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath for 30

minutes under a reflux condenser: a red-purple color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When combustion is completed, shake vigorously so as to absorb the gas evolved: the solution responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the infrared absorption spectrum of Triamcinolone, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone and Triamcinolone Reference Standard in 7 mL of a mixture of 2-propanol and water (2:1), respectively, by warming. Allow the solutions to cool in ice to effect crystals, filter, then wash the formed crystals with two 10-mL portions of water, and repeat the test on the dried crystals.

Optical rotation <2.49> $[\alpha]_D^{20}$: +65 – +71° (after drying, 0.1 g, *N,N*-dimethylformamide, 10 mL, 100 mm).

Purity Heavy metals <1.07>—Proceed with 0.5 g of Triamcinolone according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.3% (0.5 g, platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone and Triamcinolone Reference Standard, previously dried and accurately weighed, in a solution of L-ascorbic acid in methanol (1 in 1000) to make exactly 50 mL. Pipet 5 mL each of these solutions, add exactly 5 mL each of the internal standard solution, add a solution of L-ascorbic acid in methanol (1 in 1000) to make 20 mL, and use these solutions as the sample solution and standard solution. Perform the test with 10 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of triamcinolone to that of the internal standard, respectively.

$$\text{Amount (mg) of } C_{21}H_{27}FO_6 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Triamcinolone Reference Standard

Internal standard solution—Dissolve 15 mg of methyl parahydroxybenzoate in a solution of L-ascorbic acid in methanol (1 in 1000) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.0 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, triamcinolone and the internal standard are eluted in this order with the resolution between these peaks being not less than 2.0.

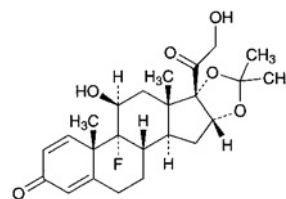
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone to that of the internal standard is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Triamcinolone Acetonide

トリアムシノロンアセトニド



$C_{24}H_{31}FO_6$: 434.50

9-Fluoro-11 β ,21-dihydroxy-16 α ,17-(1-methylethylidenedioxy)pregna-1,4-diene-3,20-dione [76-25-5]

Triamcinolone Acetonide, when dried, contains not less than 97.0% and not more than 103.0% of $C_{24}H_{31}FO_6$.

Description Triamcinolone Acetonide occurs as a white, crystalline powder. It is odorless.

It is sparingly soluble in ethanol (99.5), in acetone, and in 1,4-dioxane, slightly soluble in methanol and in ethyl ether, and practically insoluble in water and in diethyl ether.

Melting point: about 290°C (with decomposition).

Identification (1) Dissolve 2 mg of Triamcinolone Acetonide in 40 mL of ethanol (95), add 5 mL of 2,6-di-*tert*-butylcresol TS and 5 mL of sodium hydroxide TS, and heat on a water bath under a reflux condenser for 20 minutes: a green color develops.

(2) Add 5 mL of water and 1 mL of Fehling's TS to 0.01 g of Triamcinolone Acetonide, and heat: a red precipitate is produced.

(3) Proceed with 0.01 g of Triamcinolone Acetonide as directed under Oxygen Flask Combustion Method <1.06>, using a mixture of 0.5 mL of 0.01 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid. When combustion is completed, shake vigorously so as to absorb the gas evolved: the solution responds to the Qualitative Tests <1.09> for fluoride.

(4) Determine the absorption spectrum of a solution of Triamcinolone Acetonide in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>.

and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Triamcinolone Acetonide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(5) Determine the infrared absorption spectrum of Triamcinolone Acetonide, previously dried, as directed in the potassium bromide disk method under the Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of previously dried Triamcinolone Acetonide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers. If any difference appears between the spectra, dissolve 0.1 g each of Triamcinolone Acetonide and Triamcinolone Acetonide Reference Standard in 20 mL of ethanol (95), respectively, then evaporate the ethanol to dryness, and repeat the test on the dried residue.

Optical rotation <2.49> $[\alpha]_D^{20}$: +100 – +107° (after drying, 0.1 g, 1,4-dioxane, 10 mL, 100 mm).

Purity (1) Heavy metals <1.07>—Proceed with 0.5 g of Triamcinolone Acetonide according to Method 2, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 30 ppm).

(2) Related substances—Dissolve 40 mg of Triamcinolone Acetonide in 4 mL of acetone, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add acetone to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (93:7) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 2.0% (0.5 g, in vacuum, phosphorus (V) oxide, 60°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g, platinum crucible).

Assay Dissolve about 20 mg each of Triamcinolone Acetonide and Triamcinolone Acetonide Reference Standard, previously dried and accurately weighed, in methanol to make exactly 50 mL. Pipet 10 mL each of these solutions, add exactly 10 mL each of the internal standard solution, then add the mobile phase to make 50 mL, and use these solutions as the sample solution and standard solution. Perform the test with 20 μ L each of these solutions as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak height of triamcinolone acetonide to that of the internal standard, respectively.

$$\begin{aligned} &\text{Amount (mg) of } C_{24}H_{31}FO_6 \\ &= W_S \times (Q_T/Q_S) \end{aligned}$$

W_S : Amount (mg) of Triamcinolone Acetonide Reference Standard

Internal standard solution—A solution of prednisolone in methanol (1 in 50,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 30 cm in length, packed with octadecylsilanized silica gel (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of water and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of triamcinolone acetonide is about 13 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and triamcinolone acetonide are eluted in this order with the resolution between these peaks being not less than 6.

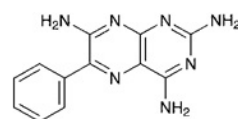
System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratios of the peak height of triamcinolone acetonide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Triamterene

トリアムテレン



$C_{12}H_{11}N_7$: 253.26

6-Phenylpteridine-2,4,7-triamine [396-01-0]

Triamterene, when dried, contains not less than 98.5% of $C_{12}H_{11}N_7$.

Description Triamterene occurs as a yellow, crystalline powder. It is odorless, and tasteless.

It is sparingly soluble in dimethylsulfoxide, very slightly soluble in acetic acid (100), and practically insoluble in water, in ethanol (95), and in diethyl ether.

It dissolves in nitric acid and in sulfuric acid, but does not dissolve in dilute nitric acid, in dilute sulfuric acid and in dilute hydrochloric acid.

Identification (1) To 0.01 g of Triamterene add 10 mL of water, heat, and filter after cooling: the filtrate shows a purple fluorescence. To 2 mL of the filtrate add 0.5 mL of hydrochloric acid: the fluorescence disappears.

(2) The filtrate obtained in (1) responds to the Qualitative Tests <1.09> for primary aromatic amines.

(3) Dissolve 0.01 g of Triamterene in 100 mL of acetic acid (100), and to 10 mL of the solution add water to make 100 mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Triamterene according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Triamterene according to Method 3, and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 0.10 g of Triamterene in 20 mL of dimethylsulfoxide. To 2 mL of this solution add methanol to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ammonia solution (28) and methanol (9:1:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (main wavelength: 365 nm): the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.10% (1 g).

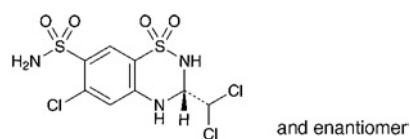
Assay Weigh accurately about 0.15 g of Triamterene, previously dried, and dissolve in 100 mL of acetic acid (100) by warming. Titrate <2.50> with 0.05 mol/L perchloric acid VS (indicator: 2 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.05 mol/L perchloric acid VS
= 12.663 mg of C₁₂H₁₁N₇

Containers and storage Containers—Well-closed containers.

Trichlormethiazide

トリクロルメチアジド



C₈H₈Cl₃N₃O₄S₂: 380.66

(3*RS*)-6-Chloro-3-dichloromethyl-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide [133-67-5]

Trichlormethiazide, when dried, contains not less than 97.5% and not more than 102.0% of C₈H₈Cl₃N₃O₄S₂.

Description Trichlormethiazide occurs as a white powder.

It is freely soluble in *N,N*-dimethylformamide and in acetone, slightly soluble in acetonitrile and in ethanol (95), and practically insoluble in water.

A solution of Trichlormethiazide in acetone (1 in 50) shows no optical rotation.

Melting point: about 270°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trichlormethiazide in ethanol (95) (3 in 250,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Trichlormethiazide Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trichlormethiazide as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Trichlormethiazide Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Perform the test with Trichlormethiazide as directed under Flame Coloration Test <1.04> (2): a green color appears.

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Trichlormethiazide in 30 mL of acetone, add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.01 mol/L hydrochloric acid VS add 30 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.036%).

(2) Sulfate <1.14>—Dissolve 1.0 g of Trichlormethiazide in 30 mL of acetone, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 1.0 mL of 0.005 mol/L sulfuric acid VS add 30 mL of acetone, 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Trichlormethiazide according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 0.6 g of Trichlormethiazide according to Method 5, using 20 mL of *N,N*-dimethylformamide, and perform the test (not more than 3.3 ppm).

(5) Related substances—Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile, and use the solution as the sample solution. Perform the test with 10 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of related substances by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 2.0%, and the total amount of the related substances is not more than 2.5%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 10	100	0
10 – 20	100 → 0	0 → 100

Flow rate: 1.5 mL per minute

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the sample solution add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that of trichlormethiazide obtained from 10 μ L of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath at 60°C for 30 minutes. When the procedure is run with 10 μ L of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide with respect to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 25 mg of Trichlormethiazide and Trichlormethiazide Reference Standard, previously dried, and dissolve separately in exactly 20 mL of the internal standard solution. To 1 mL of these solutions add acetonitrile to make 20 mL, and use these solutions as the sample solution and the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of trichlormethiazide to that of the internal standard.

$$\text{Amount (mg) of } C_8H_8Cl_3N_3O_4S_2 = W_S \times (Q_T/Q_S)$$

W_S : Amount (mg) of Trichlormethiazide Reference Standard

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 800).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Well-closed containers.

Trichlormethiazide Tablets

トリクロルメチアジド錠

Trichlormethiazide Tablets contain not less than 93.0% and not more than 107.0% of the labeled amount of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$: 380.66).

Method of preparation Prepare as directed under Tablets, with Trichlormethiazide.

Identification To an amount of pulverized Trichlormethiazide Tablets, equivalent to 4 mg of Trichlormethiazide according to the labeled amount, add 10 mL of acetone, shake vigorously for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 4 mg of Trichlormethiazide Reference Standard in 10 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and methanol (10:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the principal spots from the sample solution and the standard solution show the same R_f value.

Purity Related substances—Pulverize a suitable amount of Trichlormethiazide Tablets in an agate mortar. Take an amount of the powder, equivalent to 10 mg of Trichlormethiazide according to the labeled amount, add 20 mL of acetonitrile, shake vigorously for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with 10 μ L of the sample solution as directed under

Liquid Chromatography <2.01> according to the following conditions, determine each peak area by the automatic integration method, and calculate the amount of each related substance by the area percentage method: the amount of 4-amino-6-chlorobenzene-1,3-disulfonamide, having the relative retention time of about 0.3 with respect to trichlormethiazide, is not more than 4.0%, and the total amount of the peaks other than trichlormethiazide is not more than 5.0%.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Mobile phase B: A mixture of acetonitrile and diluted phosphoric acid (1 in 1000) (3:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 - 10	100	0
10 - 20	100 \rightarrow 0	0 \rightarrow 100

Flow rate: 1.5 mL/minute

Time span of measurement: About 2.5 times as long as the retention time of trichlormethiazide beginning after the solvent peak.

System suitability—

Test for required detectability: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. Pipet 1 mL of this solution, add acetonitrile to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 1 mL of the solution for system suitability test, and add acetonitrile to make exactly 20 mL. Confirm that the peak area of trichlormethiazide obtained from 10 μ L of this solution is equivalent to 3.5 to 6.5% of that obtained from 10 μ L of the solution for system suitability test.

System performance: To 5 mL of the solution for system suitability test add 5 mL of water, and warm in a water bath of 60°C for 30 minutes. When the procedure is run with 10 μ L of this solution, after cooling, under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide with respect to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 3 times with 10 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Uniformity of dosage units <6.02> Perform the test accord-

ing to the following method: it meets the requirement of the Content uniformity test.

Perform the test according to the following method: it meets the requirements of the Content Uniformity Test. To one tablet of Trichlormethiazide Tablets add 5 mL of diluted phosphoric acid (1 in 50) to disintegrate. Add exactly an amount of the internal standard solution, equivalent to 10 mL per 2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) according to the labeled amount, add acetonitrile to make 25 mL, shake vigorously for 15 minutes, and centrifuge. To an amount of the supernatant liquid add the mobile phase to make a solution so that it contains about 40 μ g of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) in each mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Trichlormethiazide Reference Standard, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the conditions described in the Assay, and determine the ratios, Q_T and Q_S , of the peak area of trichlormethiazide to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of trichlormethiazide } (C_8H_8Cl_3N_3O_4S_2) \\ &= W_S \times (Q_T/Q_S) \times C \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Trichlormethiazide Reference Standard

C : Labeled amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) per tablet

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Trichlormethiazide Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of water as the dissolution medium. Withdraw 20 mL or more of the dissolution medium 15 minutes after starting the test, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet the subsequent V mL of the filtrate, add diluted phosphoric acid (1 in 50) to make exactly V' mL so that each mL contains about 1.1 μ g of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of Trichlormethiazide Reference Standard, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 2 mL of this solution, add diluted phosphoric acid (1 in 50) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 40 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{Ta} and A_{Sa} , of trichlormethiazide obtained with the sample solution and the standard solution, and the area, A_{Tb} , of the peak, having the relative retention time of about 0.3 with respect to trichlormethiazide, obtained with the sample solution. The dissolution rate in 15 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of

trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$)

$$= W_S \times [(A_{Ta} + A_{Tb} \times 0.95) / A_{Sa}] \times (V' / V) \times (1 / C) \times (9 / 2)$$

W_S : Amount (mg) of Trichlormethiazide Reference Standard

C: Labeled amount (mg) of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) per tablet

Operating conditions—

Proceed as directed in the operating conditions in the Assay.

System suitability—

System performance: Dissolve 25 mg of Trichlormethiazide in 50 mL of acetonitrile. To 1 mL of this solution add acetonitrile to make 50 mL. To 5 mL of this solution add 5 mL of water, and heat at 60°C in a water bath for 30 minutes. After cooling, when the procedure is run with 10 μ L of this solution under the above operating conditions, 4-amino-6-chlorobenzene-1,3-disulfonamide and trichlormethiazide are eluted in this order, the relative retention time of 4-amino-6-chlorobenzene-1,3-disulfonamide with respect to trichlormethiazide is about 0.3, and the number of theoretical plates and the symmetry factor of the peak of trichlormethiazide are not less than 5000 and not more than 1.2, respectively.

System repeatability: When the test is repeated 6 times with 40 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichlormethiazide is not more than 2.0%.

Assay Weigh accurately the mass of not less than 20 Trichlormethiazide Tablets, and pulverize the tablets in an agate mortar. Weigh accurately an amount of the powder, equivalent to about 2 mg of trichlormethiazide ($C_8H_8Cl_3N_3O_4S_2$) according to the labeled amount, add 5 mL of diluted phosphoric acid (1 in 50) and exactly 10 mL of the internal standard solution, add 10 mL of acetonitrile, shake vigorously for 15 minutes, and centrifuge. To 2 mL of the supernatant liquid add 2 mL of the mobile phase, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of Trichlormethiazide Reference Standard, previously dried at 105°C for 3 hours, and dissolve in acetonitrile to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add 10 mL of acetonitrile and 5 mL of diluted phosphoric acid (1 in 50), and use this solution as the standard solution. Perform the test with 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of trichlormethiazide to that of the internal standard.

$$\text{Amount (mg) of trichlormethiazide (C}_8\text{H}_8\text{Cl}_3\text{N}_3\text{O}_4\text{S}_2) \\ = W_S \times (Q_T / Q_S) \times (1 / 20)$$

W_S : Amount (mg) of Trichlormethiazide Reference Standard

Internal standard solution—A solution of 3-nitrophenol in acetonitrile (1 in 5000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 268 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilanized silica gel for liquid chromatography (5 μ m in particle

diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:1).

Flow rate: Adjust the flow rate so that the retention time of trichlormethiazide is about 8 minutes.

System suitability—

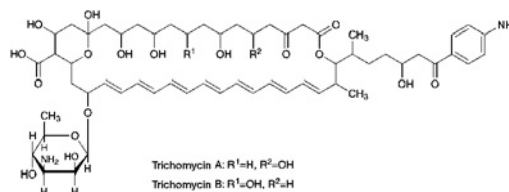
System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the internal standard and trichlormethiazide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trichlormethiazide to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trichomycin

トリコマイシン



Trichomycin A

33-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,9,11,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [12698-99-6]

Trichomycin B

33-(3-Amino-3,6-dideoxy- β -D-mannopyranosyloxy)-17-[6-(4-aminophenyl)-4-hydroxy-1-methyl-6-oxohexyl]-1,3,5,7,9,37-hexahydroxy-18-methyl-13,15-dioxo-16,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid [12699-00-2] [1394-02-1, Trichomycin]

Trichomycin is a mixture of polyene macrolide substances having antifungal and antiprotozoal activities produced by the growth of *Streptomyces hachijoensis*.

It contains not less than 7000 Units per mg, calculated on the dried basis. The potency of Trichomycin is expressed as unit based on the amount of trichomycin. One unit of Trichomycin is equivalent to 0.05 μ g of trichomycin.

Description Trichomycin occurs as a yellow to yellow-brown powder.

It is practically insoluble in water, in ethanol (99.5) and in tetrahydrofuran.

It dissolves in dilute sodium hydroxide TS.

It is hygroscopic.

Identification (1) To 2 mg of Trichomycin add 2 mL of

sulfuric acid: a blue color appears, and the color is changed to a blue-purple after allowing to stand.

(2) Dissolve 1 mg of Trichomycin in 50 mL of a solution of sodium hydroxide (1 in 200). Determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 359 nm and 365 nm, between 378 nm and 384 nm, and between 400 nm and 406 nm.

Content ratio of the active principle Conduct this procedure without exposure to daylight, using light-resistant vessels. Dissolve about 10 mg of Trichomycin in 50 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1), and use this solution as the sample solution. Perform the test with 5 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions, determine the peak areas by the automatic integration method, and calculate the amount of trichomycin A and trichomycin B by the area percentage method: the amount of trichomycin A is between 20% and 40%, and that of trichomycin B is between 15% and 25%. The relative retention time of trichomycin B with respect to trichomycin A is about 1.2.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogen phosphate and 1.7 g of sodium lauryl sulfate in a mixture of 600 mL of water and 400 mL of acetonitrile for liquid chromatography.

Flow rate: Adjust the flow rate so that the retention time of trichomycin A is about 8 minutes.

Time span of measurement: About 4 times as long as the retention time of trichomycin A.

System suitability—

Test for required detectability: Measure exactly 5 mL of the sample solution, add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 50 mL, and use this solution as the solution for system suitability test. Pipet 5 mL of the solution for system suitability test, and add a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 30 mL. Confirm that the peak area of trichomycin A obtained from 5 μ L of this solution is equivalent to 12 to 22% of that from 5 μ L of the solution for system suitability test.

System performance: When the procedure is run with 5 μ L of the solution for system suitability test under the above operating conditions, trichomycin A and trichomycin B are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 5 μ L of the solution for system suitability test under the above operating conditions, the relative standard deviation of the peak area of trichomycin A is not more than 2.0%.

Loss on drying <2.41> Not more than 5.0% (1 g, in vacuum, 60°C, 3 hours).

Assay Conduct this procedure without exposure to day-

light, using light-resistant vessels. Weigh accurately an amount of Trichomycin and Trichomycin Reference Standard, equivalent to about 150,000 units, dissolve them separately in a mixture of tetrahydrofuran for liquid chromatography and water (3:1) to make exactly 100 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of trichomycin.

$$\text{Amount (unit) of trichomycin} = W_S \times (A_T/A_S)$$

W_S : Amount (unit) of Trichomycin Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with silica gel for liquid chromatography (10 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: Dissolve 15 g of ammonium acetate in 120 mL of water, and add 1000 mL of acetonitrile for liquid chromatography and 700 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of trichomycin is about 6 minutes.

System suitability—

System performance: Dissolve 5 mg of Trichomycin and 1 mg of berberine chloride in 100 mL of a mixture of tetrahydrofuran for liquid chromatography and water (3:1). When the procedure is run with 20 μ L of this solution under the above operating conditions, berberine and trichomycin are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trichomycin is not more than 2.0%.

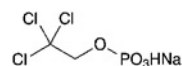
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Triclofos Sodium

Monosodium Trichloroethyl Phosphate

トリクロホスナトリウム



$C_2H_3Cl_3NaO_4P$: 251.37

Monosodium 2,2,2-trichloroethyl monohydrogenphosphate [7246-20-0]

Triclofos Sodium, when dried, contains not less than 97.0 % and not more than 102.0 % of $C_2H_3Cl_3NaO_4P$, and not less than 41.0% and not more than 43.2% of chlorine (Cl: 35.45).

Description Triclofos Sodium is a white, crystalline pow-

der.

It is freely soluble in water, slightly soluble in ethanol (95), and practically insoluble in diethyl ether.

It is hygroscopic.

Identification (1) Determine the infrared absorption spectrum of Triclofos Sodium as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) To 0.5 g of Triclofos Sodium add 10 mL of nitric acid, evaporate on a water bath to dryness, and ignite further over a flame. Dissolve the residue in 5 mL of water, and filter if necessary: the filtrate responds to Qualitative Tests <1.09> for sodium salt.

(3) To 0.1 g of Triclofos Sodium add 1 g of anhydrous sodium carbonate, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride. The remainder of the filtrate responds to the Qualitative Tests <1.09> (1) for chloride and to the Qualitative Tests <1.09> for phosphate.

pH <2.54> Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the pH of this solution is between 3.0 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Triclofos Sodium in 50 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.20 g of Triclofos Sodium. Prepare the control solution with 1.0 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.178%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Triclofos Sodium according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Triclofos Sodium according to Method 1, and perform the test (not more than 2 ppm).

(5) Free phosphoric acid—Weigh accurately about 0.3 g of Triclofos Sodium, previously dried, dissolve in water to make exactly 100 mL, and use this solution as the sample solution. Pipet 5 mL each of the sample solution and Standard Phosphoric Acid Solution, add 2.5 mL of hexaammonium heptamolybdate-sulfuric acid TS and 1 mL of 1-amino-2-naphthol-4-sulfonic acid TS, shake, add water to make exactly 25 mL, and allow to stand at 20°C for 30 minutes. Perform the test with these solutions, using a solution obtained in the same manner with 5 mL of water as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>. Determine the absorbances, A_T and A_S , of each solution from the sample solution and Standard Phosphoric Acid Solution at 740 nm: the content of the free phosphoric acid is not more than 1.0%.

$$\begin{aligned} \text{Content (\%)} & \text{ of the free phosphoric acid (H}_3\text{PO}_4\text{)} \\ & = (A_T/A_S) \times (1/W) \times 287.8 \end{aligned}$$

W : Amount (mg) of the sample taken.

Loss on drying <2.41> Not more than 5.0% (1 g, in vacuum, 100°C, 3 hours).

Assay (1) Triclofos sodium—Weigh accurately about 0.2

g of Triclofos Sodium, previously dried, place in a Kjeldhal flask, add 2 mL of sulfuric acid and 2.5 mL of nitric acid, and heat until brown gas are not evolved. After cooling, add 1 mL of nitric acid, heat until white fumes are produced, and cool. Repeat this procedure until the solution becomes colorless. Transfer this solution to a flask using 150 mL of water, add 50 mL of molybdenum (III) oxide-citric acid TS, heat gently to boil, add gradually 25 mL of quinoline TS with stirring, and heat on a water bath for 5 minutes. After cooling, filter the precipitate, and wash repeatedly with water until the washing does not indicate acidity. Transfer the precipitate to a flask using 100 mL of water, add exactly 50 mL of 0.5 mol/L sodium hydroxide VS, dissolve, and titrate <2.50> with 0.5 mol/L hydrochloric acid VS until the color of the solution changes from purple to yellow (indicator: 3 drops of phenolphthalein-thymol blue TS). Perform a blank determination.

$$\begin{aligned} \text{Each mL of 0.5 mol/L sodium hydrochloride VS} \\ = 4.834 \text{ mg of C}_2\text{H}_3\text{Cl}_3\text{NaO}_4\text{P} \end{aligned}$$

(2) Chlorine—Weigh accurately about 10 mg of Triclofos Sodium, previously dried, perform the test according to the procedure of determination for chlorine as directed under Oxygen Flask Combustion Method <1.06>, using 1 mL of 1 mol/L sodium hydroxide TS and 20 mL of water as the absorbing liquid.

Containers and storage Containers—Tight containers.

Triclofos Sodium Syrup

Monosodium Trichloroethyl Phosphate Syrup

トリクロホスナトリウムシロップ

Triclofos Sodium Syrup contains not less than 90% and not more than 110% of the labeled amount of triclofos sodium (C₂H₃Cl₃NaO₄P: 251.37).

Method of preparation Prepare as directed under Syrups, with Triclofos Sodium.

Identification (1) Weigh a portion of Triclofos Sodium Syrup, equivalent to 0.25 g of Triclofos Sodium according to the labeled amount, add 40 mL of water, shake well, add 5 mL of diluted sulfuric acid (3 in 50), and extract with 25 mL of 3-methyl-1-butanol. Take 5 mL of the extract, evaporate on a water bath to dryness, and add 1 mL of diluted sulfuric acid (1 in 2) and 1 mL of a solution of potassium permanganate (1 in 20) to the residue. Heat in a water bath for 5 minutes, add 7 mL of water, and then add a solution of oxalic acid dihydrate (1 in 20) until the color of the solution disappears. To 1 mL of this solution add 1 mL of pyridine and 1 mL of a solution of sodium hydroxide (1 in 5), and heat in a water bath, while shaking, for 1 minute: a light red color develops in the pyridine layer.

(2) Take 10 mL of the extract obtained in (1), evaporate on a water bath to dryness, add 1 g of anhydrous sodium carbonate to the residue, and heat for 10 minutes. After cooling, dissolve the residue in 40 mL of water, filter if necessary, and render the filtrate acidic with dilute nitric acid: the solution responds to the Qualitative Tests <1.09> (2) for chloride. The

remainder of the filtrate responds to the Qualitative Tests <1.09> (1) for chloride and to the Qualitative Tests <1.09> for phosphate.

pH <2.54> 6.0 – 6.5

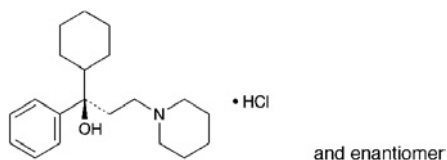
Assay Weigh accurately a portion of Triclofos Sodium Syrup, equivalent to 0.13 g of Triclofos Sodium according to the labeled amount, add 15 mL of water, 1 mL of sodium hydroxide TS and 15 mL of diethyl ether, shake for 1 minute, and separate the water layer. Wash the diethyl ether layer with 1 mL of water, and combine the washing with above water layer. To this solution add 2.5 mL of diluted sulfuric acid (3 in 50), and extract with four 10-mL portions of 3-methyl-1-butanol. Combine the 3-methyl-1-butanol extracts, and add 3-methyl-1-butanol to make exactly 50 mL. Measure exactly 10 mL each of this solution, and dilute with potassium hydroxide-ethanol TS. Place in a glass ampule, fire-seal, mix, and heat at 120°C for 2 hours in an autoclave. After cooling, transfer the contents to a flask, add 20 mL of diluted nitric acid (63 in 500) and exactly 25 mL of 0.02 mol/L silver nitrate VS, shake well, and titrate <2.50> the excess silver nitrate with 0.02 mol/L ammonium thiocyanate VS (indicator: 2 to 3 drops of ammonium iron (III) sulfate TS). Perform a blank determination.

Each mL of 0.02 mol/L silver nitrate VS
= 1.676 mg of $C_{20}H_{31}Cl_3NaO_4P$

Containers and storage Containers—Tight containers.
Storage—In a cold place.

Trihexyphenidyl Hydrochloride

トリヘキシフェニジル塩酸塩



$C_{20}H_{31}NO.HCl$: 337.93
(1*RS*)-1-Cyclohexyl-1-phenyl-3-(piperidin-1-yl)propan-1-ol
monohydrochloride [52-49-3]

Trihexyphenidyl Hydrochloride, when dried, contains not less than 98.5% of $C_{20}H_{31}NO.HCl$.

Description Trihexyphenidyl Hydrochloride occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is soluble in ethanol (95), sparingly soluble in acetic acid (100), slightly soluble in water, very slightly soluble in acetic anhydride, and practically insoluble in diethyl ether.

Melting point: about 250°C (with decomposition).

Identification (1) Dissolve 1 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool. Use this solution as the sample solution. To 5 mL of the sample solution add 1 mL of a solution of 2,4,6-trinitrophenol in chloroform (1 in 50), and shake vigorously: a yellow precipitate is formed.

(2) To 20 mL of the sample solution obtained in (1) add 2 mL of sodium hydroxide TS: a white precipitate is formed.

Collect the precipitate, wash with a small amount of water, recrystallize from methanol, and dry in a desiccator (in vacuum, silica gel) for 2 hours: the crystals so obtained melt <2.60> between 113°C and 117°C.

(3) The sample solution obtained in (1) responds to the Qualitative Tests <1.09> (2) for chloride.

pH <2.54> Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming, and cool: the pH of this solution is between 5.0 and 6.0.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Trihexyphenidyl Hydrochloride in 100 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals <1.07>—Dissolve 1.5 g of Trihexyphenidyl Hydrochloride in 60 mL of water by warming on a water bath at 80°C, cool, and filter. To 40 mL of the filtrate add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) Piperidylpropiophenone—Dissolve 0.10 g of Trihexyphenidyl Hydrochloride in 40 mL of water and 1 mL of 1 mol/L hydrochloric acid VS by warming, cool, and add water to make 100 mL. Determine the absorbance of this solution at 247 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance is not more than 0.50.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trihexyphenidyl Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (1:1), and titrate <2.50> with 0.1 mol/L perchloric acid-dioxane VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid-dioxane VS
= 33.793 mg of $C_{20}H_{31}NO.HCl$

Containers and storage Containers—Tight containers.

Trihexyphenidyl Hydrochloride Tablets

トリヘキシフェニジル塩酸塩錠

Trihexyphenidyl Hydrochloride Tablets contain not less than 93% and not more than 107% of the labeled amount of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$: 337.93).

Method of preparation Prepare as directed under Tablets, with Trihexyphenidyl Hydrochloride.

Identification (1) Weigh a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.1 g of Trihexyphenidyl Hydrochloride according to the labeled amount, add 30 mL of chloroform, shake, and filter. Evaporate the filtrate on a water bath to dryness. Dissolve the residue in 10 mL of water by warming, cool, and use this

solution as the sample solution. With 5 mL of the sample solution, proceed as directed in the Identification (1) under Trihexyphenidyl Hydrochloride.

(2) Shake a quantity of powdered Trihexyphenidyl Hydrochloride Tablets, equivalent to 0.01 g of Trihexyphenidyl Hydrochloride according to the labeled amount, with 5 mL of chloroform, filter, and use the filtrate as the sample solution. Dissolve 0.02 g of Trihexyphenidyl Hydrochloride Reference Standard in 10 mL of chloroform, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform and methanol (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly hydrogen hexachloroplatinate (IV)-potassium iodide TS on the plate: the spots from the sample solution and the standard solution show a blue-purple color and the same *R_f* value.

(3) The sample solution obtained in (1) responds to the Qualitative Tests <1.09> (2) for chloride.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To one tablet of Trihexyphenidyl Hydrochloride Tablets add 2 mL of dilute hydrochloric acid and 60 mL of water, disintegrate by vigorous shaking for 10 minutes, and warm on a water bath with occasional shaking for 10 minutes. Cool, add 2 mL of methanol, and add water to make exactly *V* mL of the solution contains about 20 μ g of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) per mL. Centrifuge, if necessary, and use the supernatant liquid as the sample solution. Separately, dissolve about 20 mg of Trihexyphenidyl Hydrochloride Reference Standard (determine previously its loss on drying <2.41> in the same manner as Trihexyphenidyl Hydrochloride) in methanol to make exactly 20 mL. Pipet 2 mL of this solution, and add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and the standard solution, transfer to glass-stoppered centrifuge tubes, add exactly 10 mL of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL of chloroform, stopper tightly, shake well, and centrifuge. Pipet 10 mL each of the chloroform layers, add chloroform to make exactly 50 mL. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

$$\begin{aligned} &\text{Amount (mg) of trihexyphenidyl hydrochloride} \\ &(\text{C}_{20}\text{H}_{31}\text{NO.HCl}) \\ &= W_S \times (A_T/A_S) \times (V/1000) \end{aligned}$$

W_S : Amount (mg) of Trihexyphenidyl Hydrochloride Reference Standard, calculated on the dried basis

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Trihexyphenidyl Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Take 30 mL or more of the dissolved solution 30 minutes after starting the test, and filter through a membrane filter with pore size of not

more than 0.8 μ m. Discard the first 10 mL of the filtrate, and use the subsequent as the sample solution. Separately, weigh accurately about 10 mg of Trihexyphenidyl Hydrochloride Reference Standard, previously dried at 105°C for 3 hours, and dissolve in 2nd fluid for dissolution test to make exactly 100 mL. Pipet 2 mL of this solution, add 2nd fluid for dissolution test to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution, the standard solution and 2nd fluid for dissolution test add exactly 1 mL of diluted acetic acid (31) (1 in 10), and immediately add 5 mL of bromocresol green-sodium hydroxide-acetic acid-sodium acetate TS, and shake. Then, add exactly 10 mL each of dichloromethane, shake well, centrifuge, and take the dichloromethane layer. Determine the absorbances, A_T , A_S and A_B , of these dichloromethane layers at 415 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using dichloromethane as a blank. The dissolution rate of Trihexyphenidyl Hydrochloride Tablets in 30 minutes should be not less than 70%.

$$\begin{aligned} &\text{Dissolution rate (\%)} \text{ with respect to the labeled amount} \\ &\text{of trihexyphenidyl hydrochloride (C}_{20}\text{H}_{31}\text{NO.HCl)} \\ &= W_S \times [(A_T - A_B)/(A_S - A_B)] \times (1/C) \times 18 \end{aligned}$$

W_S : Amount (mg) of Trihexyphenidyl Hydrochloride Reference Standard

C: Labeled amount (mg) of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$) in 1 tablet

Assay Weigh accurately and powder not less than 20 Trihexyphenidyl Hydrochloride Tablets. Weigh accurately a portion of the powder, equivalent to about 5 mg of trihexyphenidyl hydrochloride ($C_{20}H_{31}NO.HCl$), dissolve in 2 mL of dilute hydrochloric acid and 60 mL of water by warming on a water bath for 10 minutes with occasional shaking. After cooling, add 2 mL of methanol and water to make exactly 100 mL, and use this solution as the sample solution. Dissolve about 50 mg of Trihexyphenidyl Hydrochloride Reference Standard (determine previously its loss on drying <2.41> in the same manner as Trihexyphenidyl Hydrochloride), weighed accurately, in methanol, add methanol to make exactly 20 mL. Pipet 2 mL of this solution, add 2 mL of dilute hydrochloric acid and water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and the standard solution into glass-stoppered centrifuge tubes, add exactly 10 mL each of bromocresol purple-dipotassium hydrogenphosphate-citric acid TS and 15 mL each of chloroform, stopper tightly, shake thoroughly, and centrifuge. Pipet 10 mL each of the chloroform layers, and add chloroform to make exactly 50 mL. Determine the absorbances, A_T and A_S , of the subsequent solutions of the sample solution and standard solution at 408 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

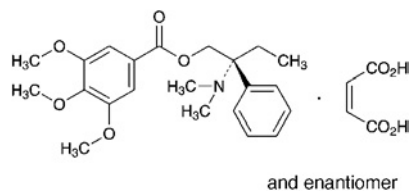
$$\begin{aligned} &\text{Amount (mg) of trihexyphenidyl hydrochloride} \\ &(\text{C}_{20}\text{H}_{31}\text{NO.HCl}) \\ &= W_S \times (A_T/A_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of Trihexyphenidyl Hydrochloride Reference Standard, calculated on the dried basis

Containers and storage Containers—Tight containers.

Trimebutine Maleate

トリメブチンマレイン酸塩



$C_{22}H_{29}NO_5 \cdot C_4H_4O_4$; 503.54
(*2RS*)-2-Dimethylamino-2-phenylbutyl 3,4,5-trimethoxybenzoate monomaleate [34140-59-5]

Trimebutine Maleate, when dried, contains not less than 98.5% and not more than 101.0% of trimebutine maleate ($C_{22}H_{29}NO_5 \cdot C_4H_4O_4$).

Description Trimebutine Maleate occurs as white, crystals or crystalline powder.

It is freely soluble in *N,N*-dimethylformamide and in acetic acid (100), soluble in acetonitrile, and slightly soluble in water and in ethanol (99.5).

It dissolves in 0.01 mol/L hydrochloric acid TS.

A solution of it in *N,N*-dimethylformamide (1 in 20) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Trimebutine Maleate in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimebutine Maleate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 131 – 135°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Trimebutine Maleate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 2.0 g of Trimebutine Maleate according to Method 3, and perform the test (not more than 1 ppm).

(3) Related substances—Dissolve 0.10 g of Trimebutine Maleate in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 250 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than maleic acid and trimebutine from the sample solution is not more than 1/2 times the peak area of trimebutine from the stan-

dard solution, and the total area of the peaks other than maleic acid and trimebutine is not more than the peak area of trimebutine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: To 650 mL of diluted perchloric acid (17 in 20,000), previously adjusted the pH to 3.0 with a solution of ammonium acetate (1 in 1000), add 1 g of sodium 1-pentanesulfonate to dissolve. To 650 mL of this solution add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of trimebutine is about 9 minutes.

Time span of measurement: About 2 times as long as the retention time of trimebutine beginning after the peak of maleic acid.

System suitability—

Test for required detectability: Measure exactly 5 mL of the standard solution, and add a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7) to make exactly 20 mL. Confirm that the peak area of trimebutine obtained from 20 μ L of this solution is equivalent to 20 to 30% of that from 20 μ L of the standard solution.

System performance: Dissolve 40 mg of Trimebutine Maleate and 20 mg of imipramine hydrochloride in 100 mL of a mixture of 0.01 mol/L hydrochloric acid TS and acetonitrile (13:7). When the procedure is run with 20 μ L of this solution under the above operating conditions, trimebutine and imipramine are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimebutine is not more than 5%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

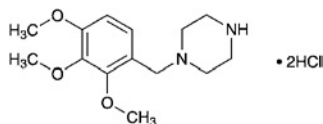
Assay Weigh accurately about 0.8 g of Trimebutine Maleate, previously dried, dissolve in 70 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS until the color of the solution changes from purple through blue to blue-green (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 50.35 mg of $C_{22}H_{29}NO_5 \cdot C_4H_4O_4$

Containers and storage Containers—Well-closed containers.

Trimetazidine Hydrochloride

トリメタジジン塩酸塩



$C_{14}H_{22}N_2O_3 \cdot 2HCl$: 339.26

1-(2,3,4-Trimethoxybenzyl)piperazine dihydrochloride
[13171-25-0]

Trimetazidine Hydrochloride contains not less than 98.0% and not more than 101.0% of $C_{14}H_{22}N_2O_3 \cdot 2HCl$, calculated on the dried basis.

Description Trimetazidine Hydrochloride occurs as a white, crystalline powder.

It is very soluble in water and in formic acid, sparingly soluble in methanol, and slightly soluble in ethanol (99.5).

The pH of a solution of Trimetazidine Hydrochloride (1 in 20) is between 2.3 and 3.3.

Melting point: about 227°C (with decomposition).

Identification (1) Determine the absorption spectrum of a solution of Trimetazidine Hydrochloride in 0.1 mol/L hydrochloric acid TS (1 in 6250) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetazidine Hydrochloride as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetazidine Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Trimetazidine Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 0.5 g of Trimetazidine Hydrochloride in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of diethylamine and cyclohexane (1:1) to a distance of about 10 cm, air-dry the plate, and then dry at 110°C for 1 hour. After cooling, examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot and the spot at the starting point are not more intense than the spots from the standard solution.

Water <2.48> Not more than 1.5% (2 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.12 g of Trimetazidine Hydrochloride, dissolve in 5 mL of formic acid, add exactly 15 mL of 0.1 mol/L perchloric acid VS, and heat at 90–100°C for 30 minutes. After cooling, add 45 mL of acetic acid (100), and titrate <2.50> the excess perchloric acid with 0.1 mol/L sodium acetate VS (potentiometric titration). Perform a blank determination in the same manner.

Each mL of 0.1 mol/L perchloric acid VS
= 16.96 mg of $C_{14}H_{22}N_2O_3 \cdot 2HCl$

Containers and storage Containers—Tight containers.

Trimetazidine Hydrochloride Tablets

トリメタジジン塩酸塩錠

Trimetazidine Hydrochloride Tablets contain not less than 94.0% and not more than 106.0% of the labeled amount of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3 \cdot 2HCl$: 339.26).

Method of preparation Prepare as directed under Tablets, with Trimetazidine Hydrochloride.

Identification Shake a quantity of powdered Trimetazidine Hydrochloride Tablets, equivalent to 10 mg of Trimetazidine Hydrochloride according to the labeled amount, with 10 mL of a mixture of ethanol (95) and water (3:1), and filter. Evaporate the filtrate on a water bath, add 2 mL of water to the residue, and shake. To 1 mL of this solution add 1 mL of *p*-benzoquinone TS, boil gently for 2 to 3 minutes, and cool: a red color develops.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Trimetazidine Hydrochloride Tablets add 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1), and treat with ultrasonic waves for 10 minutes. Shake the solution for 10 minutes, and add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL. Centrifuge, pipet *V* mL of the supernatant liquid, equivalent to about 0.75 mg of trimetazidine hydrochloride ($C_{14}H_{22}N_2O_3 \cdot 2HCl$), add exactly 2.5 mL of the internal standard solution, add 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay, separately determined the water content <2.48> in the same manner as Trimetazidine Hydrochloride, and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Proceed as directed in the Assay.

Amount (mg) of trimetazidine hydrochloride
($C_{14}H_{22}N_2O_3 \cdot 2HCl$) = $W_S \times (Q_T/Q_S) \times (1/2V)$

W_S : Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxyben-

zoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Trimetazidine Hydrochloride Tablets at 50 revolutions per minute according to the Paddle method using 900 mL of water as the dissolution medium. Withdraw not less than 20 mL of the dissolved solution 45 minutes after starting the test, and filter through a membrane filter with a pore size of not more than 0.45 μm . Discard the first 10 mL of the filtrate, pipet V mL of the subsequent filtrate, and add water to make exactly V' mL so that each mL contains about 3.3 μg of trimetazidine hydrochloride ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$) according to the labeled amount. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the sample solution. Separately, weigh accurately about 17 mg of trimetazidine hydrochloride for assay, separately determined the water content in the same manner as Trimetazidine Hydrochloride, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, and add water to make exactly 25 mL. Pipet 3 mL of this solution, add exactly 3 mL of 0.1 mol/L hydrochloric acid TS, and use this solution as the standard solution. Perform the test with exactly 50 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of trimetazidine. The dissolution rate in 45 minutes is not less than 80%.

Dissolution rate (%) with respect to the labeled amount of trimetazidine hydrochloride ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 18$$

W_S : Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

C : Labeled amount (mg) of trimetazidine hydrochloride ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$) in 1 tablet

Operating conditions—

Proceed as directed in the Assay.

System suitability—

System performance: When the procedure is run with 50 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of trimetazidine are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 50 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetazidine is not more than 1.5%.

Assay Weigh accurately not less than 20 tablets of Trimetazidine Hydrochloride Tablets, and powder. Weigh accurately a portion of the powder, equivalent to about 3 mg of trimetazidine hydrochloride ($\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}$), add about 15 mL of a mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1), and treat with ultrasonic waves for 10 minutes. Then shake for 10 minutes, add the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 20 mL, and centrifuge. To exactly 5 mL of the supernatant liquid add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL,

and use this solution as the sample solution. Separately, weigh accurately about 30 mg of trimetazidine hydrochloride for assay, separately determined the water content <2.48> in the same manner as Trimetazidine Hydrochloride, and dissolve in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) to make exactly 200 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and 0.1 mol/L hydrochloric acid TS to make 50 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of trimetazidine to that of the internal standard.

$$\text{Amount (mg) of trimetazidine hydrochloride} \\ (\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3 \cdot 2\text{HCl}) = W_S \times (Q_T/Q_S) \times 1/10$$

W_S : Amount (mg) of trimetazidine hydrochloride for assay, calculated on the anhydrous basis

Internal standard solution—A solution of parahydroxybenzoic acid in the mixture of 0.1 mol/L hydrochloric acid TS and ethanol (99.5) (1:1) (7 in 40,000).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 230 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L potassium dihydrogen phosphate TS, pH 3.0 and methanol (17:3).

Flow rate: Adjust the flow rate so that the retention time of trimetazidine is about 7 minutes.

System suitability—

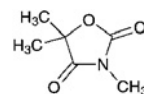
System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, trimetazidine and the internal standard are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the peak area of trimetazidine to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Trimethadione

トリメタジオン



$\text{C}_6\text{H}_9\text{NO}_3$: 143.14

3,5,5-Trimethyl-1,3-oxazolidine-2,4-dione [127-48-0]

Trimethadione, when dried, contains not less than 98.0% of $\text{C}_6\text{H}_9\text{NO}_3$.

Description Trimethadione occurs as white crystals or crystalline powder. It has a camphor-like odor.

It is very soluble in ethanol (95) and in chloroform, freely soluble in diethyl ether, and soluble in water.

Identification (1) To 5 mL of a solution of Trimethadione (1 in 50) add 2 mL of barium hydroxide TS: a precipitate is formed immediately.

(2) Determine the infrared absorption spectrum of a solution of Trimethadione in chloroform (1 in 50) as directed in the solution method under Infrared Spectrophotometry <2.25>, using a 0.1-mm fixed sodium chloride cell, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 45 – 47°C

Purity Heavy metals <1.07>—Proceed with 2.0 g of Trimethadione according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

Loss on drying <2.41> Not more than 0.5% (1 g, silica gel, 6 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Trimethadione, previously dried, in a glass-stoppered conical flask, dissolve in 5 mL of ethanol (95), add exactly measured 50 mL of 0.1 mol/L sodium hydroxide VS, stopper, and allow to stand for 15 minutes with occasional shaking. Titrate <2.50> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium hydroxide VS
= 14.31 mg of C₆H₉NO₃

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Trimethadione Tablets

トリメタジオン錠

Trimethadione Tablets contain not less than 94% and not more than 106% of the labeled amount of trimethadione (C₆H₉NO₃: 143.14).

Method of preparation Prepare as directed under Tablets, with Trimethadione.

Identification (1) Weigh a portion of powdered Trimethadione Tablets, equivalent to 1 g of Trimethadione according to the labeled amount, add 10 mL of petroleum benzin, and shake frequently for 15 minutes. Decant, remove the petroleum benzin, add another 10 mL of petroleum benzin, and repeat the extraction in the same manner. To the residue add 25 mL of diethyl ether, allow to stand for 20 minutes with occasional shaking, filter, evaporate the filtrate at room temperature, and dry the residue in a desiccator (silica gel) for 6 hours: the residue melts <2.60> between 44°C and 47°C. Proceed with this residue as directed in the Identifica-

tion (1) under Trimethadione.

(2) Determine the infrared absorption spectrum of a solution of the residue obtained in (1) in chloroform (1 in 50) in a 0.1-mm fixed sodium chloride cell, as directed in the solution method under Infrared Spectrophotometry <2.25>: it exhibits absorption at the wave numbers of about 2960 cm⁻¹, 1814 cm⁻¹, 1735 cm⁻¹, 1445 cm⁻¹, 1394 cm⁻¹, 1290 cm⁻¹, 1100 cm⁻¹ and 1055 cm⁻¹.

Assay Weigh accurately and powder not less than 20 Trimethadione Tablets. Weigh accurately a portion of the powder, equivalent to about 1 g of trimethadione (C₆H₉NO₃), add 50 mL of ethanol (95), and boil gently for 15 minutes under a reflux condenser. Filter the warm ethanol (95) solution into a 100-mL volumetric flask through a glass filter (G4), and wash the residue with three 10-mL portions of warm ethanol (95). Combine the washings with the filtrate in the flask, cool, and add ethanol (95) to make exactly 100 mL. Pipet 25 mL of the solution into a glass-stoppered conical flask, add 25 mL of water and exactly 30 mL of 0.1 mol/L sodium hydroxide VS, stopper, allow to stand for 15 minutes with occasional shaking, and titrate <2.50> the excess sodium hydroxide with 0.1 mol/L hydrochloric acid VS (indicator: 4 drops of cresol red TS). Perform a blank determination.

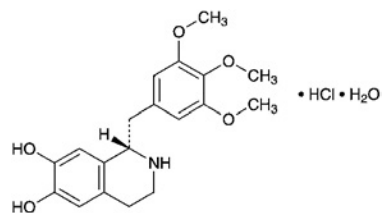
Each mL of 0.1 mol/L sodium hydroxide VS
= 14.31 mg of C₆H₉NO₃

Containers and storage Containers—Tight containers.
Storage—Not exceeding 30°C.

Trimetoquinol Hydrochloride Hydrate

Tretoquinol Hydrochloride

トリメトキノール塩酸塩水和物



C₁₉H₂₃NO₅·HCl·H₂O: 399.87

(1S)-1-(3,4,5-Trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol monohydrochloride monohydrate [18559-59-6, anhydride]

Trimetoquinol Hydrochloride Hydrate contains not less than 98.5% and not more than 101.0% of trimetoquinol hydrochloride (C₁₉H₂₃NO₅·HCl: 381.85), calculated on the anhydrous basis.

Description Trimetoquinol Hydrochloride Hydrate occurs as white, crystals or crystalline powder.

It is freely soluble in methanol, and sparingly soluble in water and in ethanol (99.5).

Melting point: about 151°C (with decomposition, after drying in vacuum, 105°C, 4 hours).

Identification (1) Determine the absorption spectrum of a

solution of Trimetoquinol Hydrochloride Hydrate in 0.01 mol/L hydrochloric acid TS (1 in 20,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Trimetoquinol Hydrochloride Hydrate as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Trimetoquinol Hydrochloride Hydrate (1 in 50) responds to the Qualitative Tests <1.09> (1) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: $-16 - -19^\circ$ (0.25 g, calculated on the anhydrous basis, water, after warming and cooling, 25 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Trimetoquinol Hydrochloride Hydrate in 100 mL of water by warming, and cool: the pH of this solution is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Trimetoquinol Hydrochloride Hydrate in 10 mL of water by warming: the solution is clear and colorless.

(2) Sulfate <1.14>—Perform the test with 0.5 g of Trimetoquinol Hydrochloride Hydrate. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.038%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Trimetoquinol Hydrochloride Hydrate according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Related substances—Dissolve 50 mg of Trimetoquinol Hydrochloride Hydrate in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution, as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than that of trimetoquinol from the sample solution is not larger than the peak area of trimetoquinol from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2 g of potassium dihydrogen phosphate and 2 g of sodium 1-pentane sulfonate in 1000 mL of water. Adjust with phosphoric acid to a pH between 2.8 and 3.2, and filter through a membrane filter with pore size of 0.4 μ m. Add 200 mL of acetonitrile to 800 mL of the filtrate.

Flow rate: Adjust the flow rate so that the retention time of trimetoquinol is about 7 minutes.

Time span of measurement: About twice as long as the

retention time of trimetoquinol beginning after the solvent peak.

System suitability—

Test for required detection: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of trimetoquinol obtained from 20 μ L of this solution is equivalent to 7 to 13% of that of trimetoquinol obtained from 20 μ L of the standard solution.

System performance: Dissolve 5 mg of Trimetoquinol Hydrochloride Hydrate and 1 mg of procaine hydrochloride in 50 mL of the mobile phase. When the procedure is run with 20 μ L of this solution under the above operating conditions, procaine and trimetoquinol are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of trimetoquinol is not more than 2.0%.

Water <2.48> 3.5 – 5.5% (0.3 g, volumetric titration, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Trimetoquinol Hydrochloride Hydrate, dissolve in 2 mL of 0.1 mol/L hydrochloric acid VS and 70 mL of ethanol (99.5) with thorough shaking, and titrate <2.50> with 0.1 mol/L potassium hydroxide-ethanol VS (potentiometric titration). Calculate the consumed volume of 0.1 mol/L potassium hydroxide-ethanol VS between the first inflection point and of the second inflection point.

Each mL of 0.1 mol/L potassium hydroxide-ethanol VS
= 38.19 mg of $C_{19}H_{23}NO_5 \cdot HCl$

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Dental Triozinc Paste

歯科用トリオジンクパスタ

Dental Triozinc Paste consists of a powder containing Paraformaldehyde, Thymol, anhydrous zinc sulfate and Zinc Oxide, and a solution containing Cresol, Potash Soap and Glycerin. Suitable amounts of the two components are triturated before use.

Method of preparation

(1) The powder	
Paraformaldehyde, finely powdered	10 g
Thymol, finely powdered	3 g
Zinc Sulfate Hydrate	9 g
Zinc Oxide	82 g

To make about 100 g

Heat Zinc Sulfate Hydrate at about 250°C to obtain anhydrous zinc sulfate, cool, and pulverize to a fine powder. Mix homogeneously this powder with Thymol, Paraformaldehyde, and Zinc Oxide.

(2) The solution

Cresol	40 g
Potash Soap	40 g
Glycerin	20 g
To make 100 g	

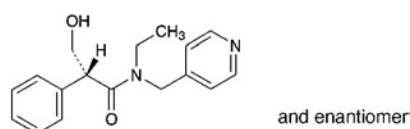
Dissolve Potash Soap in a mixture of Cresol and Glycerin.

Description The powder occurs as a fine, white powder, having a characteristic odor. The solution is a clear, yellow-brown to red-brown, viscous liquid, having the odor of cresol.

Containers and storage Containers—Tight containers.

Tropicamide

トロピカミド



$C_{17}H_{20}N_2O_2$: 284.35
(2*RS*)-*N*-Ethyl-3-hydroxy-2-phenyl-*N*-(pyridin-4-ylmethyl)propanamide [1508-75-4]

Tropicamide, when dried, contains not less than 98.5% of $C_{17}H_{20}N_2O_2$.

Description Tropicamide occurs as a white, crystalline powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95) and in chloroform, slightly soluble in water and in diethyl ether, and practically insoluble in petroleum ether.

It dissolves in dilute hydrochloric acid.

The pH of a solution of Tropicamide (1 in 500) is between 6.5 and 8.0.

Identification (1) To 5 mg of Tropicamide add 0.5 mL of a solution of ammonium vanadate (V) in sulfuric acid, (1 in 200), and heat: a blue-purple color develops.

(2) Dissolve 5 mg of Tropicamide in 1 mL of ethanol (95) and 1 mL of water, add 0.1 g of 1-chloro-2,4-dinitrobenzene, and heat on a water bath for 5 minutes. Cool, and add 2 to 3 drops of a solution of sodium hydroxide (1 in 10) and 3 mL of ethanol (95): a red-purple color develops.

Absorbance <2.24> $E_{1\text{cm}}^{1\%}$ (255 nm): 166 – 180 (after drying, 5 mg, 2 mol/L hydrochloric acid TS, 200 mL).

Melting point <2.60> 96 – 99°C

Purity (1) Chloride <1.03>—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 6 mL of dilute nitric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.45 mL of 0.01 mol/L hydrochloric acid VS, 30 mL of ethanol (95), 6 mL of dilute nitric acid, and add water to make 50 mL (not more than 0.016%).

(2) Heavy metals <1.07>—Dissolve 1.0 g of Tropicamide in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 30 mL of ethanol (95), 2 mL of

dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(3) *N*-Ethyl- γ -picolylamine—Dissolve 0.10 g of Tropicamide in 5 mL of water by heating, add 1 mL of a solution of acetaldehyde (1 in 20), and shake well. Add 1 to 2 drops of sodium pentacyanonitrosylferrate (III) TS and 1 to 2 drops of sodium hydrogen carbonate TS, and shake: no blue color develops.

(4) Tropic acid—To 10 mg of Tropicamide add 5 mg of sodium borate and 7 drops of 4-dimethylaminobenzaldehyde TS, and heat in a water bath for 3 minutes. Cool in ice water, and add 5 mL of acetic anhydride: no red-purple color develops.

Loss on drying <2.41> Not more than 0.30% (1 g, in vacuum, silica gel, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tropicamide, previously dried, dissolve in 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (indicator: 3 drops of crystal violet TS). Perform a blank determination, and make any necessary correction.

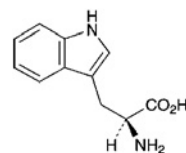
Each mL of 0.1 mol/L perchloric acid VS
= 28.44 mg of $C_{17}H_{20}N_2O_2$

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

L-Tryptophan

L-トリプトファン



$C_{11}H_{12}N_2O_2$: 204.23
(2*S*)-2-Amino-3-(indol-3-yl)propanoic acid [73-22-3]

L-Tryptophan, when dried, contains not less than 98.5% of $C_{11}H_{12}N_2O_2$.

Description L-Tryptophan occurs as white to yellowish white crystals or crystalline powder. It is odorless, and has a slightly bitter taste.

It is freely soluble in formic acid, slightly soluble in water, and very slightly soluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Tryptophan, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> $[\alpha]_D^{20}$: –30.0 – –33.0° Weigh accurately about 0.25 g of L-Tryptophan, previously dried, and dissolve in 20 mL of water by warming. After cooling, add water to make exactly 25 mL, and determine the optical rotation of the solution in a 100-mm cell.

pH <2.54> Dissolve 1.0 g in 100 mL of water by warming, and cool: the pH of this solution is between 5.4 and 6.4.

Purity (1) Clarity of solution—Dissolve 0.20 g of L-Tryptophan in 10 mL of 2 mol/L hydrochloric acid TS: the solution is clear.

(2) Chloride <1.03>—Dissolve 0.5 g of L-Tryptophan in 6 mL of dilute nitric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Dissolve 0.6 g of L-Tryptophan in 40 mL of water and 1 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Tryptophan. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Tryptophan according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Dissolve 1.0 g of L-Tryptophan in 3 mL of 1 mol/L hydrochloric acid TS and 2 mL of water by heating, and perform the test with this solution as the test solution (not more than 2 ppm).

(7) Related substances—Dissolve 0.30 g of L-Tryptophan in 1 mL of 1 mol/L hydrochloric acid TS, add water to make 50 mL, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of L-Tryptophan, previously dried, dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 20.42 mg of $C_{37}H_{41}ClN_2O_6$

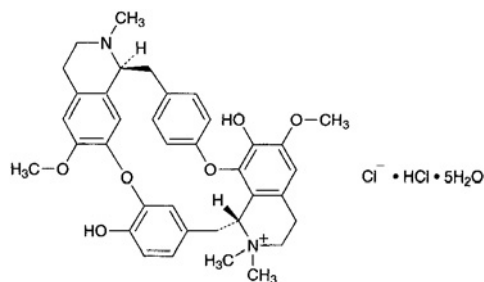
Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Tubocurarine Chloride Hydrochloride Hydrate

Tubocurarine Hydrochloride

ツボクラリン塩化物塩酸塩水和物



$C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$: 771.72
7',12'-Dihydroxy-6,6'-dimethoxy-2,2',2'-trimethyltubocuraranium chloride monohydrochloride pentahydrate [41354-45-4]

Tubocurarine Chloride Hydrochloride Hydrate contains not less than 98.0% of tubocurarine chloride hydrochloride ($C_{37}H_{41}ClN_2O_6 \cdot HCl$: 681.65), calculated on the dried basis.

Description Tubocurarine Chloride Hydrochloride Hydrate occurs as white crystals or crystalline powder. It is odorless.

It is sparingly soluble in water and in ethanol (95), slightly soluble in acetic acid (100), and practically insoluble in diethyl ether and in chloroform.

The pH of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 100) is between 4.0 and 6.0.

Melting point: about 270°C (with decomposition).

Identification (1) To 20 mL of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 2000) add 0.2 mL of sulfuric acid and 2 mL of a solution of potassium iodate (1 in 100), shake, and heat on a water bath for 30 minutes: a yellow color is produced.

(2) To 1 mL of a solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 100) add 1 mL of a solution of Reinecke salt (1 in 25): a red precipitate is formed.

(3) Determine the absorption spectrum of a solution of Tubocurarine Chloride Hydrochloride Hydrate (3 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Tubocurarine Chloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) A solution of Tubocurarine Chloride Hydrochloride Hydrate (1 in 50) responds to Qualitative Tests <1.09> (2) for chloride.

Optical rotation <2.49> $[\alpha]_D^{20}$: +210 – +220° (0.1 g, calculated on the dried basis, water, 10 mL, after allowing to stand for 3 hours, 100 mm).

Purity (1) Clarity and color of solution—Dissolve 0.10 g of Tubocurarine Chloride Hydrochloride Hydrate in 10 mL

of ethanol (95): the solution is clear and colorless.

(2) Chloroform-soluble substances—Weigh accurately about 0.2 g of Tubocurarine Chloride Hydrochloride Hydrate, calculated on the dried basis, add 200 mL of water and 1 mL of a saturated solution of sodium hydrogen carbonate, and extract with three 20-mL portions of chloroform. Combine the chloroform extracts, wash with 10 mL of water, filter the chloroform solution through absorbent cotton into a tared beaker, wash the absorbent cotton with two 5-mL portions of chloroform, and combine the filtrate and the washings. Evaporate the chloroform on a water bath, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 2.0% of the mass of Tubocurarine Chloride Hydrochloride Hydrate taken. Add 10 mL of water to the residue: the residue does not dissolve. Add 1 mL of hydrochloric acid, and stir: the residue dissolves.

Loss on drying <2.41> 9 – 12% (0.5 g, in vacuum, phosphorus (V) oxide, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.2% (0.5 g).

Assay Weigh accurately about 0.5 g of Tubocurarine Chloride Hydrochloride Hydrate, add 20 mL of acetic acid (100), and dissolve by warming on a water bath. After cooling, add 60 mL of acetic anhydride, and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 34.08 mg of $C_{37}H_{41}ClN_2O_6 \cdot HCl$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Tubocurarine Chloride Hydrochloride Injection

Tubocurarine Hydrochloride Injection

ツボクラリン塩化物塩酸塩注射液

Tubocurarine Chloride Hydrochloride Injection is an aqueous solution for injection. It contains not less than 93% and not more than 107% of the labeled amount of tubocurarine chloride hydrochloride hydrate ($C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$: 771.72).

Method of preparation Prepare as directed under Injections, with Tubocurarine Chloride Hydrochloride Hydrate.

Description Tubocurarine Chloride Hydrochloride Injection is a clear, colorless liquid.

Identification (1) To a volume of Tubocurarine Chloride Hydrochloride Injection, equivalent to 0.01 g of Tubocurarine Chloride Hydrochloride Hydrate according to the labeled amount, add water to make 20 mL, and proceed as directed in the Identification (1) under Tubocurarine Chloride Hydrochloride Hydrate.

(2) Proceed with a volume of Tubocurarine Chloride Hydrochloride Injection, equivalent to 3 mg of Tubocurarine Chloride Hydrochloride Hydrate according to the labeled amount, as directed in the Identification (2) under Tubocurarine Chloride Hydrochloride Hydrate.

rine Chloride Hydrochloride Hydrate.

(3) To a volume of Tubocurarine Chloride Hydrochloride Injection, equivalent to 3 mg of Tubocurarine Chloride Hydrochloride Hydrate according to the labeled amount, add water to make 100 mL, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 279 nm and 281 nm, and a minimum between 253 nm and 257 nm.

Optical rotation <2.49> α_D^{20} : +0.35 – +0.42° (200 mm), calculated with reference to the value of solution containing 1 mg of Tubocurarine Chloride Hydrochloride Hydrate per mL, according to the labeled amount of Tubocurarine Chloride Hydrochloride Injection.

pH <2.54> 3.0 – 6.0

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Tubocurarine Chloride Hydrochloride Injection, equivalent to about 15 mg of tubocurarine chloride hydrochloride hydrate ($C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$), add water to make exactly 500 mL, and use this solution as the sample solution. Separately, weigh accurately about 15 mg of Tubocurarine Chloride Hydrochloride Reference Standard (separately determine the loss on drying <2.41> in the same manner as Tubocurarine Chloride Hydrochloride Hydrate), dissolve in water to make exactly 500 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and the standard solution at 280 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, respectively.

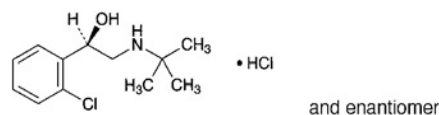
Amount (mg) of tubocurarine chloride hydrochloride hydrate ($C_{37}H_{41}ClN_2O_6 \cdot HCl \cdot 5H_2O$)
= $W_S \times (A_T/A_S) \times 1.1321$

W_S : Amount (mg) of Tubocurarine Chloride Hydrochloride Reference Standard, calculated on the dried basis

Containers and storage Containers—Hermetic containers.
Storage—Light-resistant, and under Nitrogen atmosphere.

Tulobuterol Hydrochloride

ツロブテロール塩酸塩



$C_{12}H_{18}ClNO \cdot HCl$: 264.19
(1*RS*)-1-(2-Chlorophenyl)-2-(1,1-dimethylethyl)aminoethanol monohydrochloride
[56776-01-3]

Tulobuterol Hydrochloride, when dried, contains not less than 98.5% of $C_{12}H_{18}ClNO \cdot HCl$.

Description Tulobuterol Hydrochloride occurs as white crystals or crystalline powder.

It is very soluble in methanol, freely soluble in water, in ethanol (95) and in acetic acid (100), sparingly soluble in acetic anhydride, and very slightly soluble in diethyl ether.

A solution of Tulobuterol Hydrochloride (1 in 20) shows no optical rotation.

Melting point: about 163°C

Identification (1) Determine the absorption spectrum of a solution of Tulobuterol Hydrochloride (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Tulobuterol Hydrochloride, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Tulobuterol Hydrochloride (1 in 20) responds to the Qualitative Tests <1.09> for chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Tulobuterol Hydrochloride in 10 mL of water: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 2.0 g of Tulobuterol Hydrochloride according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Related substances—Dissolve 0.30 g of Tulobuterol Hydrochloride in 5 mL of methanol, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Use a plate previously developed with the upper-layer of a mixture of ethyl acetate and ammonia solution (28) (200:9) to the top of the plate and air-dried. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with the upper layer of a mixture of ethyl acetate and ammonia solution (28) (200:9) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spots other than the principal spot and the spot of starting point from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (0.5 g, in vacuum, 60°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Tulobuterol Hydrochloride, previously dried, dissolve in 80 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.42 mg of C₁₂H₁₈ClNO.HCl

Containers and storage Containers—Tight containers.

Turpentine Oil

Oleum Terebinthinae

テレピン油

Turpentine Oil is the essential oil distilled with steam from the wood or balsam of *Pinus* species (*Pinaceae*).

Description Turpentine Oil is a clear, colorless to pale yellow liquid. It has a characteristic odor and a pungent, bitter taste.

Turpentine Oil (1 mL) is miscible with 5 mL of ethanol (95) and this solution is neutral.

Refractive index <2.45> n_D^{20} : 1.465 – 1.478

Specific gravity <1.13> d_{20}^{20} : 0.860 – 0.875

Purity (1) Foreign matter—Turpentine Oil has no offensive odor. Shake 5 mL of Turpentine Oil with 5 mL of a solution of potassium hydroxide (1 in 6): the aqueous layer does not show a yellow-brown to dark brown color.

(2) Hydrochloric acid-coloring substances—Shake 5 mL of Turpentine Oil with 5 mL of hydrochloric acid, and allow to stand for 5 minutes: the hydrochloric acid layer is light yellow and not brown in color.

(3) Mineral oil—Place 5 mL of Turpentine Oil in a Cassia flask, cool to a temperature not exceeding 15°C, add dropwise 25 mL of fuming sulfuric acid while shaking, warm between 60°C and 65°C for 10 minutes, and add sulfuric acid to raise the lower level of the oily layer to the graduated portion of the neck: not more than 0.1 mL of oil separates.

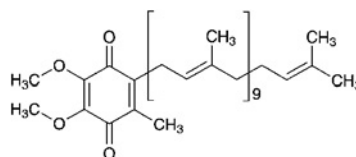
Distilling range <2.57> 150 – 170°C, not less than 90 vol%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ubidecarenone

ユビデカレノン



C₅₉H₉₀O₄: 863.34

(2*E*,6*E*,10*E*,14*E*,18*E*,22*E*,26*E*,30*E*,34*E*,38*E*)-2-(3,7,11,15,19,23,27,31,35,39-Decamethyltetraconta-2,6,10,14,18,22,26,30,34,38-decaen-1-yl)-5,6-dimethoxy-3-methyl-1,4-benzoquinone [303-98-0]

Ubidecarenone contains not less than 98.0% of C₅₉H₉₀O₄, calculated on the anhydrous basis.

Description Ubidecarenone occurs as a yellow to orange crystalline powder.

It is odorless and has no taste.

It is soluble in diethyl ether, very slightly soluble in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed and colored by light.

Melting point: about 48°C

Identification (1) Dissolve 0.05 g of Ubidecarenone in 1 mL of diethyl ether, and add 10 mL of ethanol (99.5). To 2 mL of this solution add 3 mL of ethanol (99.5) and 2 mL of dimethyl malonate, then add dropwise 1 mL of a solution of potassium hydroxide (1 in 5), and mix: a blue color appears.

(2) Determine the infrared absorption spectrum of Ubidecarenone as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Ubidecarenone Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Ubidecarenone according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.05 g of Ubidecarenone in 50 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling use this solution as the sample solution. To exactly 1 mL of the sample solution add ethanol (99.5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of both solutions by the automatic integration method: the total area of the peaks other than the peak of ubidecarenone from the sample solution is not larger than the peak area of ubidecarenone from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, flow rate, and selection of column: Proceed as directed in the operating conditions in the Assay.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of ubidecarenone obtained from 5 μ L of the standard solution is between 20 mm and 40 mm.

Time span of measurement: About 2 times of the retention time of ubidecarenone beginning after the solvent peak.

Water <2.48> Not more than 0.20% (1 g, direct titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 50 mg each of Ubidecarenone and Ubidecarenone Reference Standard (separately determined the water content <2.48> in the same manner as Ubidecarenone) dissolve each in 40 mL of ethanol (99.5) by warming at about 50°C for 2 minutes, and after cooling add ethanol (99.5) to make exactly 50 mL each, and use these solutions as the sample solution and the standard solution. Perform the test with exact 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine peak areas, A_T and A_S , of ubidecarenone of these solution.

$$\begin{aligned} &\text{Amount (mg) of } C_{59}H_{90}O_4 \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ubidecarenone Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 275 nm).

Column: A stainless steel column about 5 mm in inside diameter and about 15 cm in length, packed with octadecylsilylanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 35°C.

Mobile phase: A mixture of methanol and ethanol (99.5) (13:7).

Flow rate: Adjust the flow rate so that the retention time of ubidecarenone is about 10 minutes.

Selection of column: Dissolve 0.01 g each of Ubidecarenone and ubiquinone-9 in 20 mL of ethanol (99.5) by warming at about 50°C for 2 minutes. After cooling, proceed with 5 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of ubiquinone-9 and ubidecarenone in this order with the resolution between these peaks being not less than 4.

System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak areas of ubidecarenone is not more than 0.8%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Ulinastatin

ウリナスタチン

Ulinastatin is a solution of a glycoprotein having trypsin inhibiting activity, which is separated and purified from human urine.

It contains ulinastatin of not less than 45,000 Units per mL and not less than 2500 Units per mg protein.

Description Ulinastatin occurs as a light brown to brown, clear liquid.

Identification (1) Dilute a suitable amount of Ulinastatin with water to make a solution containing 4000 Units of ulinastatin per mL. To 1 mL of this solution add 1 mL of a solution of phenol (1 in 20), then carefully add 5 mL of sulfuric acid, and mix: an orange to red-orange color develops.

(2) Dilute a suitable quantity of Ulinastatin with water to make a solution containing 2000 units per mL. Determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Dilute a suitable amount of Ulinastatin with pH 7.8 2,2',2''-nitrilotrisethanol buffer solution to make a solution containing 500 Units of ulinastatin per mL, and use this solution as the sample solution. Use the same buffer solution as the control solution. To 0.1 mL each of the sample solution and the control solution add 1.6 mL of the buffer solution and 0.2 mL of trypsin TS for test of ulinastatin, mix, and allow them to stand in a water bath at 25°C for 1 minute. Then add 1 mL of *N*- α -benzoyl-L-arginine-4-nitroanilide TS, mix, and allow them to stand at 25°C for 2 minutes: the solution obtained with the sample solution develops no color while that obtained with the control solution develops a yellow

color.

(4) To 1.5 g of Powdered Agar add 100 mL of pH 8.4 boric acid-sodium hydroxide buffer solution, dissolve by warming in a water bath, then pour immediately into a Petri dish placed horizontally so that the agar layer is about 2 mm in thickness. After the agar becomes hard, bore two wells about 2.5 mm in diameter with a separation of 6 mm from each other. In one of the wells place 10 μ L of a solution of Ulinastatin containing 500 Units per mL in pH 8.4 boric acid-sodium hydroxide buffer solution, and in the other well place 10 μ L of anti-ulinastatin rabbit serum, cover the dish to avoid drying of the agar, and allow to stand for overnight at a room temperature: a clear precipitin line appears between the wells.

pH <2.54> 6.0 – 8.0

Specific activity When calculated from the results obtained by the Assay and the following method, the specific activity is not less than 2500 Units per 1 mg protein.

(i) Sample solution—To an exactly measured volume of Ulinastatin, equivalent to about 10,000 Units according to the labeled amount, add water to make exactly 20 mL.

(ii) Standard solutions—Weigh accurately about 10 mg of bovine serum albumin for test of ulinastatin, and dissolve in water to make exactly 20 mL. To a suitable volume of this solution add water to make four solutions containing exactly 300, 200, 100 and 50 μ g of the albumin per mL, respectively.

(iii) Procedure—Pipet 0.5 mL each of the sample solution and standard solutions, put them in glass test tubes about 18 mm in internal diameter and about 130 mm in length, add exactly 5 mL of alkaline copper TS, mix, and allow the tubes to stand in a water bath at 30°C for 10 minutes. Then add exactly 0.5 mL of diluted Folin's TS (1 in 2), mix, and warm in the water bath for 20 minutes. Determine the absorbances of these solutions at 750 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a solution obtained in the same manner with 0.5 mL of water as the blank.

Plot the absorbances of the standard solutions on the vertical axis and their protein concentrations on the horizontal axis to prepare a calibration curve, and determine the protein content of the sample solution from its absorbance by using this curve. Then calculate the amount of protein per mL of Ulinastatin.

Purity (1) Heavy metals <1.07>—Proceed with 10 mL of Ulinastatin according to Method 2, and perform the test. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 1 ppm).

(2) Related substances—To a suitable volume of Ulinastatin add water to make a solution containing exactly 12,500 Units per mL, and use this solution as the sample stock solution. To exactly 0.25 mL of the sample stock solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the sample solution. Separately, to exactly 1 mL of the sample stock solution add water to make exactly 100 mL. To exactly 0.25 mL of this solution add exactly 0.2 mL of glycerin and exactly 0.05 mL of 0.05% bromophenol blue TS, mix, and use this solution as the standard solution. Perform the following test with the sample solution and the standard solution: the bands other than the principal band obtained from the sample solution are not more intense than the band from the standard solution in the electrophoretogram.

(i) Tris buffer solution for polyacrylamide gel electrophoresis A Dissolve 18.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(ii) Tris buffer solution for polyacrylamide gel electrophoresis B Dissolve 6.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 80 mL of water, adjust to pH 8.8 with 6 mol/L hydrochloric acid TS, and add water to make 100 mL.

(iii) Tris buffer solution for polyacrylamide gel electrophoresis C Dissolve 3.0 g of 2-amino-2-hydroxymethyl-1,3-propanediol and 14.4 g of glycine in water to make 1000 mL.

(iv) Acrylamide solution for polyacrylamide gel electrophoresis Dissolve 30 g of acrylamide and 0.8 g of *N,N'*-methylenebisacrylamide in water to make 100 mL.

(v) Gel for separation Mix gently 15 mL of tris buffer solution for polyacrylamide gel electrophoresis A, 20 mL of acrylamide solution for polyacrylamide gel electrophoresis, 24.5 mL of water, 0.022 mL of *N,N,N',N'*-tetramethylethylenediamine, 0.32 mL of 10% ammonium peroxodisulfate TS and 0.3 mL of 1 mol/L sodium sulfite TS, pour into a plate for slab gel preparation, then cover the gel mixture with a layer of water, and allow to set for 1 hour.

(vi) Gel for concentration Remove the water layer on the gel for separation, and pour a mixture of 2.5 mL of tris buffer solution for polyacrylamide gel electrophoresis B, 2.66 mL of acrylamide solution for polyacrylamide gel electrophoresis, 14.6 mL of water, 0.01 mL of *N,N,N',N'*-tetramethylethylenediamine, 0.2 mL of 10% ammonium peroxodisulfate TS and 0.04 mL of 1 mol/L sodium sulfite TS on the gel. Then position a plastic sample well former so that the height of the gel for concentration is about 15 mm, and allow to set for 2 hours.

(vii) Procedure

Electrophoresis—Set the gel in an apparatus for slab gel electrophoresis, and fill the upper and lower reservoirs with tris buffer solution for polyacrylamide gel electrophoresis C. Introduce carefully 10 μ L each of the sample solution and standard solution into the wells using a different well for each solution, and allow electrophoresis to proceed using the electrode of the lower reservoir as the anode. Switch off the power supply when the bromophenol blue band has migrated to about 10 mm from the bottom of the gel.

Staining—Dissolve 2.0 g of Coomassie brilliant blue R-250 in 400 mL of a mixture of methanol and 100 mL of acetic acid (100), add water to make 1000 mL, and use this solution as the staining solution. Stain the gel for 2 hours in the staining solution warmed to 40°C.

Decolorization—To 100 mL of methanol and 75 mL of acetic acid (100) add water to make 1000 mL, and use this solution as the rinsing solution. Immerse the gel removed from the staining solution in the rinsing solution to decolorise.

(3) Kallidinogenase—Dilute a suitable volume of Ulinastatin with water so that each mL of the solution contains about 50,000 Units, and use this solution as the sample solution. Take exactly 0.4 mL of the sample solution into a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in a water bath at 37 \pm 0.2°C for 5 minutes. Add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, allow the tube to stand in the water bath of 37 \pm 0.2°C for exactly 30 minutes, then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, and use this solution as the test solution. Separately, take exactly

0.4 mL of the sample solution in a test tube, add exactly 0.5 mL of pH 8.2 tris buffer solution, mix, and allow the tube to stand in the water bath of $37 \pm 0.2^\circ\text{C}$ for 35 minutes. Then add exactly 0.1 mL of diluted acetic acid (100) (1 in 2), mix, add exactly 0.1 mL of substrate TS for kallidinogenase assay (4), mix, and use this solution as the control solution. Determine the absorbances of the test solution and the control solution at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank, and calculate the difference between them: the difference is not more than 0.050.

Molecular mass Dilute a suitable volume of Ulinastatin with the mobile phase so that each mL of the solution contains about 6500 Units, and use this solution as the sample solution. Separately, dissolve 1.0 mg each of γ -globulin (mol. mass: 160,000), bovine serum albumin for test of ulinastatin (mol. mass: 67,000), and myoglobin (mol. mass: 17,000) in about 1 mL of the mobile phase, and use this solution as the molecular mass reference solution. Perform the test with 50 μL each of the sample solution and molecular mass reference solution as directed under Liquid Chromatography <2.01> according to the following conditions. Prepare a calibration curve by plotting the logarithm of molecular masses on the vertical axis and the retention times (min) of the molecular mass reference substances on the horizontal axis, and determine the molecular mass of the sample using the calibration curve and the retention time obtained with the sample solution: the molecular mass is $67,000 \pm 5000$.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column about 7 mm in inside diameter and about 60 cm in length, packed with porous silica gel for liquid chromatography (10–12 μm in particle diameter).

Column temperature: A constant temperature of about 25°C .

Mobile phase: Dissolve 16.33 g of potassium dihydrogenphosphate and 124.15 g of ethylene glycol in water to make 1000 mL. If necessary, adjust to pH 4.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of bovine serum albumin is about 36 minutes.

Selection of column: Proceed with 50 μL of the molecular mass reference solution according to the above operating conditions, and calculate the resolution. Use a column from which γ -globulin, bovine serum albumin and myoglobin are eluted in this order with the resolution between their peaks being not less than 1.5, respectively.

Antigenicity Dilute a suitable volume of Ulinastatin with isotonic sodium chloride solution so that each mL of the solution contains 15,000 Units, and use this solution as the sample solution. Inject 0.10 mL of the sample solution on 3 occasions at intervals of 2 days into the peritoneal cavity of each of 4 well-nourished, healthy guinea pigs weighing 250 to 300 g. Inject 0.10 mL of horse serum into the peritoneal cavity of each of 4 guinea pigs of another group as a control. Inject 0.20 mL of the sample solution intravenously into each of 2 guinea pigs of the first group 14 days after the first intraperitoneal injection and into each of the remaining 2 guinea pigs 21 days after the injection, and inject 0.20 mL of horse serum intravenously in the same manner into each

guinea pig of the second group. Observe the signs of respiratory distress, collapse or death of the animals for 30 minutes after each intravenous injection and 24 hours later: the animals of the first group exhibit none of the signs mentioned above, and all the animals of the second group exhibit symptoms of respiratory distress or collapse and not less than 3 animals are killed.

Toxicity Inject intravenously 0.50 mL of Ulinastatin into each of five well-fed, healthy albino mice weighing 18 to 25 g: no mouse dies within 48 hours after injection. If any mouse dies within 48 hours, repeat the test using 5 albino mice weighing 19 to 21 g: all the animals survive for 48 hours.

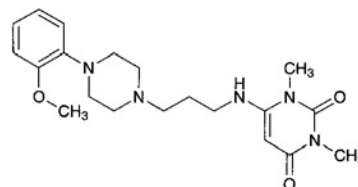
Assay Measure exactly a suitable volume of Ulinastatin, dilute with 2,2',2''-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains about 150 Units according to the labeled amount, and use this solution as the sample solution. Separately, dilute a suitable volume of Ulinastatin Reference Standard with 2,2',2''-nitrilotrisethanol buffer solution, pH 7.8 so that each mL of the solution contains exactly 300, 200, 100, 50 or 0 Units, and use these solutions as the standard solutions. 2,2',2''-Nitrilotrisethanol buffer solution, pH 7.8 and *N*- α -benzoyl-L-arginine-4-nitroanilide TS are warmed in a water bath of $25 \pm 1^\circ\text{C}$ for use as described below. Take exactly 0.1 mL each of the sample solution and the standard solutions in test tubes, add exactly 1.6 mL of 2,2',2''-nitrilotrisethanol buffer solution, pH 7.8 mix, and put the tubes in the water bath of $25 \pm 1^\circ\text{C}$. One minute after addition of the buffer solution add exactly 0.2 mL of ice-cooled trypsin TS for test of ulinastatin, mix, and put the tubes again in the water bath. One minute later add exactly 1 mL of *N*- α -benzoyl-L-arginine-4-nitroanilide TS, mix, and then put the tubes in the water bath. Exactly 2 minutes later add exactly 0.1 mL of diluted acetic acid (100) (1 in 2) to stop the enzyme reaction, and determine the absorbances of the solutions so obtained at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using water as the blank. Prepare a calibration curve using the absorbances obtained with the standard solutions, and calculate ulinastatin Units in the sample solution from its absorbance by using this curve.

Containers and storage Containers—Tight containers.

Storage—Preserve at -20°C or lower.

Urapidil

ウラピジル



$\text{C}_{20}\text{H}_{29}\text{N}_5\text{O}_3$: 387.48

6-[3-[4-(2-Methoxyphenyl)piperazin-1-yl]propylamino]-1,3-dimethyluracil [34661-75-1]

Urapidil, when dried, contains not less than 98.0% and not more than 101.0% of $\text{C}_{20}\text{H}_{29}\text{N}_5\text{O}_3$.

Description Urapidil occurs as white to pale yellowish, white, crystals or crystalline powder. It has a bitter taste.

It is freely soluble in acetic acid (100), sparingly soluble in ethanol (95) and in acetone, and very slightly soluble in water.

Identification (1) Determine the absorption spectrum of a solution of Urapidil in ethanol (95) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Urapidil as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 156 – 161°C

Purity (1) Chloride <1.03>—Dissolve 3.0 g of Urapidil in 40 mL of acetone and 6 mL of dilute nitric acid, add water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows. To 0.25 mL of 0.01 mol/L hydrochloric acid VS add 40 mL of acetone, 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.003%).

(2) Heavy metals <1.07>—Proceed with 1.0 g of Urapidil according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Related substances—Dissolve 40 mg of Urapidil in 5 mL of ethanol (95), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add ethanol (95) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop with a mixture of ethyl acetate, ethanol (95) and ammonia water (28) (22:13:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): the spot other than the principal spot appears not more than one and it is not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

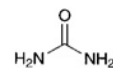
Assay Weigh accurately about 70 mg of Urapidil, previously dried, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 12.92 mg of C₂₀H₂₉N₃O₃

Containers and storage Containers—Tight containers.

Urea

尿素



CH₄N₂O: 60.06

Urea [57-13-6]

Urea contains not less than 99.0% of CH₄N₂O.

Description Urea occurs as colorless to white crystals or crystalline powder. It is odorless, and has a cooling, saline taste.

It is very soluble in water, freely soluble in boiling ethanol (95), soluble in ethanol (95), and very slightly soluble in diethyl ether.

A solution of Urea (1 in 100) is neutral.

Identification (1) Heat 0.5 g of Urea: it liquefies and the odor of ammonia is perceptible. Continue heating until the liquid becomes turbid, then cool. Dissolve the resulting lump in a mixture of 10 mL of water and 2 mL of sodium hydroxide TS, and add 1 drop of copper (II) sulfate TS: a reddish purple color develops.

(2) Dissolve 0.1 g of Urea in 1 mL of water, and add 1 mL of nitric acid: a white, crystalline precipitate is formed.

Melting point <2.60> 132.5 – 134.5°C

Purity (1) Chloride <1.03>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.007%).

(2) Sulfate <1.14>—Perform the test with 2.0 g of Urea. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Heavy metals <1.07>—Proceed with 1.0 g of Urea according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(4) Ethanol-insoluble substances—Dissolve 5.0 g of Urea in 50 mL of warm ethanol (95), filter through a tared glass filter (G4), wash the residue with 20 mL of warm ethanol (95), and dry at 105°C for 1 hour: the mass of the residue is not more than 2.0 mg.

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Urea, dissolve in water, and make exactly 200 mL. Measure exactly 5 mL of this solution into a Kjeldahl flask, and proceed as directed under Nitrogen Determination <1.08>.

Each mL of 0.005 mol/L sulfuric acid VS
= 0.3003 mg of CH₄N₂O

Containers and storage Containers—Well-closed containers.

Urokinase

ウロキナーゼ

[9010-53-1]

Urokinase is an enzyme, obtained from human urine, that activates plasminogen, and has the molecular mass of about 54,000.

It is a solution using a suitable buffer solution as the solvent.

It contains not less than 60,000 Units per mL, and not less than 120,000 Units per mg of protein.

Description Urokinase is a clear and colorless liquid.

The pH is between 5.5 and 7.5.

Identification (1) Dissolve 0.07 g of fibrinogen in 10 mL of phosphate buffer solution, pH 7.4. To this solution add 1 mL of a solution of thrombin containing 10 Units per mL in isotonic sodium chloride solution, mix, place in a Petri dish about 90 mm in inside diameter, and keep horizontally until the solution is coagulated. On the surface drop 10 μ L of a solution of Urokinase containing 100 Units per mL in gelatin-tris buffer solution, and stand for overnight: lysis circle is appeared.

(2) Dissolve 1.0 g of Powdered Agar in 100 mL of boric acid-sodium hydroxide buffer solution, pH 8.4, by warming, and pour the solution into a Petri dish until the height come to about 2 mm. After cooling, make two wells of 2.5 mm in diameter with the space of 6 mm. To each well place separately 10 μ L of a solution of Urokinase containing 30,000 Units per mL in isotonic sodium chloride solution and 10 μ L of anti-urokinase serum, and stand for overnight: a clear precipitin line is appeared.

Purity (1) Heavy metals <1.07>—Proceed with 2.0 mL of Urokinase according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Blood group substances—Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. To anti-A type antibody for blood typing add isotonic sodium chloride solution to dilute each 32, 64, 128, 256, 512 and 1024 times, place separately 25 μ L each of these solutions in six wells on the first and second lane of a V-shaped 96-wells microplate. Next, add 25 μ L of the sample solution into the six wells on the first lane and 25 μ L of isotonic sodium chloride solution into the six wells of the second lane, mix, and allow to stand for 30 min. To each well add 50 μ L of A-type erythrocyte suspension, mix, allow to stand for 2 hours, and compare the agglutination of erythrocyte in both lanes: dilution factor of anti-A type antibody of the wells which show the agglutination is equal in both lanes.

Perform the same test by using anti-B type antibody for blood typing and B-type erythrocyte suspension.

Abnormal toxicity Dilute Urokinase with isotonic sodium chloride solution so that each mL of the solution contains 12,000 Units, and use this solution as the sample solution. Inject 5.0 mL of the sample solution into the peritoneal cavity

of each of 2 or more of well-nourished, healthy guinea pigs weighing about 350 g, and inject 0.5 mL of the sample solution into the peritoneal cavity of each of 2 or more of well-nourished, healthy mice aged about 5 weeks. Observe the conditions of the animals for more than 7 days: all the animals exhibit no abnormalities.

High molecular mass urokinase Dilute Urokinase with gelatin-phosphate buffer solution so that each mL of the solution contains 10,000 Units, and use this solution as the sample solution. Perform the test with 100 μ L of the sample solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas of two peaks eluted closely at about 35 minutes having smaller retention time, A_a , and larger retention time, A_b , by the automatic integration method: the value, $A_a/(A_a + A_b)$, is not less than 0.85.

Operating conditions—

Apparatus: Use a pumping system for the mobile phase, a sample injection port, a column, a pumping system for the reaction reagent, a reaction coil, a reaction chamber, a spectrofluorometer and a recorder. Attach a 3-way tube to the outlet for the mobile phase of the column, connect the pumping system for the reaction reagent and the reaction coil, and join outlet of the reaction coil to the spectrofluorometer.

Detector: Spectrofluorometer (excitation wavelength: 365 nm, fluorescence wavelength: 460 nm).

Column: A stainless steel column 7.5 mm in inside diameter and 60 cm in length, packed with porous silica gel for liquid chromatography (10 to 12 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Reaction coil: A stainless steel column 0.25 mm in inside diameter and 150 cm in length.

Reaction coil temperature: 37°C

Mobile phase: Gelatin-phosphate buffer solution.

Flow rate of mobile phase: 0.5 mL per minute.

Reaction reagent: 7-(Glutarylglucyl-L-arginylamino)-4-methylcoumarin TS.

Flow rate of reaction reagent: 0.75 mL per minute.

Selection of column: Adjust the pH of Urokinase to 7.5 with sodium hydroxide TS, allow to stand at 37°C for over 24 hours, and add gelatin-phosphate buffer solution to make the solution containing 20,000 Units per mL. Proceed with 100 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of high molecular mass urokinase (mol. wt.: 54,000) and low molecular mass urokinase (mol. wt.: 33,000) in this order with the resolution between these peaks being not less than 1.0.

Assay (1) Urokinase—Pipet 1 mL of Urokinase, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the sample solution. Add exactly 2 mL of gelatin-tris buffer solution to contents of one ampoule of High Molecular Mass Urokinase Reference Standard to dissolve, pipet 1 mL of this solution, dilute exactly with gelatin-tris buffer solution so that each mL of the solution contains about 30 Units, and use this solution as the standard solution. Place 1.0 mL of L-pyroglutamylglucyl-L-arginine-*p*-nitroaniline hydrochloride TS in two silicon-coated test tubes about 10 mm in inside diameter, warm them in a water bath at $35 \pm 0.2^\circ\text{C}$ for 5 minutes, add separately 0.50 mL each of the sample solution and the standard solution, warm in a water bath at $35 \pm$

0.2°C for exactly 30 minutes, then add 0.50 mL of diluted acetic acid (100) (2 in 5). Determine the absorbances, A_T and A_S , of these solutions at 405 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using water as the blank. Separately place 1.0 mL of L-pyroglutamylglycyl-L-arginine-*p*-nitroaniline hydrochloride TS in two test tubes, add 0.50 mL of diluted acetic acid (100) (2 in 5), and 0.50 mL each of the sample solution and the standard solution. Determine the absorbances, A_{T0} and A_{S0} , of these solutions at 405 nm as the same manner, using water as the blank.

$$\begin{aligned} \text{Amount (Units) of Urokinase} \\ = \{(A_T - A_{T0}) / (A_S - A_{S0})\} \times a \times b \end{aligned}$$

a: Amount (Units) of urokinase in 1 mL of the standard solution

b: Total volume (mL) of the sample solution

(2) Protein—Measure exactly a volume of Urokinase, equivalent to about 15 mg of protein, and perform the test as directed under Nitrogen Determination <1.08>.

$$\begin{aligned} \text{Each mL of 0.005 mol/L sulfuric acid VS} \\ = 0.8754 \text{ mg of protein} \end{aligned}$$

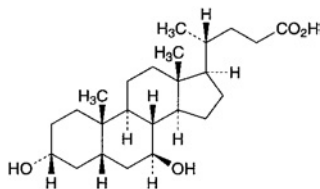
Containers and storage Containers—Tight containers.

Storage—Not exceeding -20°C .

Ursodeoxycholic Acid

Ursodesoxycholic Acid

ウルソデオキシコール酸



$\text{C}_{24}\text{H}_{40}\text{O}_4$: 392.57

3 α ,7 β -Dihydroxy-5 β -cholan-24-oic acid [128-13-2]

Ursodeoxycholic Acid, when dried, contains not less than 98.5% of $\text{C}_{24}\text{H}_{40}\text{O}_4$.

Description Ursodeoxycholic Acid occurs as white crystals or powder. It is odorless, and has a bitter taste.

It is freely soluble in ethanol (95), in ethanol (99.5) and in acetic acid (100), slightly soluble in chloroform, very slightly soluble in diethyl ether, and practically insoluble in water.

It dissolves in sodium hydroxide TS.

Identification Dissolve 0.01 g of Ursodeoxycholic Acid in 1 mL of sulfuric acid, add 1 drop of formaldehyde solution, and allow to stand for 5 minutes. To the solution add 5 mL of water: a blue-green suspended substance is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: $+59.0 - +62.0^\circ$ (after drying, 1.0 g, ethanol (99.5), 25 mL, 100 mm).

Melting point <2.60> $200 - 204^\circ\text{C}$

Purity (1) Odor—To 2.0 g of Ursodeoxycholic Acid add 100 mL of water, and boil for 2 minutes: no odor is perceptible.

(2) Chloride <1.03>—Dissolve 2.0 g of Ursodeoxycholic Acid in 20 mL of acetic acid (100) with shaking, add water to make 200 mL, shake thoroughly, and allow to stand for 10 minutes. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. To 40 mL of the sample solution add 6 mL of dilute nitric acid and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.25 mL of 0.01 mol/L hydrochloric acid VS add 4 mL of acetic acid (100), 6 mL of dilute nitric acid and water to make 50 mL (not more than 0.022%).

(3) Sulfate <1.14>—To 40 mL of the sample solution obtained in (2) add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 0.40 mL of 0.005 mol/L sulfuric acid VS add 4 mL of acetic acid (100), 1 mL of dilute hydrochloric acid and water to make 50 mL (not more than 0.048%).

(4) Heavy metals <1.07>—Proceed with 1.0 g of Ursodeoxycholic Acid according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(5) Barium—To the solution obtained in (1) add 2 mL of hydrochloric acid, boil for 2 minutes, cool, filter, and wash with water until the last washing makes 100 mL. To 10 mL of the solution add 1 mL of dilute sulfuric acid: no turbidity is produced.

(6) Arsenic <1.11>—Prepare the test solution with 1.0 g of Ursodeoxycholic Acid according to Method 3, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 50 mg of Ursodeoxycholic Acid in a mixture of chloroform and ethanol (95) (9:1) to make exactly 25 mL, and use this solution as the sample solution. Separately, dissolve 75 mg of chenodeoxycholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 100 mL. To exactly 2 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL, and use this solution as the standard solution (1). Dissolve 25 mg of lithocholic acid for thin-layer chromatography in a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL. To exactly 1 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 50 mL. To exactly 2 mL of this solution add a mixture of chloroform and ethanol (95) (9:1) to make exactly 10 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Dry the plate at 120°C for 30 minutes, spray evenly a solution of phosphomolybdic acid *n*-hydrate in ethanol (95) (1 in 5) immediately, and heat at 120°C for 2 to 3 minutes: the spot from the sample solution, corresponding to that from the standard solution (1), is not more intense than the spot from the standard solution (1), and the spot other than the principal spot and the above spots from the sample solution are not more intense than the spot from the standard solution (2).

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C , 2 hours).

Residue on ignition <2.44> Not more than 0.2% (1 g).

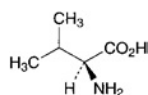
Assay Weigh accurately about 0.5 g of Ursodeoxycholic Acid, previously dried, and dissolve in 40 mL of neutralized ethanol and 20 mL of water. Add 2 drops of phenolphthalein TS, titrate with 0.1 mol/L sodium hydroxide VS, and titrate <2.50> again after adding 100 mL of freshly boiled and cooled water near the end point.

Each mL of 0.1 mol/L sodium hydroxide VS
= 39.26 mg of C₂₄H₄₀O₄

Containers and storage Containers—Well-closed containers.

L-Valine

L-バリン



C₅H₁₁NO₂: 117.15

(2*S*)-2-Amino-3-methylbutanoic acid [72-18-4]

L-Valine, when dried, contains not less than 98.5% of C₅H₁₁NO₂.

Description L-Valine occurs as white crystals or crystalline powder. It is odorless or has a faint characteristic odor, and has a slightly sweet taste, which becomes bitter.

It is freely soluble in formic acid, soluble in water, and practically insoluble in ethanol (95).

It dissolves in dilute hydrochloric acid.

Identification Determine the infrared absorption spectrum of L-Valine, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Optical rotation <2.49> [α]_D²⁰: +26.5 – +29.0° (after drying, 2 g, 6 mol/L hydrochloric acid TS, 25 mL, 100 mm).

pH <2.54> Dissolve 0.5 g of L-Valine in 20 mL of water: the pH of this solution is between 5.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 0.5 g of L-Valine in 20 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 0.5 g of L-Valine. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.021%).

(3) Sulfate <1.14>—Perform the test with 0.6 g of L-Valine. Prepare the control solution with 0.35 mL of 0.005 mol/L sulfuric acid VS (not more than 0.028%).

(4) Ammonium <1.02>—Perform the test with 0.25 g of L-Valine. Prepare the control solution with 5.0 mL of Standard Ammonium Solution (not more than 0.02%).

(5) Heavy metals <1.07>—Proceed with 1.0 g of L-Valine according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(6) Arsenic <1.11>—Proceed with 1.0 g of L-Valine, prepare the test solution according to Method 2, and perform the test (not more than 2 ppm).

(7) Related substances—Dissolve 0.10 g of L-Valine in 25 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add water to make exactly 50 mL. Pipet 5 mL of this solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (3:1:1) to a distance of about 10 cm, and dry the plate at 80°C for 30 minutes. Spray evenly a solution of ninhydrin in acetone (1 in 50) on the plate, and heat at 80°C for 5 minutes: the spots other than the principal spot from the sample solution are not more intense than the spot from the standard solution.

Loss on drying <2.41> Not more than 0.30% (1 g, 105°C, 3 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

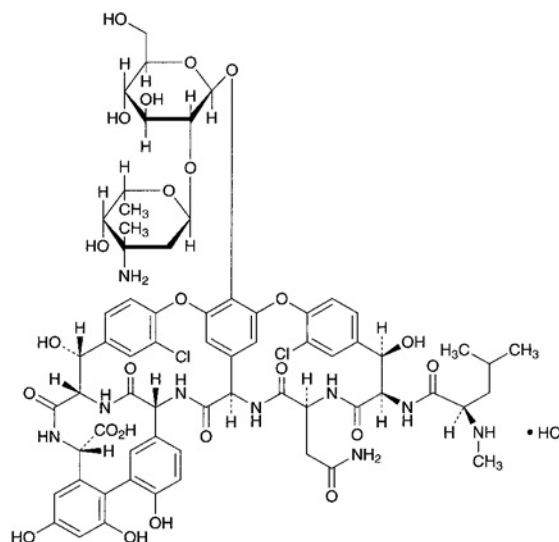
Assay Weigh accurately about 0.12 g of L-Valine, previously dried, and dissolve in 3 mL of formic acid, add 50 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 11.72 mg of C₅H₁₁NO₂

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride

バンコマイシン塩酸塩



C₆₆H₇₅Cl₂N₉O₂₄·HCl: 1485.71

(1*S*,2*R*,18*R*,19*R*,22*S*,25*R*,28*R*,40*S*)-50-[3-Amino-2,3,6-trideoxy-3-*C*-methyl- α -L-lyxo-hexopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyloxy]-22-carbamoylmethyl-5,15-dichloro-2,18,32,35,37-pentahydroxy-19-[(2*R*)-

4-methyl-2-(methylamino)pentanoylamino]-20,23,26,42,44-pentaoxa-7,13-dioxa-21,24,27,41,43-pentaazaocatacyclo[26.14.2.2^{3,6}.2^{14,17}.1^{8,12}.1^{29,33}.0^{10,25}.0^{34,39}]pentaconta-3,5,8,10,12(50),14,16,29,31,33(49),34,36,38,45,47-pentadecaene-40-carboxylic acid monohydrochloride [1404-93-9]

Vancomycin Hydrochloride is the hydrochloride of a glycopeptide substance having antibacterial activity produced by the growth of *Streptomyces orientalis*.

It contains not less than 1000 μg (potency) and not more than 1200 μg (potency) per mg, calculated on the anhydrous basis. The potency of Vancomycin Hydrochloride is expressed as mass (potency) of vancomycin ($\text{C}_{66}\text{H}_{75}\text{Cl}_2\text{N}_9\text{O}_{24}$: 1449.25).

Description Vancomycin Hydrochloride occurs as a white powder.

It is freely soluble in water, soluble in formamide, slightly soluble in methanol, very slightly soluble in ethanol (95), and practically insoluble in acetonitrile.

It is hygroscopic.

Identification (1) Determine the absorption spectrum of a solution of Vancomycin Hydrochloride (1 in 10,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vancomycin Hydrochloride Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vancomycin Hydrochloride as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Vancomycin Hydrochloride Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) Dissolve 20 mg of Vancomycin Hydrochloride in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

Optical rotation <2.49> $[\alpha]_D^{20}$: -30 – -40° (0.2 g calculated on the anhydrous basis, water, 20 mL, 100 mm).

pH <2.54> The pH of a solution obtained by dissolving 0.25 g of Vancomycin Hydrochloride in 5 mL of water is between 2.5 and 4.5.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Vancomycin Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Related substances—Dissolve 0.10 g of Vancomycin Hydrochloride in 10 mL of the mobile phase A, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mobile phase A to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under the Liquid Chromatography <2.01> according to the following conditions. If necessary, proceed with 20 μL of the mobile phase A in the same manner to compensate for the base line. Determine each peak area by the automatic integration method: the area of each peak other than

vancomycin from the sample solution is not more than the peak area of vancomycin from the standard solution, and the total area of the peaks other than vancomycin is not more than 3 times of the peak area of vancomycin from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 280 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase A: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (92:7:1). Adjust the amount of acetonitrile so that the retention time of vancomycin is 7.5 to 10.5 minutes.

Mobile phase B: A mixture of triethylamine buffer solution, pH 3.2, acetonitrile and tetrahydrofuran (70:29:1).

Flowing of the mobile phase: Control the gradient by mixing the mobile phases A and B as directed in the following table.

Time after injection of sample (min)	Mobile phase A (vol%)	Mobile phase B (vol%)
0 – 12	100	0
12 – 20	100 → 0	0 → 100
20 – 22	0	100

Flow rate: 1.5 mL per minute.

Time span of measurement: As long as about 2.5 times of the retention time of vancomycin beginning after the solvent peak.

System suitability—

Test for required detectability: Confirm that the peak area of vancomycin obtained from 20 μL of the standard solution is equivalent to 3 to 5% of that from 20 μL of the sample solution.

System performance: Dissolve 5 mg of Vancomycin Hydrochloride in 10 mL of water, heat at 65°C for 48 hours, and cool to the ordinal temperature. When the procedure is run with 20 μL of this solution under the above operating conditions, related substance 1, vancomycin and related substance 2 are eluted in this order, the resolution between the peaks of the related substance 1 and vancomycin is not less than 3, the number of theoretical plates of the peak of vancomycin is not less than 1500, and the related substance 2 is eluted between 15 and 18 minutes.

System repeatability: When the test is repeated 5 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vancomycin is not more than 2.0%.

Water <2.48> Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for water determination and methanol for water determination (3:1)).

Residue on ignition <2.44> Not more than 1.0% (1 g).

Assay Perform the test according to the Cylinder-plate method as directed under the Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism—*Bacillus subtilis* ATCC 6633

(ii) Culture medium—Use the medium i in 1) Medium for test organism [5] under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 6.2 to 6.4 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Vancomycin Hydrochloride Reference Standard, equivalent to about 25 mg (potency), dissolve in water to make exactly 25 mL, and use this solution as the standard stock solution. Keep the standard stock solution at 5°C or below, and use within 7 days. Take exactly a suitable amount of the standard stock solution before use, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 µg (potency) and 25 µg (potency), and use these solutions as the high concentration standard solution and low concentration standard solution, respectively.

(iv) Sample solutions—Weigh accurately an amount of Vancomycin Hydrochloride, equivalent to about 25 mg (potency), and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 µg (potency) and 25 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Tight containers.

Vancomycin Hydrochloride for Injection

注射用バンコマイシン塩酸塩

Vancomycin Hydrochloride for Injection is a preparation for injection which is dissolved before use.

It contains not less than 90.0% and not more than 115.0% of the labeled amount of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$: 1449.25).

Method of preparation Prepare as directed under Injections, with Vancomycin Hydrochloride.

Description Vancomycin Hydrochloride for Injection occurs as white masses or a white powder.

Identification (1) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 5 mg (potency) of Vancomycin Hydrochloride, in 50 mL of water, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 279 and 283 nm.

(2) Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 20 mg (potency) of Vancomycin Hydrochloride, in 10 mL of water, and add 1 drop of silver nitrate TS: a white turbidity is produced.

pH <2.54> The pH of a solution prepared by dissolving an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water is between 2.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.5 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of water: the solution is

clear and colorless to pale yellow, and the absorbance of the solution, determined at 465 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, is not more than 0.05.

(2) Related substances—Dissolve an amount of Vancomycin Hydrochloride for Injection, equivalent to 0.1 g (potency) of Vancomycin Hydrochloride according to the labeled amount, in 10 mL of the mobile phase A, and use this solution as the sample solution.

Proceed as directed in the Purity (2) under Vancomycin Hydrochloride.

Water <2.48> Not more than 5.0% (0.1 g, volumetric titration, direct titration. Use a mixture of formamide for Karl Fisher method and methanol for Karl Fisher method (3:1)).

Bacterial endotoxins <4.01> Less than 0.25 EU/mg (potency).

Uniformity of dosage unit <6.02> It meets the requirement of the Mass Variation Test.

Foreign insoluble matter <6.06> Perform the test according to the Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to the Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions.

(i) Test organism, culture medium, and standard solutions—Proceed as directed in the Assay under Vancomycin Hydrochloride.

(ii) Sample solutions—Weigh accurately the contents of not less than 10 Vancomycin Hydrochloride for Injection. Weigh accurately an amount of the content, equivalent to about 25 mg (potency) of Vancomycin Hydrochloride according to the labeled amount, and dissolve in water to make exactly 25 mL. Take exactly a suitable amount of this solution, add 0.1 mol/L phosphate buffer solution, pH 4.5 to make solutions so that each mL contains 100 µg (potency) and 25 µg (potency), and use these solutions as the high concentration sample solution and low concentration sample solution, respectively.

Containers and storage Containers—Hermetic containers.

Vasopressin Injection

バソプレシン注射液

Vasopressin Injection is an aqueous solution for injection.

It contains synthetic vasopressin or the pressor principle, vasopressin, obtained from the posterior lobe of the pituitary of healthy cattles and pigs, from which the majority of the oxytocic principle, oxytocin, has been removed.

It contains not less than 85% and not more than 120% of the labeled vasopressin Units.

Method of preparation Prepare as directed under Injec-

tions, with vasopressin prepared by synthesis or obtained from the posterior lobe of the pituitary.

Description Vasopressin Injection is a clear and colorless liquid. It is odorless or has a slight, characteristic odor.

pH: 3.0 – 4.0

Purity Oxytocic principle—When tested by the following procedure, Vasopressin Injection contains not more than 0.6 oxytocin Units for each determined 10 vasopressin Units.

(i) Standard stock solution: Dissolve 200 Units of Oxytocin Reference Standard, according to the labeled Units, in exactly 10 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of preparation.

(ii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that each mL of the solution contains 0.020 oxytocin Units.

(iii) Sample solution: Assume oxytocin Units as equivalent to 6/100 of the determined vasopressin Units. Dilute Vasopressin Injection with isotonic sodium chloride solution so that each mL of the resulting solution is expected to contain 0.020 oxytocin Unit.

(iv) Apparatus: Use the apparatus for the uterus contraction test, equipped with a thermostatic bath. Maintain a temperature of the bath at 37°C to 38°C with a variation of not more than 0.1°C during the course of the test. Use a 100-mL Magnus' chamber for suspending the uterus.

(v) Test animal: Use healthy, virgin and metestrus guinea pigs weighing between 175 g and 350 g. They have been bred under conditions where they have been completely isolated from the sight and smell of males since the time of weaning.

(vi) Procedure: Immerse the Magnus' chamber in the bath maintained at a constant temperature, add Locke-Ringer's solution to the chamber, and introduce oxygen into the solution at a moderate rate. Sacrifice a guinea pig by means of a blow on the head, immediately remove the uterus from the body, suspend it in the chamber, and connect one horn of the uterus to the lever with a thread. If necessary, weigh the lever provided that the mass is not changed throughout the assay. Start the assay after 15 to 30 minutes when the uterus is completely relaxed. Administer the same quantities, 0.1 to 0.5 mL, of the standard solution and the sample solution to the Magnus' chamber alternately twice with regular intervals of between 10 and 20 minutes to contract the uterus, finally administer the standard solution in a quantity which is 25% larger than the preceding doses, and measure the height of every contraction. The mean height of uterus contraction caused by the standard solution is equal to or higher than that caused by the sample solution. The height of contraction caused by the increased dose of the standard solution is distinctly higher than those caused by the preceding doses of the standard solution.

Extractable volume <6.05> It meets the requirement.

Assay (i) Test animals: Use healthy male rats weighing between 200 g and 300 g.

(ii) Standard stock solution: Dissolve 2000 Units of Vasopressin Reference Standard, according to the labeled Units, in exactly 100 mL of diluted acetic acid (100) (1 in 400). Pipet 1 mL of this solution, and add diluted acetic acid (100) (1 in 400) to make exactly 10 mL. Store in a cold place, avoiding freezing. Use within 6 months from the date of

preparation.

(iii) Standard solution: Dilute the standard stock solution with isotonic sodium chloride solution so that 0.2 mL of the obtained solution causes blood pressure increases of between 35 mmHg and 60 mmHg in test animals when injected according to (vi), and designate this solution as the high-dose standard solution (S_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose standard solution (S_L).

(iv) Sample solution: Dilute an accurately measured volume of Vasopressin Injection with isotonic sodium chloride solution so that the obtained solution contains the same concentration in Units as the high-dose standard solution based on the labeled Units, and designate it as the high-dose sample solution (T_H). Then dilute this solution with isotonic sodium chloride solution 1.5 to 2.0 times by volume, and designate it as the low-dose sample solution (T_L). Make the concentration ratio of S_H to S_L equal to the ratio of T_H to T_L . When the sensitivity of an animal is changed, adjust the concentration of S_H and T_H before the next set of assay is started. However, keep the same ratio of S_H to S_L and T_H to T_L as in the primary set.

(v) Dose of injection: Although 0.2 mL of each solution is usually injected, the dose of injection can be determined based from preliminary tests or experiences. Inject the same volume throughout a set of tests.

(vi) Procedure: Inject subcutaneously 0.7 mL of a solution of ethyl carbamate (1 in 4) per 100 g of body mass to anesthetize the test animals and cannulate the trachea. Under artificial respiration (about 60 strokes per minute), remove a part of the second cervical vertebra, cut off the spinal cord and destroy the brain through the foramen magnum. Insert a cannula filled with isotonic sodium chloride solution into a femoral vein. Through this cannula, inject the solution prepared by dissolving 200 heparin Units of heparin sodium in 0.1 mL of isotonic sodium chloride solution, and then immediately inject 0.3 mL of isotonic sodium chloride solution. Insert a cannula into a carotid artery, and connect the cannula to a manometer for blood pressure measurement with a vinyl tube. The cannula and the vinyl tube have previously been filled with isotonic sodium chloride solution. Inject the standard and the sample solutions at regular intervals of 10 to 15 minutes into the femoral vein through the cannula followed by 0.3 mL of the isotonic solution when the blood pressure increases caused by each solution returns to the original level. Measure the height of blood pressure increases within 1 mmHg on the kymogram. Maintain a constant temperature between 20°C and 25°C during the assay. In advance, make four pairs from S_H , S_L , T_H , T_L as follows. Randomize the order of injection for pairs, but keep the order of injection within pairs as indicated.

Pair 1: S_H , T_L Pair 2: S_L , T_H Pair 3: T_H , S_L Pair 4: T_L , S_H

Carry out this assay using the same animals throughout a set of four pairs of observations. Perform this assay with two sets. If necessary, however, use the different animals for both sets of tests.

(vii) Calculation: Subtract increases of blood pressure caused by the low dose from those caused by the high dose in the Pair 1, 2, 3 and 4 of each set, and obtain the responses y_1 , y_2 , y_3 and y_4 , respectively. Sum up y_1 , for each set to obtain Y_1 , and obtain Y_2 , Y_3 and Y_4 in the same way.

Units in each mL of Vasopressin Injection

= antilog $M \times$ (Units in each mL of the S_H) \times (b/a)

$$M = (IY_a/Y_b)$$

$$I = \log (S_H/S_L) = \log (T_H/T_L)$$

$$Y_a = -Y_1 + Y_2 + Y_3 - Y_4$$

$$Y_b = Y_1 + Y_2 + Y_3 + Y_4$$

a : Volume (mL) of Vasopressin Injection sampled.

b : Total volume (mL) of the high-dose sample solution prepared by diluting with isotonic sodium chloride solution.

Compute L ($P = 0.95$) by the following equation, and confirm L to be 0.15 or less. If L exceeds 0.15, repeat the test, improving the conditions of the assay or increasing the number of sets until L reaches 0.15 or less.

$$L = 2\sqrt{(C-1)(CM^2 + F^2)}$$

$$C = [Y_b^2/(Y_a^2 - 4fs^2t^2)]$$

f : Number of sets

$$s^2 = [\Sigma y^2 - (Y/f) - (Y'/4) + (Y_b^2/4f)]/n$$

Σy^2 : The sum of the squares of y_1, y_2, y_3 and y_4 .

$$Y = Y_1^2 + Y_2^2 + Y_3^2 + Y_4^2$$

Y' : The sum of the squares of the sum of y_1, y_2, y_3 and y_4 in each set.

$$n = 3(f - 1)$$

t^2 : Value shown in the following table against n for which s^2 is calculated.

n	$t^2 = F_1$	n	$t^2 = F_1$	n	$t^2 = F_1$
1	161.45	13	4.667	25	4.242
2	18.51	14	4.600	26	4.225
3	10.129	15	4.543	27	4.210
4	7.709	16	4.494	28	4.196
5	6.608	17	4.451	29	4.183
6	5.987	18	4.414	30	4.171
7	5.591	19	4.381	40	4.085
8	5.318	20	4.351	60	4.001
9	5.117	21	4.325	120	3.920
10	4.965	22	4.301	∞	3.841
11	4.844	23	4.279		
12	4.747	24	4.260		

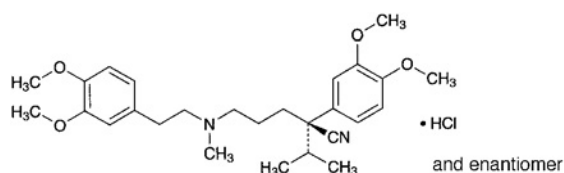
Containers and storage Containers—Hermetic containers.
Storage—In a cold place, and avoid freezing.

Expiration date 36 months after preparation.

Verapamil Hydrochloride

Iproveratril Hydrochloride

ベラパミル塩酸塩



$C_{27}H_{38}N_2O_4 \cdot HCl$: 491.06

(2*RS*)-5-[(3,4-Dimethoxyphenethyl)methylamino]-2-(3,4-dimethoxyphenyl)-2-(1-methylethyl)pentanenitrile monohydrochloride [152-11-4]

Verapamil Hydrochloride, when dried, contains not less than 98.5% of $C_{27}H_{38}N_2O_4 \cdot HCl$.

Description Verapamil Hydrochloride occurs as a white, crystalline powder. It is odorless.

It is freely soluble in methanol, in acetic acid (100) and in chloroform, soluble in ethanol (95) and in acetic anhydride, sparingly soluble in water, and practically insoluble in diethyl ether.

Identification (1) To 2 mL of a solution of Verapamil Hydrochloride (1 in 50) add 5 drops of Reinecke salt TS: a light red precipitate is produced.

(2) Determine the absorption spectrum of a solution of Verapamil Hydrochloride in 0.01 mol/L hydrochloric acid TS (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Verapamil Hydrochloride, previously dried, as directed in the potassium chloride disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Verapamil Hydrochloride (1 in 50) responds to the Qualitative Tests <1.09> for chloride.

Melting point <2.60> 141 – 145°C

pH <2.54> Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of freshly boiled and cooled water by warming, and cool: the pH of this solution is between 4.5 and 6.5.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Verapamil Hydrochloride in 20 mL of water by warming: the solution is clear and colorless.

(2) Heavy metals <1.07>—Proceed with 1.0 g of Verapamil Hydrochloride according to Method 2, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of Verapamil Hydrochloride according to Method 3, and perform the test (not more than 2 ppm).

(4) Related substances—Dissolve 0.50 g of Verapamil Hydrochloride in exactly 10 mL of chloroform, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add chloroform to make exactly 100 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add chloroform to make exactly 100 mL, and use this solution as the standard solution (1). Separately, pipet 5 mL of standard stock solution, add chloroform to make exactly 50 mL, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on two plates of silica gel for thin-layer chromatography. With the one plate, develop the plate with a mixture of cyclohexane and diethylamine (17:3) to a distance of about 15 cm, air-dry the plate, heat at 110°C for 1 hour, and cool. Examine immediately after spraying evenly iron (III) chloride-io-

dine TS on the plate: the three spots, having more intense color in the spots other than the principal spot and the original point from the sample solution, are not more intense than the spot from the standard solution (2) in color. The remaining spots from the sample solution are not more intense than the spot from the standard solution (1) in color. With another plate, develop the plate with a mixture of toluene, methanol, acetone and acetic acid (100) (14:4:1:1), and perform the test in the same manner.

Loss on drying <2.41> Not more than 1.0% (1 g, 105°C, 2 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.7 g of Verapamil Hydrochloride, previously dried, dissolve in 50 mL of a mixture of acetic anhydride and acetic acid (100) (7:3), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 49.11 mg of C₂₇H₃₈N₂O₄.HCl

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Verapamil Hydrochloride Tablets

ベラパミル塩酸塩錠

Verapamil Hydrochloride Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl: 491.06).

Method of preparation Prepare as directed under Tablets, with Verapamil Hydrochloride.

Identification (1) To a quantity of pulverized Verapamil Hydrochloride Tablets, equivalent to 0.2 g of Verapamil Hydrochloride according to the labeled amount, add 70 mL of 0.02 mol/L hydrochloric acid TS, and shake occasionally in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make 100 mL, and filter. To 3 mL of the filtrate add several drops of Reinecke's salt TS: a light red precipitate is formed.

(2) To 2 mL of the filtrate obtained in (1) add 0.02 mol/L hydrochloric acid TS to make 100 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm, and between 276 nm and 280 nm.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Verapamil Hydrochloride Tablets add 70 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablet by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate,

take exactly *V* mL of the subsequent filtrate, add 0.02 mol/L hydrochloric acid TS to make exactly *V'* so that each mL contains about 40 mg of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl), and use this solution as the sample solution. Hereafter, proceed as directed in the Assay.

Amount (mg) of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/25)$

W_S: Amount (mg) of verapamil hydrochloride for assay

Assay To 10 tablets of Verapamil Hydrochloride Tablets add 140 mL of 0.02 mol/L hydrochloric acid TS, disintegrate the tablets by occasional shaking in a water bath at 60°C for about 30 minutes, and then shake for 5 minutes. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 200 mL, and filter. Discard the first 20 mL of the filtrate, take a volume of the subsequent filtrate, equivalent to about 4 mg of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl), add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.1 g of verapamil hydrochloride for assay, previously dried at 105°C for 2 hours, and dissolve in 70 mL of 0.02 mol/L hydrochloric acid TS by occasional shaking in a water bath at 60°C. After cooling, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 4 mL of this solution, add 0.02 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, *A_T* and *A_S*, of the sample solution and the standard solution at 278 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

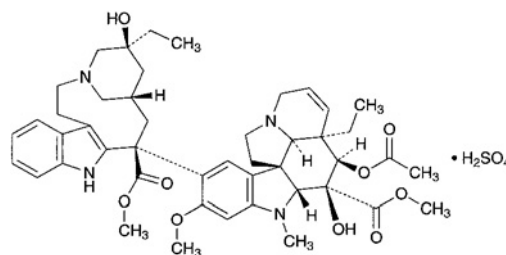
Amount (mg) of verapamil hydrochloride (C₂₇H₃₈N₂O₄.HCl)
= $W_S \times (A_T/A_S) \times (V'/V) \times (1/25)$

W_S: Amount (mg) of verapamil hydrochloride for assay

Containers and storage Containers—Tight containers.

Vinblastine Sulfate

ビンブラスチン硫酸塩



C₄₆H₅₈N₄O₉.H₂SO₄: 909.05

Methyl (3*aR*,4*R*,5*S*,5*aR*,10*bR*,13*aR*)-4-acetoxy-3*a*-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-5-hydroxy-8-methoxy-6-methyl-3*a*,4,5,5*a*,6,11,12,13*a*-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [143-67-9]

Vinblastine Sulfate contains not less than 96.0% and not more than 102.0% of C₄₆H₅₈N₄O₉.H₂SO₄, calculated on the dried basis.

Description Vinblastine Sulfate occurs as a white to pale yellow powder.

It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol (99.5).

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: -28 – -35° (0.20 g calculated on the dried basis, methanol, 10 mL, 100 mm).

Identification (1) Determine the absorption spectrum of a solution of Vinblastine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Vinblastine Sulfate Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Vinblastine Sulfate as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Vinblastine Sulfate Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Vinblastine Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 15 mg of Vinblastine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 5.0.

Purity (1) Clarity and color of solution—Dissolve 50 mg of Vinblastine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve about 4 mg of Vinblastine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area of these solutions by the automatic integration method: the area of peak other than the main peak is not larger than 1/4 of the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 3/4 of the peak area of vinblastine from the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 4 times as long as the retention time of vinblastine beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2.5 mL of the standard solution add water to make exactly 100 mL. Confirm that the peak area of vinblastine obtained from 200 μ L of this solution is equivalent to 1.7 to 3.3% of that from 200 μ L of the standard solution.

System performance: Proceed as directed in the system suitability in the Assay.

System repeatability: When the test is repeated 5 times with 200 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.5%.

Loss on drying Perform the test with about 10 mg of Vinblastine Sulfate as directed in Method 2 under the Thermal Analysis <2.52> according to the following conditions: not more than 15.0%.

Operating conditions—

Heating rate: 5°C/minute

Temperature range: room temperature to 200°C

Atmospheric gas: dried Nitrogen

Flow rate of atmospheric gas: 40 mL/minute

Assay Weigh accurately about 10 mg each of Vinblastine Sulfate and Vinblastine Sulfate Reference Standard (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use these solutions as the sample solution and the standard solution, respectively. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of vinblastine.

$$\text{Amount (mg) of } C_{46}H_{58}N_4O_9 \cdot H_2SO_4 = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Vinblastine Sulfate Reference Standard, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 262 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: To 7 mL of diethylamine add water to make 500 mL, and adjust the pH to 7.5 with phosphoric acid. To 380 mL of this solution add 620 mL of a mixture of methanol and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of vinblastine is about 8 minutes.

System suitability—

System performance: Dissolve 10 mg each of Vinblastine Sulfate and vincristine sulfate in 25 mL of water. When the procedure is run with 20 μ L of this solution under the above operating conditions, vincristine and vinblastine are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 5 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of vinblastine is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, at not exceeding -20°C .

Vinblastine Sulfate for Injection

注射用ビンブラスチン硫酸塩

Vinblastine Sulfate for Injection is a preparation for injection, which is dissolved before use.

It contains not less than 90.0% and not more than

110.0% of the labeled amount of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$: 909.05).

Method of preparation Prepare as directed under Injections, with Vinblastine Sulfate.

Description Vinblastine Sulfate for Injection occurs as white to pale yellow, light masses or powder.

It is freely soluble in water.

The pH of a solution (1 in 1000) is 3.5 – 5.0.

Identification Proceed as directed in the Identification (1) under Vinblastine Sulfate.

Purity Related substances—Dissolve 4 mg of Vinblastine Sulfate for Injection in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 200 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than the main peak from the sample solution is not larger than 1/2 of the peak area of vinblastine from the standard solution, and the total area of the peaks other than the main peak is not larger than 2 times the peak area of vinblastine from the standard solution.

Operating conditions—

Perform as directed in the operating conditions in Purity (2) under Vinblastine Sulfate.

System suitability—

Perform as directed in the system suitability in Purity (2) under Vinblastine Sulfate.

Bacterial endotoxins <4.01> Less than 10 EU/mg.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Dissolve 1 Vinblastine Sulfate for Injection in water to make exactly V mL so that each mL contains about 0.4 mg of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$) according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate Reference Standard (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9 \cdot H_2SO_4) \\ = W_S \times (A_T/A_S) \times (25/V)$$

W_S : Amount (mg) of Vinblastine Sulfate Reference Standard, calculated on the dried basis

Foreign insoluble matter <6.06> Perform the test according to Method 2: it meets the requirement.

Insoluble particulate matter <6.07> Perform the test according to Method 1: it meets the requirement.

Sterility <4.06> Perform the test according to the Membrane filtration method: it meets the requirement.

Assay Take an amount of Vinblastine Sulfate for Injection, equivalent to 0.10 g of vinblastine sulfate ($C_{46}H_{58}N_4O_9 \cdot H_2SO_4$), dissolve each content with a suitable

amount of water, transfer into a 100-mL volumetric flask, wash each container with water, transfer the washings into the volumetric flask, and add water to make exactly 100 mL. Pipet 10 mL of this solution, add water to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Vinblastine Sulfate Reference Standard (previously determine the loss on drying in the same manner as Vinblastine Sulfate), dissolve in water to make exactly 25 mL, and use this solution as the standard solution. Proceed as directed in the Assay under Vinblastine Sulfate.

$$\text{Amount (mg) of vinblastine sulfate } (C_{46}H_{58}N_4O_9 \cdot H_2SO_4) \\ = W_S \times (A_T/A_S) \times 10$$

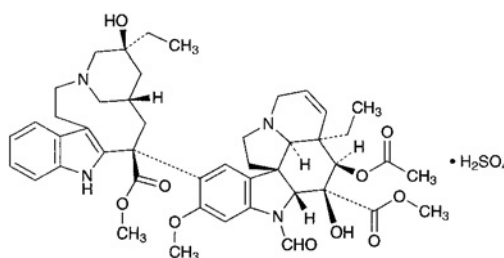
W_S : Amount (mg) of Vinblastine Sulfate Reference Standard, calculated on the dried basis

Containers and storage Containers—Hermetic containers, and colored containers may be used.

Storage—Light-resistant, at 2 to 8°C.

Vincristine Sulfate

ビンクリスチン硫酸塩



$C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$: 923.04

Methyl (3*a*R,4*R*,5*S*,5*a*R,10*b*R,13*a*R)-4-acetoxy-3*a*-ethyl-9-[(5*S*,7*S*,9*S*)-5-ethyl-5-hydroxy-9-methoxycarbonyl-1,4,5,6,7,8,9,10-octahydro-3,7-methano-3-azacycloundecino[5,4-*b*]indol-9-yl]-6-formyl-5-hydroxy-8-methoxy-3*a*,4,5,5*a*,6,11,12,13*a*-octahydro-1*H*-indolizino[8,1-*cd*]carbazole-5-carboxylate monosulfate [2068-78-2]

Vincristine Sulfate contains not less than 95.0% and not more than 105.0% of $C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$, calculated on the dried basis.

Description Vincristine Sulfate occurs as a white to light yellowish white powder.

It is very soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

It is hygroscopic.

Optical rotation $[\alpha]_D^{20}$: +28.5 – +35.5° (0.2 g, calculated on the dried basis, water, 10 mL, 100 mm).

Identification (1) Dissolve 5 mg of Vincristine Sulfate in 2 mL of cerium (IV) tetraammonium sulfate-phosphoric acid TS: a blue-purple color develops.

(2) Determine the absorption spectrum of a solution of Vincristine Sulfate (1 in 50,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar in-

tensities of absorption at the same wavelengths.

(3) Dissolve 0.02 g of Vincristine Sulfate in 10 mL of sodium chloride TS, adjust the pH to between 9 and 10 with ammonia TS, and extract with two 5-mL portions of chloroform. Wash the combined chloroform extracts with a small quantity of sodium chloride TS, add a small quantity of anhydrous sodium sulfate, and allow to stand for several minutes. Filter through a pledget of absorbent cotton, evaporate the filtrate to dryness under reduced pressure, and dissolve the residue in a small quantity of chloroform. Determine the infrared absorption spectrum of the solution as directed in the solution method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(4) A solution of Vincristine Sulfate (1 in 100) responds to the Qualitative Tests <1.09> for sulfate.

pH <2.54> Dissolve 10 mg of Vincristine Sulfate in 10 mL of water: the pH of this solution is between 3.5 and 4.5.

Purity (1) Clarity and color of solution—Dissolve 25 mg of Vincristine Sulfate in 10 mL of water: the solution is clear and colorless.

(2) Related substances—Dissolve 25 mg of Vincristine Sulfate in 10 mL of water, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add water to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine each peak area of these solutions by the automatic integration method: the total area of the peaks other than the principal peak of the sample solution is not larger than the peak area of vincristine from the standard solution, and the area of any peak other than the principal peak of the sample solution is not larger than 2/5 of the peak area of vincristine from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 297 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 25 cm in length, packed with octylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: Use methanol as the mobile phase A, and a mixture of water and diethylamine (197:3) adjusted with phosphoric acid to a pH of 7.5 as the mobile phase B. Run a mixture of the mobile phase A and the mobile phase B (31:19) for 24 minutes after injection of the sample, and run a mixture of the mobile phase A and the mobile phase B for subsequent 30 minutes, increasing the composition ratio of the mobile phase A by 1% per minute. For subsequent 4 minutes, run a mixture of the mobile phase A and the mobile phase B, decreasing the composition ratio of the mobile phase A by 7.5% per minute, then continue running a mixture of the mobile phase A and the mobile phase B (31:19).

Flow rate: Adjust the flow rate so that the retention time of vincristine is about 19 minutes.

Selection of column: Dissolve 10 mg each of Vincristine Sulfate and vinblastine sulfate in 100 mL of water. Proceed with 20 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elu-

tion of vincristine and vinblastine in this order with the resolution between these peaks being not less than 4.

Detection sensitivity: Adjust the detection sensitivity so that the peak height of vincristine from 20 μ L of the standard solution is between 5 mm and 15 mm.

Time span of measurement: About 3 times as long as the retention time of vincristine beginning after the solvent peak.

Loss on drying <2.41> Not more than 12.0% (50 mg, in vacuum, 105°C, 2 hours).

Assay Weigh accurately about 10 mg of Vincristine Sulfate, dissolve in acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Pipet 5 mL of this solution, add acetic acid-sodium acetate buffer solution, pH 5.0, to make exactly 50 mL. Determine the absorbance *A* of this solution at the maximum wavelength at about 296 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>.

$$\begin{aligned} \text{Amount (mg) of } C_{46}H_{56}N_4O_{10} \cdot H_2SO_4 \\ = (A/177) \times 5000 \end{aligned}$$

Containers and storage Containers—Hermetic containers. Storage—Light-resistant, and in a cold place.

Vitamin A Oil

ビタミンA油

Vitamin A Oil is synthetic vitamin A esters diluted with fixed oils.

It contains not less than 30,000 vitamin A Units per g.

It may contain suitable antioxidants.

It contains not less than 90.0% and not more than 120.0% of the labeled amount of vitamin A.

Description Vitamin A Oil is a yellow to yellow-brown, clear or slightly turbid oil. It is odorless or has a faint, characteristic odor.

It is decomposed upon exposure to air or light.

Identification Dissolve Vitamin A Oil, Retinol Acetate Reference Standard and Retinol Palmitate Reference Standard, equivalent to 15,000 Units, in 5 mL of petroleum ether, and use these solutions as the sample solution, the standard solution (1) and the standard solution (2), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop with a mixture of cyclohexane and diethyl ether (12:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly antimony (III) chloride TS: the principal spot obtained from the sample solution has the same color tone and the same *R_f* value with the blue spot obtained from the standard solution (1) or the standard solution (2).

Purity (1) Acidity—Dissolve 1.2 g of Vitamin A Oil in 30 mL of a mixture of neutralized ethanol and diethyl ether (1:1), boil gently for 10 minutes under a reflux condenser, cool, and add 5 drops of phenolphthalein TS and 0.60 mL of 0.1 mol/L sodium hydroxide VS: a red color develops.

(2) Rancidity—No unpleasant odor of rancid oil is perceptible by warming Vitamin A Oil.

Assay Proceed as directed in Method 1-1 under Vitamin A Assay <2.55>.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under Nitrogen atmosphere.

Vitamin A Oil Capsules

Vitamin A Capsules

ビタミンA油カプセル

Vitamin A Oil Capsules contain not less than 90.0% and not more than 130.0% of the labeled Units of vitamin A.

Method of preparation Prepare as directed under Capsules, with Vitamin A Oil.

Description The content of Vitamin A Oil Capsules conforms to the requirements of Description under Vitamin A Oil.

Identification Proceed the test with the content of Vitamin A Oil Capsules as directed in the Identification under Vitamin A Oil.

Assay Weigh accurately 20 Vitamin A Oil Capsules, and open the capsules to take out the content. Wash the capsules well with a small amount of diethyl ether, allow the capsules to stand at ordinal temperature to vaporize the diethyl ether, and weigh accurately. Perform the test with the content as directed under Vitamin A Assay <2.55>, and calculate the units of vitamin A per capsule. Before applying Method 1-1, it is necessary to know which the sample is, retinol acetate or retinol palmitate.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Compound Vitamin B Powder

複方ビタミンB散

Method of preparation

Thiamine Nitrate	10 g
Riboflavin	10 g
Pyridoxine Hydrochloride	10 g
Nicotinamide	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
	To make 1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Vitamin B Powder is orange-yellow in color. It has a slightly bitter taste.

It is slowly affected by light.

Identification (1) Shake 2 g of Compound Vitamin B

Powder with 100 mL of water, filter, and to 5 mL of the filtrate add 2.5 mL of sodium hydroxide TS and 0.5 mL of potassium hexacyanoferrate (III) TS. Then add 5 mL of 2-methyl-1-propanol, shake the mixture vigorously for 2 minutes, allow to stand, and observe under ultraviolet light: the 2-methyl-1-propanol layer shows a blue-purple fluorescence. This fluorescence disappears when the mixture is acidified, but reappears when it is again made alkaline (thiamine).

(2) Shake 0.1 g of Compound Vitamin B Powder with 100 mL of water, and filter. Perform the following tests with the filtrate (riboflavin).

(i) The filtrate is light yellow-green in color and has an intense yellow-green fluorescence. This color and fluorescence of the solution disappears upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the filtrate, and again appears by shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(ii) To 10 mL of the filtrate placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, after illuminating with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake thoroughly with 5 mL of chloroform: the chloroform layer shows yellow-green fluorescence.

(3) Shake 1 g of Compound Vitamin B Powder with 100 mL of diluted ethanol (7 in 10), filter, and to 5 mL of the filtrate add 2 mL of sodium hydroxide TS and 40 mg of manganese dioxide. Heat on a water bath for 30 minutes, cool, and filter. Add 5 mL of 2-propanol to 1 mL of the filtrate, and use the solution as the sample solution. To 3 mL of the sample solution add 2 mL of bartibal buffer solution, 4 mL of 2-propanol and 2 mL of a freshly prepared solution of 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine in ethanol (95) (1 in 4000) prepared when required for use: a blue color develops. To 1 mL of the sample solution add 1 mL of a saturated boric acid solution, and proceed as directed in the same manner as above: no blue color develops (pyridoxine).

(4) Shake 0.5 g of Compound Vitamin B Powder with 10 mL of ethanol (95), filter, and evaporate 1 mL of the filtrate on a water bath to dryness. Add 0.01 g of 2,4-dinitrochlorobenzene to the residue, heat gently for 5–6 seconds to fuse, and after cooling, add 4 mL of potassium hydroxide-ethanol TS: a red color develops (nicotinamide).

(5) Shake 1 g of Compound Vitamin B Powder with 5 mL of diluted ethanol (7 in 10), filter, and use the filtrate as the sample solution. Separately, dissolve 0.01 g each of thiamine mononitrate, riboflavin, pyridoxine hydrochloride and nicotinamide in 1 mL, 50 mL, 1 mL and 1 mL of water, respectively, and use these solutions as standard solutions (1), (2), (3) and (4). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solutions (1), (2), (3) and (4) on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, ethanol (95) and acetic acid (100) (100:50:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): four spots from the sample solution show the same color tone and the same R_f value as the corresponding spots from standard solutions (1), (2), (3) and (4).

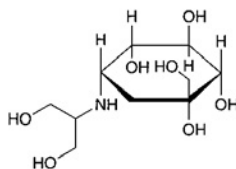
Containers and storage Containers—Well-closed contain-

ers.

Storage—Light-resistant.

Voglibose

ボグリボース



$C_{10}H_{21}NO_7$; 267.28

3,4-Dideoxy-4-[2-hydroxy-1-(hydroxymethyl)ethylamino]-
2-C-(hydroxymethyl)-D-*epi*-inositol
[83480-29-9]

Voglibose contains not less than 99.5% and not more than 101.0% of $C_{10}H_{21}NO_7$, calculated on the anhydrous basis.

Description Voglibose occurs as white crystals or crystalline powder.

It is very slightly soluble in water, freely soluble in acetic acid (100), slightly soluble in methanol, and very slightly soluble in ethanol (99.5).

It dissolves in 0.1 mol/L hydrochloric acid TS.

Identification (1) Determine the infrared absorption spectrum of Voglibose as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

(2) Determine the spectrum of a solution of Voglibose in heavy water for nuclear magnetic resonance spectroscopy (3 in 70) as directed under Nuclear Magnetic Resonance Spectroscopy <2.21> (1H), using sodium 3-trimethylsilylpropionate- d_4 for nuclear magnetic resonance spectroscopy as an internal reference compound: it exhibits 2 double signals A at about δ 1.5 ppm, 2 double signals B at about δ 2.1 ppm, a multiple signal C at about δ 2.9 ppm, and a multiple signal D between δ 3.4 ppm and δ 3.9 ppm. The area intensity ratio of each signal, A:B:C:D, is about 1:1:1:10.

Optical rotation <2.49> $[\alpha]_D^{20}$: +45 – +48° (0.2 g calculated on the anhydrous basis, 0.1 mol/L hydrochloric acid TS, 20 mL, 100 mm).

pH <2.54> Dissolve 1.0 g of Voglibose in 10 mL of water: the pH of the solution is between 9.8 and 10.4.

Melting point <2.60> 163 – 168°C

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Voglibose according to Method 1, and perform the test. Adjust the pH of the test solution between 3.0 and 3.5 with dilute hydrochloric acid instead of dilute acetic acid. Prepare the control solution with 1.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Related substances—Dissolve 50 mg of Voglibose in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mo-

bile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following operating conditions, and determine each peak area by the automatic integration method: the total area of the peaks other than voglibose is not larger than 1/5 times the peak area of voglibose from the standard solution. For the calculate of the total area, use the area of the peaks, having the relative retention time of about 1.7, about 2.0 and about 2.3 after multiplying by their relative response factors, 2, 2 and 2.5, respectively.

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with pentaethylenehexaaminated polyvinyl alcohol polymer bead for liquid chromatography.

Column temperature: A constant temperature of about 25°C.

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 370 mL of this solution add 630 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C

Cooling temperature: A constant temperature of about 15°C

Flow rate of the mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of the reaction reagent: Same as the flow rate of the mobile phase

Time span of measurement: About 2.5 times as long as the retention time of voglibose, beginning after the solvent peak
System suitability—

Test for required detectability: Pipet 10 mL of the standard solution, and add the mobile phase to make 100 mL. Confirm that the peak area of voglibose obtained from 50 μ L of this solution is equivalent to 7 to 13% of that of voglibose obtained from 50 μ L of the standard solution.

System performance: When the procedure is run with 50 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of voglibose are not less than 7000 and between 0.8 and 1.2, respectively.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 3.0%.

Water <2.48> Not more than 0.2% (0.5 g, coulometric titration).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.4 g of Voglibose, dissolve in 80 mL of acetic acid (100), and titrate <2.50> with 0.1 mol/L perchloric acid VS (potentiometric titration). Perform a blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L perchloric acid VS
= 26.73 mg of $C_{10}H_{21}NO_7$

Containers and storage Containers—Tight containers.

Voglibose Tablets

ボグリボース錠

Voglibose Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of voglibose ($C_{10}H_{21}NO_7$; 267.28).

Method of preparation Prepare as directed under Tablets, with Voglibose.

Identification Shake vigorously an amount of pulverized Voglibose Tablets, equivalent to 5 mg of Voglibose according to the labeled amount, with 40 mL of water, and centrifuge. Transfer the supernatant liquid to a chromatographic column [prepared by pouring 1.0 mL of strongly acidic ion-exchange resin (H type) for column chromatography (100 to 200 μ m in particle diameter) into a chromatographic column 8 mm in inside diameter and 130 mm in height], and allow to flow at a rate of about 5 mL per minute. Then wash the column with 200 mL of water, and allow to flow with 10 mL of diluted ammonia TS (1 in 4) at a rate of about 5 mL per minute. Filter the effluent solution two times through a membrane filter with pore size of not more than 0.22 μ m. Evaporate the filtrate to dryness at 50°C under reduced pressure, dissolve the residue with 0.5 mL of a mixture of water and methanol (1:1), and use this solution as the sample solution. Separately, dissolve 20 mg of voglibose for assay in 2 mL of the mixture of water and methanol (1:1), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ammonia water (28) and water (5:3:1) to a distance of about 12 cm, air-dry the plate, and allow to stand in iodine vapors: the principal spot from the sample solution and the spot from the standard solution show a yellow-brown color, and the same *R_f* value.

Uniformity of dosage unit <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Voglibose Tablets add exactly *V* mL of the mobile phase so that the solution contains about 40 μ g of voglibose ($C_{10}H_{21}NO_7$) per mL according to the labeled amount, disintegrate the tablet completely by shaking, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.45 μ m. Discard the

first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Hereinafter, proceed as directed in the Assay.

$$\begin{aligned} & \text{Amount (g) of voglibose (C}_{10}\text{H}_{21}\text{NO}_7) \\ & = W_S \times (A_T/A_S) \times (V/500) \end{aligned}$$

W_S: Amount (mg) of voglibose for assay, calculated on the anhydrous basis

Assay To 20 tablets of Voglibose Tablets add 80 mL of the mobile phase, and completely disintegrate by shaking. To an exact volume of the solution, equivalent to about 4 mg of voglibose ($C_{10}H_{21}NO_7$) according to the labeled amount, add the mobile phase to make exactly 100 mL, and centrifuge. Filter the supernatant liquid through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 1 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 20 mg of voglibose for assay (previously determine the water <2.48> in the same manner as Voglibose), and dissolve in the mobile phase to make exactly 25 mL. Pipet 5 mL of this solution, add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 50 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of voglibose.

$$\begin{aligned} & \text{Amount (mg) of voglibose (C}_{10}\text{H}_{21}\text{NO}_7) \\ & = W_S \times (A_T/A_S) \times (1/500) \end{aligned}$$

W_S: Amount of voglibose for assay, calculated on the dried basis

Operating conditions—

Apparatus: Use an apparatus consisting of 2 pumps for the mobile phase and reaction reagent transportation, sample injection port, column, reaction coil, cooling coil, detector and recording device, and the reaction coil and cooling coil maintained at a constant temperature.

Detector: Fluorophotometer (excitation wavelength: 350 nm, fluorescence wavelength: 430 nm)

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with aminopropylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C

Reaction coil: A polytetrafluoroethylene tube 0.5 mm in inside diameter and 20 m in length

Cooling coil: A polytetrafluoroethylene tube 0.3 mm in inside diameter and 2 m in length

Mobile phase: To 1.56 g of sodium dihydrogen phosphate dihydrate add water to make 500 mL. To this solution add a solution, prepared by dissolving 3.58 g of disodium hydrogen phosphate dodecahydrate in water to make 500 mL, to adjust to pH 6.5. To 300 mL of this solution add 600 mL of acetonitrile.

Reaction reagent: Dissolve 6.25 g of taurine and 2.56 g of sodium periodate in water to make 1000 mL.

Reaction temperature: A constant temperature of about 100°C

Cooling temperature: A constant temperature of about 15°C

Flow rate of mobile phase: Adjust the flow rate so that the retention time of voglibose is about 20 minutes.

Flow rate of reaction reagent: Same as the flow rate of the mobile phase

System suitability—

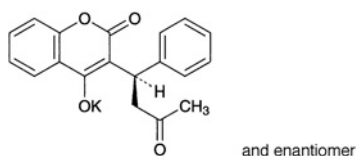
System performance: Dissolve 2 mg of voglibose for assay and 0.2 g of lactose monohydrate in 5 mL of water, and add the mobile phase to make 50 mL. When the procedure is run with 50 μ L of this solution under the above operating conditions, lactose and voglibose are eluted in this order with the resolution between these peaks being not less than 4.

System repeatability: When the test is repeated 6 times with 50 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of voglibose is not more than 2.0%.

Containers and storage Containers—Tight containers.

Warfarin Potassium

ワルファリンカリウム



$C_{19}H_{15}KO_4$: 346.42

Monopotassium (1*RS*)-2-oxo-3-(3-oxo-1-phenylbutyl)chromen-4-olate [2610-86-8]

Warfarin Potassium, when dried, contains not less than 98.0% and not more than 102.0% of $C_{19}H_{15}KO_4$.

Description Warfarin Potassium occurs as a white, crystalline powder.

It is very soluble in water, and freely soluble in ethanol (95).

It dissolves in sodium hydroxide TS.

The pH of a solution prepared by dissolving 1.0 g of Warfarin Potassium in 100 mL of water is 7.2 – 8.3.

It is colored to light yellow by light.

A solution of Warfarin Potassium (1 in 10) shows no optical rotation.

Identification (1) Determine the absorption spectrum of a solution of Warfarin Potassium in 0.02 mol/L potassium hydroxide TS (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Warfarin Potassium Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

(2) Determine the infrared absorption spectrum of Warfarin Potassium, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum or the spectrum of Warfarin Potassium Reference Standard: both spectra exhibit similar intensities of absorption at the same wave numbers.

(3) A solution of Warfarin Potassium (1 in 250) responds to the Qualitative Tests <1.09> (1) for potassium salt.

Purity (1) Alkaline colored substances—Dissolve 1.0 g of Warfarin Potassium in a solution of sodium hydroxide (1 in

20) to make exactly 10 mL, and determine the absorbance at 385 nm within 15 minutes as directed under Ultraviolet-visible Spectrophotometry <2.24>, using a solution of sodium hydroxide (1 in 20) as a blank: it does not exceed 0.20.

(2) Heavy metals <1.07>—Dissolve 2.0 g of Warfarin Potassium in 30 mL of ethanol (95), add 2 mL of dilute acetic acid and ethanol (95) to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 2.0 mL of Standard Lead Solution, 2 mL of dilute acetic acid and ethanol (95) to make 50 mL (not more than 10 ppm).

(3) Related substances—Dissolve 0.10 g of Warfarin Potassium in 100 mL of a mixture of water and methanol (3:1), and use this solution as the sample solution. Pipet 1 mL of the sample solution, add the mixture of water and methanol (3:1) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: each peak area other than warfarin is not larger than 1/10 times the peak area of warfarin obtained with the standard solution, and the total area of the peaks other than warfarin is not larger than 1/2 times the peak area of warfarin with the standard solution.

Operating conditions—

Detector, column, column temperature, mobile phase, and flow rate: Proceed as directed in the operating conditions in the Assay.

Time span of measurement: About 2 times as long as the retention time of warfarin beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 1 mL of the standard solution add the mixture of water and methanol (3:1) to make exactly 20 mL. Confirm that the peak area of warfarin obtained with 20 μ L of this solution is equivalent to 3.5 to 6.5% of that with 20 μ L of the standard solution.

System performance: Dissolve 20 mg of propyl parahydroxybenzoate in 50 mL of methanol, and add water to make 200 mL. To 5 mL of this solution add 4 mL of a solution of Warfarin Potassium in the mixture of water and methanol (3:1) (1 in 2000), and add the mixture of water and methanol (3:1) to make 100 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, propyl parahydroxybenzoate and warfarin are eluted in this order with the resolution between these peaks being not less than 7.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 2.0%.

Loss on drying <2.41> Not more than 4.5% (1 g, 105°C, 3 hours).

Assay Weigh accurately about 25 mg each of Warfarin Potassium and Warfarin Potassium Reference Standard, previously dried, and dissolve separately in the mixture of water and methanol (3:1) to make exactly 50 mL. Pipet 10 mL each of these solutions, add the mixture of water and methanol (3:1) to make exactly 50 mL, and use these solutions as the sample solution and the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>.

matography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of warfarin.

$$\text{Amount (mg) of } C_{19}H_{15}KO_4 = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Warfarin Potassium Reference Standard

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 260 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with cyanopropyl-silanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (68:32:1).

Flow rate: Adjust the flow rate so that the retention time of warfarin is about 10 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of warfarin are not less than 8000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of warfarin is not more than 1.0%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Warfarin Potassium Tablets

ワルファリンカリウム錠

Warfarin Potassium Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of warfarin potassium ($C_{19}H_{15}KO_4$; 346.42).

Method of preparation Prepare as directed under Tablets, with Warfarin Potassium.

Identification (1) Determine the absorption spectrum of the solution T_2 obtained in the Assay, using 0.02 mol/L potassium hydroxide TS as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum between 306 nm and 310 nm, and a minimum between 258 nm and 262 nm. Separately, determine the absorption spectrum of the solution T_1 obtained in the Assay, using 0.02 mol/L hydrochloric acid TS as the blank, as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 281 nm and 285 nm and between 303 nm and 307 nm, and a minimum between 243 nm and 247 nm.

(2) Weigh a quantity of Warfarin Potassium Tablets, equivalent to 0.01 g of Warfarin Potassium according to the labeled amount, add 10 mL of acetone, shake, and filter. Heat the filtrate on a water bath to evaporate the acetone. To the residue add 10 mL of diethyl ether and 2 mL of dilute hydrochloric acid, and shake: the aqueous layer responds to the Qualitative Tests <1.09> (1) for potassium salt.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

Powder 1 tablet of Warfarin Potassium Tablets, add 40 mL of water, and shake vigorously for 30 minutes. Add water to make exactly V mL of this solution containing about 20 μ g of warfarin potassium ($C_{19}H_{15}KO_4$) per mL. Filter this solution, discard the first 5 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 40 mg of Warfarin Potassium Reference Standard, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L hydrochloric acid TS to make exactly 25 mL, and use these solutions as the solution T_1 and the solution S_1 , respectively. Separately, pipet 20 mL each of the sample solution and the standard solution, add 0.05 mol/L potassium hydroxide TS to make exactly 25 mL, and use these solutions as the solution T_2 and the solution S_2 , respectively. Determine the absorbances, A_T and A_S , of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution T_2 and the solution S_2 as the blank, respectively.

$$\begin{aligned} \text{Amount (mg) of warfarin potassium (} C_{19}H_{15}KO_4 \text{)} \\ = W_S \times (A_T/A_S) \times (V/2000) \end{aligned}$$

W_S : Amount (mg) of Warfarin Potassium Reference Standard

Assay Weigh accurately and powder not less than 20 Warfarin Potassium Tablets. Weigh accurately a portion of the powder, equivalent to about 4 mg of warfarin potassium ($C_{19}H_{15}KO_4$), add 80 mL of water, shake vigorously for 15 minutes, and add water to make exactly 100 mL. Filter this solution, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 80 mg of Warfarin Potassium Reference Standard, previously dried at 105°C for 3 hours, and dissolve in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 mL each of the sample solution and the standard solution, add 0.02 mol/L hydrochloric acid TS to make exactly 20 mL, and use these solutions as the solution T_1 and the solution S_1 , respectively. Separately, pipet 10 mL each of the sample solution and the standard solution, add 0.02 mol/L potassium hydroxide TS to make exactly 20 mL, and use these solutions as the solution T_2 and the solution S_2 , respectively. Determine the absorbances, A_T and A_S , of the solution T_1 and the solution S_1 at 272 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using the solution T_2 and the solution S_2 as the blank, respectively.

$$\begin{aligned} \text{Amount (mg) of warfarin potassium (} C_{19}H_{15}KO_4 \text{)} \\ = W_S \times (A_T/A_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of Warfarin Potassium Reference Standard

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Water

常水

H₂O: 18.02

Water meets the quality standards of water supplies under Article 4 of the Water Supply Law (the Ministry of Health, Labour and Welfare Ministerial Ordinance No. 101, May 30, 2003), and also meets the following requirement:

Purity Ammonium <1.02>—Perform the test with 30 mL of Water as directed under Ammonium Limit Test. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

Water for Injection

注射用水

Water for Injection is water either prepared by distillation of Water or Purified Water, or by the Reverse Osmosis-Ultrafiltration (a reverse osmosis membrane, an ultrafiltration membrane or a combined purification system using these membranes) of Purified Water, and used for the preparation of injections, or for an alternative usage as a packed Water for Injection, which is preserved in suitable containers and sterilized.

When Water for Injection is prepared by the Reverse Osmosis-Ultrafiltration, take precaution against microbial contamination of the purifying system to get comfortable quality being equivalent to that of water prepared by distillation.

Water for Injection for the preparation of injections must be used immediately after preparation. However, it may be stored for a certain period of time, if the purifying system of water is established for avoiding microbial contamination and its growth in preserved period.

Water for Injection preserved in containers and sterilized is used mainly as solvent for injections to be dissolved or suspended before use.

Water for Injection prepared by distillation and packed in containers as sterilized products may be labeled “Distilled Water for Injection” as a commonly used name.

Purity (1) Acidity or alkalinity—To 20 mL of Water for Injection add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. Separately, to 20 mL of Water for Injection add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Water for Injection add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Water for Injection add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Water for Injection to a 50-mL beaker, add 1 mL of sodium salicylate-sodium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Water for Injection to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate TS: no pale red color develops.

(6) Ammonium <1.02>—Perform the test with 30 mL of Water for Injection as the test solution. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

(7) Heavy metals—To 40 mL of Water for Injection add 2 mL of dilute acetic acid and 1 drop of sodium sulfide TS: no change occurs.

(8) Potassium permanganate-reducing substances—To 100 mL of Water for Injection add 10 mL of dilute sulfuric acid, boil, add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color does not disappear.

(9) Residue on evaporation—Evaporate 100 mL of Water for Injection on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

For Water for Injection prepared by the Reverse Osmosis-Ultrafiltration for the preparation of injections, perform the test for (8) Total organic carbon described below, instead of (8) Potassium permanganate-reducing substances. For Water for Injection preserved in containers and sterilized, perform the test for (1) Acid or alkali, (2) Chloride, (6) Ammonium and (9) Residue on evaporation according to the following methods:

(1) Acidity or alkalinity—Shake gently 20 mL of Water for Injection with 0.05 mL of phenol red TS and 0.13 mL of 0.01 mol/L sodium hydroxide VS, and allow to stand for 30 seconds: a red color develops. Separately, shake gently 20 mL of Water for Injection with 0.05 mL of bromothymol blue TS and 0.13 mL of 0.01 mol/L hydrochloric acid VS, and allow to stand for 30 seconds: a yellow color develops.

(2) Chloride—For Water for Injection in containers holding a volume not more than 10 mL, add 2.0 mL of dilute nitric acid to 15 mL of Water for Injection, and use this solution as the test solution. Separately, to 0.20 mL of 0.001 mol/L hydrochloric acid VS add water to make 15 mL, then add 2.0 mL of dilute nitric acid, and use this solution as the control solution. Mix the test solution and the control solution separately with 0.30 mL each of silver nitrate TS, allow to stand for 5 minutes under the protection from sunlight, and compare the turbidity of the solutions on a black background: the turbidity of the test solution is not thicker than that of the control solution (not more than 0.00005%). For Water for Injection in containers holding a volume exceeding 10 mL, add 3 drops of nitric acid and 0.5 mL of silver nitrate TS to 50 mL of Water for Injection: the solution remains un-

changed.

(6) Ammonium <1.02>—Perform the test with 30 mL of Water for Injection as the test solution. Prepare the control solution as follows: To 0.6 mL of Standard Ammonium Solution for Water for Injection in containers holding a volume not more than 10 mL, and 0.3 mL of Standard Ammonium Solution for Water for Injection in a volume exceeding 10 mL, add purified water for ammonium limit test to make 30 mL (not more than 0.2 mg/L for Water for Injection in a volume not more than 10 mL, and not more than 0.1 mg/L for that exceeding 10 mL).

(8) Total organic carbon <2.59>—Apply the test to Water for Injection prepared by the Reverse Osmosis-Ultrafiltration for the preparation of injections: it contains not more than 0.50 mg/L of total organic carbon.

(9) Residue on evaporation—Evaporate 100 mL of Water for Injection, and dry the residue at 105°C for 1 hour: the residue weighs not more than 4.0 mg for Water for Injection in a volume not more than 10 mL, and not more than 3.0 mg for that exceeding 10 mL.

Bacterial endotoxins <4.01> Less than 0.25 EU/mL.

Extractable volume <6.05> It meets the requirement.

Sterility <4.06> Apply the test to Water for Injection preserved in containers as sterilized products: it meets the requirement.

Containers and storage Containers—(1) For the preparation of injections, suitable containers, protected from microbial contamination.

(2) Hermetic containers for Water for Injection preserved in the containers as sterilized products. Plastic containers for aqueous infusions may be used.

Purified Water

精製水

Purified Water is purified Water by hyperfiltration (reverse osmosis, ultrafiltration), ion-exchange treatment, distillation or combination of these methods. When prepare Purified Water, be careful to prevent microbial contamination.

Use immediately after purification. It may be stored for a certain period, if it is in suitable containers preventing microbial growth.

Description Purified Water is a clear, colorless liquid. It is odorless and tasteless.

Purity (1) Acidity or alkalinity—To 20 mL of Purified Water add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. To 20 mL of Purified Water add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Purified Water add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Purified Water add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Purified Water to a 50-mL beaker, add 1 mL of sodium salicylate-so-

dium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Purified Water to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N*-(1-naphthyl)-*N'*-diethylethylenediamine oxalate TS: no pale red color develops.

(6) Ammonium <1.02>—Perform the test with 30 mL of Purified Water as the test solution. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

(7) Heavy metals—The 40 mL of Purified Water add 2 mL of dilute acetic acid and 1 drop of sodium sulfide TS: no change occurs.

(8) Potassium permanganate-reducing substances—To 100 mL of Purified Water add 10 mL of dilute sulfuric acid, boil, add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color does not disappear.

(9) Residue on evaporation—Evaporate 100 mL of Purified Water on a water bath to dryness, and dry the residue at 105°C for 1 hour: the amount of the residue is not more than 1.0 mg.

Containers and storage Containers—Tight containers.

Sterile Purified Water

滅菌精製水

Sterile Purified Water is sterilized Purified Water.

Description Sterile Purified Water is a clear, colorless liquid. It is odorless and tasteless.

Purity (1) Acidity or alkalinity—To 20 mL of Sterile Purified Water add 0.1 mL of methyl red TS for acid or alkali test: a yellow to orange color develops. To 20 mL of Sterile Purified Water add 0.05 mL of bromothymol blue TS: no blue color develops.

(2) Chloride—To 50 mL of Sterile Purified Water add 3 drops of nitric acid and 0.5 mL of silver nitrate TS: no change occurs.

(3) Sulfate—To 50 mL of Sterile Purified Water add 0.5 mL of barium chloride TS: no change occurs.

(4) Nitrogen from nitrate—Transfer 2.0 mL of Sterile Purified Water to a 50-mL beaker, add 1 mL of sodium salicylate-sodium hydroxide TS, 1 mL of a solution of sodium chloride (1 in 500) and 1 mL of a solution of ammonium amidosulfate (1 in 1000), and evaporate on a water bath to dryness. Cool, dissolve in 2 mL of sulfuric acid, allow to stand for 10 minutes, with occasional shaking, add 10 mL of water, and transfer to a Nessler tube. Cool, add 10 mL of a solution of sodium hydroxide (2 in 5) slowly, and add water to make 25 mL: no yellow color develops.

(5) Nitrogen from nitrite—Transfer 10 mL of Sterile Purified Water to a Nessler tube, and add 1 mL of a solution of sulfanilamide in dilute hydrochloric acid (1 in 100) and 1 mL of *N*-(1-Naphthyl)-*N'*-diethylethylenediamine oxalate TS; no pale red color develops.

(6) Ammonium <1.02>—Perform the test with 30 mL of Sterile Purified Water as the test solution. Prepare the control solution as follows: to 0.15 mL of Standard Ammonium Solution add purified water for ammonium limit test to make 30 mL (not more than 0.05 mg/L).

(7) Heavy metals—To 40 mL of Sterile Purified Water add 2 mL of dilute acetic acid and 1 drop of sodium sulfide TS; no change occurs.

(8) Potassium permanganate-reducing substances—To 100 mL of Sterile Purified Water add 10 mL of dilute sulfuric acid, boil, add 0.10 mL of 0.02 mol/L potassium permanganate VS, and boil again for 10 minutes: the red color does not disappear.

(9) Residue on evaporation—Evaporate 100 mL of Sterile Purified Water on a water bath to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Sterility <4.06> Take 500 mL of Sterile Purified Water, and perform the test by the Membrane filtration method: it meets the requirements.

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Weil's Disease and Akiyami Combined Vaccine

ワイル病秋やみ混合ワクチン

Weil's Disease and Akiyami Combined Vaccine is a liquid for injection containing inactivated Weil's disease leptospira, Akiyami A leptospira, Akiyami B leptospira and Akiyami C leptospira. The product lacking more than a kind of Akiyami leptospira may be prepared, if necessary.

It conforms to the requirements of Weil's Disease and Akiyami Combined Vaccine in the Minimum Requirements for Biological Products.

Description Weil's Disease and Akiyami Combined Vaccine is a white-turbid liquid.

Wheat Starch

Amylum Triticum

コムギデンプン

This monograph is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆)

Wheat Starch consists of the starch granules obtained from the seeds of *Triticum aestivum* Linné

(*Gramineae*).

♦ **Description** Wheat Starch occurs as white masses or powder.

It is practically insoluble in water and in ethanol (99.5).◆

Identification (1) Under a microscope <5.01>, Wheat Starch, preserved in a mixture of water and glycerin (1:1), appears as large and small sized simple grains, or quite rarely median sized simple grains; usually, large sized grains about 10–60 μm in diameter, from upper view, disc like or quite rarely reniform, centric hilum and striation indistinct or hardly distinct, often cleft on marginal portion visible; from lateral view, narrowly ellipsoid or fusiform, hilum recognized as a long and slender cleft along with long axis; small sized grains 2–10 μm in diameter, spherical or polygonal; a black cross, its intersection point on hilum, is observed when grains are put between two nicol prisms fixed at right angle to each other.

(2) To 1 g of Wheat Starch add 50 mL of water, boil for 1 minute, and allow to cool: a subtle white-turbid, pasty liquid is formed.

(3) To 1 mL of the pasty liquid obtained in (2) add 0.05 mL of diluted iodine TS (1 in 10): a deep blue color is formed, and the color disappears by heating.

pH <2.54> Put 5.0 g of Wheat Starch in a non-metal vessel, add 25.0 mL of freshly boiled and cooled water, mix gently for 1 minute, and allow to stand for 15 minutes: the pH of the solution is between 4.5 and 7.0.

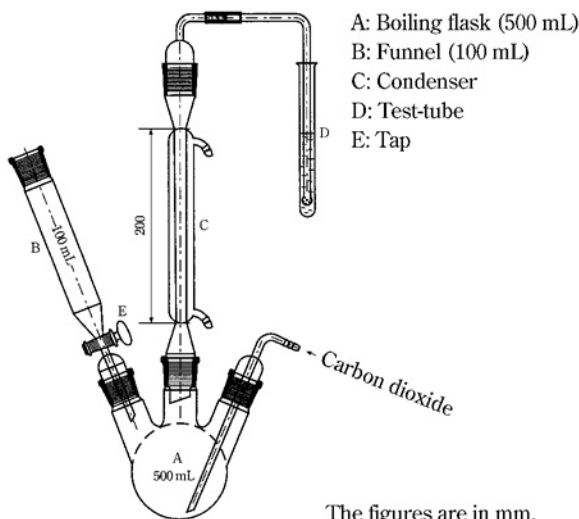
Purity (1) Iron—To 1.5 g of Wheat Starch add 15 mL of 2 mol/L hydrochloric acid TS, mix, filter, and use the filtrate as the test solution. To 2.0 mL of Standard Iron Solution add water to make 20 mL, and use as the control solution. Put 10 mL each of the test solution and the control solution in test tubes, add 2 mL of a solution of citric acid (2 in 10) and 0.1 mL of mercapto acetic acid, and mix. Alkalize with ammonia solution (28) to litmus paper, add water to make 20 mL, and mix. Transfer 10 mL each of these solutions into test tubes, allow to stand for 5 minutes, and compare the color of these solutions against a white background: the color of the test solution is not more intense than that of the control solution (not more than 10 ppm).

(2) Oxidizing substances—To 4.0 g of Wheat Starch add 50.0 mL of water, shake for 5 minutes, and centrifuge. To 30.0 mL of the supernatant liquid add 1 mL of acetic acid (100) and 0.5 to 1.0 g of potassium iodide, shake, and allow to stand for 25 to 30 minutes at a dark place. Add 1 mL of starch TS, and titrate <2.50> with 0.002 mol/L sodium thiosulfate VS until the color of the solution disappears. Perform a blank determination and make any necessary correction: the volume of 0.002 mol/L sodium thiosulfate VS consumed is not more than 1.4 mL (not more than 20 ppm, calculated as hydrogen peroxide).

(3) Sulfur dioxide—

(i) Apparatus Use as shown in the figure.

(ii) Procedure Introduce 150 mL of water into the boiling flask, close the tap of the funnel, and pass carbon dioxide through the whole system at a rate of 100 ± 5 mL per minute. Pass cooling water through the condenser, and place 10 mL of hydrogen peroxide-sodium hydroxide TS in the test-tube. After 15 minutes, remove the funnel without interrupting the stream of carbon dioxide, and introduce through the opening into the flask about 25 g of Wheat Starch, accurately



weighed, with the aid of 100 mL of water. Apply tap grease to the outside of the connection part of the funnel, and load the funnel. Close the tap of the funnel, pour 80 mL of 2 mol/L hydrochloric acid TS into the funnel, open the tap to introduce the hydrochloric acid into the flask, and close the tap while several mL of the hydrochloric acid remains, in order to avoid losing sulfur dioxide. Place the flask in a water bath, and heat the mixture for 1 hour. Transfer the contents of the test-tube with the aid of a little water to a wide-necked conical flask. Heat in a water bath for 15 minutes, and cool. Add 0.1 mL of bromophenol blue TS, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS until the color changes from yellow to violet-blue lasting for at least 20 seconds. Perform a blank determination and make any necessary correction. Calculate the amount of sulfur dioxide by applying the following formula: it is not more than 50 ppm.

$$\text{Amount (ppm) of sulfur dioxide} = (V/W) \times 1000 \times 3.203$$

W : Amount (g) of the sample

V : Amount (mL) of 0.1 mol/L sodium hydroxide VS consumed

Loss on drying <2.41> Not more than 15.0% (1 g, 130°C, 90 minutes).

Residue on ignition <2.44> Not more than 0.6% (1 g).

◆**Containers and storage** Containers—Well-closed containers. ◆

White Ointment

白色軟膏

Method of preparation

White Beeswax	50 g
Sorbitan Sesquioleate	20 g
White Petrolatum	a sufficient quantity
To make 1000 g	

Prepare as directed under Ointments, with the above materials.

Description White Ointment is white in color. It has a

slight, characteristic odor.

Containers and storage Containers—Tight containers.

Whole Human Blood

人全血液

Whole Human Blood is a liquid for injection which is prepared by mixing human blood cells and an anticoagulant solution for storage.

It conforms to the requirements of Whole Human Blood in the Minimum Requirements for Biological Products.

Description Whole Human Blood is a deep red liquid from which the erythrocytes settle upon standing, leaving a yellow supernatant layer. A gray layer which mainly consists of leucocytes may appear on the surface of the settled erythrocyte layer. The supernatant layer may become turbid in the presence of fat, or may show the faint color of hemoglobin.

Wine

ブドウ酒

Wine is an alcoholic liquid obtained by fermenting the juice of the fruits of *Vitis vinifera* Linné (*Vitaceae*) or allied plants.

It contains not less than 11 vol% and not more than 14 vol% of ethanol (C_2H_6O : 46.07) (by specific gravity), and not less than 0.10 w/v% and not more than 0.40 w/v% of L-tartaric acid ($C_4H_6O_6$: 150.09).

It contains no artificial sweetener and no artificial coloring agent.

Description Wine is a light yellow or reddish purple to red-purple liquid. It has a characteristic and aromatic odor. It has a slightly astringent and faintly irritating taste.

Specific gravity <2.56> d_{20}^{20} : 0.990 – 1.010

Optical rotation <2.49> Boil 160 mL of Wine, neutralize with potassium hydroxide TS, and concentrate to 80 mL on a water bath. Cool, dilute with water to 160 mL, add 16 mL of lead subacetate TS, shake well, and filter. To 100 mL of the filtrate add 10 mL of a saturated solution of sodium sulfate decahydrate, shake well, filter, and use the filtrate as the sample solution. Allow 20 mL of the sample solution to stand for 24 hours, add 0.5 g of activated charcoal, shake, stopper, and allow to stand for 10 minutes. Filter, and observe the optical rotation of the filtrate in a 200-mm cell. Multiply the optical rotation observed by 1.21, and designate as the optical rotation of Wine: it is between -0.3° and $+0.3^\circ$.

Purity (1) Total acid [as L-tartaric acid ($C_4H_6O_6$)]—To exactly 10 mL of Wine add 250 mL of freshly boiled and cooled water, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (indicator: 1 mL of phenolphthalein TS).

Each mL of 0.1 mol/L sodium hydroxide VS
= 7.504 mg of $C_4H_6O_6$

Total acid is not less than 0.40 w/v% and not more than 0.80 w/v%.

(2) Volatile acid [as acetic acid ($C_2H_4O_2$: 60.05)]—Transfer 100 mL of Wine to a beaker, add 1 mL of 1 mol/L sodium hydroxide VS and the same volume of 1 mol/L sodium hydroxide VS as that of 0.1 mol/L sodium hydroxide VS titrated in (1) to make the solution alkaline, and concentrate to 50 mL on a water bath. Cool, add water to make 100 mL, transfer to a 1000-mL distillation flask, containing previously added 100 g of sodium chloride. Wash the beaker with 100 mL of water, and combine the washings in the distillation flask. Add 5 mL of a solution of L-tartaric acid (3 in 20), and distil with steam cautiously to maintain the volume of the solution in the flask until 450 mL of the distillate is obtained for 45 minutes. Dilute the distillate to exactly 500 mL with water, and use this solution as the sample solution. Titrate <2.50> a 250-mL portion of the sample solution with 0.1 mol/L sodium hydroxide VS (indicator: 5 drops of phenolphthalein TS). Perform a blank determination, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 6.005 mg of $C_2H_4O_2$

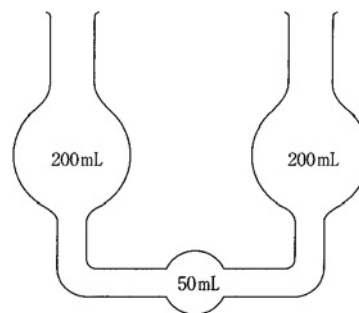
The volatile acid is not more than 0.15 w/v%.

(3) Sulfur dioxide—Stopper a 750-mL round-bottomed flask with a stopper having two holes. Through one hole, insert a glass tube A extending nearly to the bottom of the flask. Through the other hole, insert a glass tube B ending to the neck of the flask. Connect the tube B to a Liebig's condenser, and the end of the condenser to a joint of which inner diameter is 5 mm at the lower end. Connect the other end of the joint with a holed rubber stopper to a U tube having three bulbs as shown in the Figure. Pass carbon dioxide washed with a solution of potassium permanganate (3 in 100) through the tube A. Displace the air in the apparatus by carbon dioxide, and place 50 mL of a freshly prepared and diluted starch TS (1 in 5) and 1 g of potassium iodide in the U tube. From the other end of the U tube, add 1 to 2 drops of 0.01 mol/L iodine VS from a burette. While passing carbon dioxide, remove the stopper of the flask a little, add exactly 25 mL of Wine, 180 mL of freshly boiled and cooled water, 0.2 g of tannic acid, and 30 mL of phosphoric acid, and stopper again. Pass carbon dioxide for further 15 minutes, heat the distillation flask with caution so that 40 to 50 drops of the distillate may be obtained in 1 minute. When the color of starch TS in the U tube is discharged, add 0.01 mol/L iodine VS dropwise from a burette so that the color of the starch TS remains light blue to blue during the distillation. Read the volume of 0.01 mol/L iodine VS consumed when exactly 60 minutes have passed after the beginning of distillation. In this case, however, the coloration of starch TS produced by 1 drop of 0.01 mol/L iodine VS should persist at least for 1 minute.

Each mL of 0.01 mol/L iodine VS = 0.6406 mg of SO_2

The amount of sulfur dioxide (SO_2 : 64.06) does not exceed 7.5 mg.

(4) Total sulfuric acid—Transfer 10 mL of Wine to a beaker, boil, and add 50 mL of a solution prepared by dissolving 5.608 g of barium chloride dihydrate in 50 mL of hydrochloric acid and water to make 1000 mL. Cover the beaker, and heat on a water bath for 2 hours, supplying the



water lost by distillation. Cool, centrifuge, and decant the supernatant liquid in another beaker. To this solution add 1 to 2 drops of dilute sulfuric acid, and allow to stand for 1 hour: a white precipitate is formed.

(5) Arsenic <1.11>—Evaporate 10 mL of Wine on a water bath to dryness. Prepare the test solution with the residue according to Method 3, and perform the test (not more than 0.2 ppm).

(6) Glycerin—Pipet 100 mL of Wine into a 150-mL porcelain dish, and concentrate on a water bath to 10 mL. Add 1 g of sea sand (No. 1), and make the solution strongly alkaline by adding a solution prepared by dissolving 4 g of calcium hydroxide in 6 mL of water. Heat on a water bath with constant stirring and pushing down any material adhering to the wall of the dish until the contents of the dish become soft masses. Cool, add 5 mL of ethanol (99.5), and grind to a grue-like substance. Heat on a water bath, add 10 to 20 mL of ethanol (99.5) while agitating, boil, and transfer to a 100-mL volumetric flask. Wash the dish with seven 10-mL portions of hot ethanol (99.5), combine the washings with the contents of the flask, cool, and add ethanol (99.5) to make exactly 100 mL. Filter through a dry filter paper, evaporate 90 mL of the filtrate on a water bath, taking care not to boil the solution during the evaporation. Dissolve the residue in a small amount of ethanol (99.5), transfer to a 50-mL glass-stoppered volumetric cylinder, wash with several portions of ethanol (99.5), and add the washings to the solution in the cylinder to make 15 mL. Add three 7.5-mL portions of dehydrated diethyl ether, shake vigorously each time, and allow to stand. When the solution becomes quite clear, transfer to a tared, flat weighing bottle. Wash the volumetric cylinder with 5 mL of a mixture of dehydrated diethyl ether and ethanol (99.5) (3:2). Transfer the washings to the weighing bottle, and evaporate carefully on a water bath. When the liquid becomes sticky, dry at 105°C for 1 hour, and cool in a desiccator (silica gel), and weigh: the mass of the residue is not less than 0.45 g and not more than 0.90 g.

(7) Reducing sugars—To a 25-mL portion of the sample solution obtained in the Optical rotation add 50 mL of boiling Fehling's TS, and heat for exactly 2 minutes. Filter the separated precipitates by a tared glass filter by suction, wash successively with hot water, with ethanol (95) and with diethyl ether, and continue to dry the precipitates by suction. Heat the filter gently at first, and then strongly until the precipitates become completely black. Cool the precipitates in a desiccator (silica gel), and weigh as copper (II) oxide: the mass of cupric oxide does not exceed 0.325 g.

(8) Sucrose—Transfer a 50-mL portion of the sample solution obtained in the Optical rotation to a 100-mL flask, neutralize with diluted hydrochloric acid (1 in 30), followed by further addition of 5 mL of diluted hydrochloric acid (1 in

30). Heat in a water bath for 30 minutes, cool, neutralize with a solution of potassium hydroxide (1 in 100), add 4 drops of sodium carbonate TS, filter into a 100-mL volumetric flask, wash with water, combine the washings with the filtrate, and add water to make 100 mL. To 25 mL of this solution add 50 mL of boiling Fehling's TS, and proceed as directed in (7), and weigh as copper (II) oxide. From the number obtained by multiplying the mass (g) of copper (II) oxide by 2, deduct the amount (g) of copper (II) oxide determined in (7), and multiply again the number so obtained by 1.2: the number obtained does not exceed 0.104 (g).

(9) Benzoic acid, cinnamic acid and salicylic acid—Transfer exactly 50 mL of the sample solution obtained in (2) to a separator, add 10 g of sodium chloride and 2 mL of dilute hydrochloric acid, and extract with three 10-mL portions of diethyl ether. Combine the diethyl ether extracts, wash with two 5-mL portions of water, and extract with three 10-mL portions of 0.1 mol/L sodium hydroxide VS. Combine the alkaline extracts, evaporate the diethyl ether by warming on a water bath, cool, neutralize with 1 mol/L hydrochloric acid VS, and add 5 mL of potassium chloride-hydrochloric acid buffer solution and water to make exactly 50 mL. Perform the test as directed under Ultraviolet-visible Spectrophotometry <2.24> with this solution, using a solution prepared in the same manner instead of the sample solution as the blank: the absorbance does not exceed 0.15 at a wavelength between 220 nm and 340 nm.

(10) Boric acid—Transfer 50 mL of Wine to a porcelain dish, add 5 mL of sodium carbonate TS, evaporate on a water bath to dryness, and ignite: a half portion of the residue does not respond to Qualitative Tests <1.09> (1) for borate. Dissolve another half portion of the residue in 5 mL of hydrochloric acid: it does not respond to Qualitative Tests <1.09> (2) for borate.

(11) Methanol—Wine meets the requirements of the Methanol Test <1.12>, when proceeding with exactly 1 mL of ethanol layer obtained by Method 1 of the Alcohol Number Determination <1.01> and distilling without adding water after shaking with 0.5 g of calcium carbonate.

(12) Formaldehyde—To 25 mL of Wine add 5 g of sodium chloride and 0.2 g of L-tartaric acid, distil, and obtain 15 mL of the distillate. To 5 mL of the distillate add 5 mL of acetyl acetone TS, mix, and heat on a water bath for 10 minutes: the solution has no more color than that of the following control solution.

Control solution: Using 5 mL of water instead of the distillate, perform the test in the same manner.

Extract content 1.9 – 3.5 w/v% Pipet 25 mL of Wine to a 200-mL tared beaker containing 10 g of sea sand (No. 1), previously dried at 105°C for 2.5 hours, and evaporate to dryness on a water bath. Dry the residue at 105°C for 2 hours, cool in a desiccator (silica gel), and weigh.

Total ash 0.13 – 0.40 w/v% Pipet 50 mL of Wine to a tared porcelain dish, and evaporate to dryness on a water bath. Ignite the residue to the constant mass, cool, and weigh.

Assay (1) Ethanol—Pipet Wine into a 100-mL volumetric flask at 15°C, transfer to a 300- to 500-mL flask, and wash this volumetric flask with two 15-mL portions of water. Add the washings to the sample in the flask, connect the flask to a distillation tube having a trap, and distil using the volumetric

flask as a receiver. When about 80 mL of the distillate is obtained (it takes about 20 minutes), stop the distillation, allow to stand in water at 15°C for 30 minutes, and add water to make exactly 100 mL. Shake well, and determine the specific gravity at 15°C according to Method 3 under Specific Gravity <2.56>: the specific gravity d_{15}^{15} is between 0.982 and 0.985.

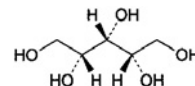
(2) L-Tartaric acid—Pipet 100 mL of Wine, add 2 mL of acetic acid (100), 0.5 mL of a solution of potassium acetate (1 in 5) and 15 g of powdered potassium chloride, and shake vigorously to dissolve as much as possible. Add 10 mL of ethanol (95), rub the inner wall of the beaker strongly for 1 minute to induce the crystallization, and allow to stand between 0°C and 5°C for more than 15 hours. Filter the crystals by suction, wash successively the beaker and the crystals with 3-mL portions of a solution prepared by dissolving 15 g of powdered potassium chloride in 120 mL of diluted ethanol (1 in 6), and repeat the washings five times. Transfer the crystals together with the filter paper to a beaker, wash the filter with 50 mL of hot water, combine the washings in the beaker, and dissolve the crystals by heating. Titrate <2.50> the solution with 0.2 mol/L sodium hydroxide VS immediately (indicator: 1 mL of phenolphthalein TS). The number obtained by adding 0.75 to the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed represents the amount (mL) of 0.2 mol/L sodium hydroxide VS consumed.

Each mL of 0.2 mol/L sodium hydroxide VS
= 30.02 mg of C₄H₆O₆

Containers and storage Containers—Tight containers.

Xylitol

キシリトール



C₅H₁₂O₅: 152.15
meso-Xylitol [87-99-0]

Xylitol, when dried, contains not less than 98.0% of C₅H₁₂O₅.

Description Xylitol occurs as white crystals or powder. It is odorless and has a sweet taste.

It is very soluble in water, slightly soluble in ethanol (95).

It is hygroscopic.

Identification (1) To 1 mL of a solution of Xylitol (1 in 2) add 2 mL of iron (II) sulfate TS and 1 mL of a solution of sodium hydroxide (1 in 5): blue-green color is produced without turbidity.

(2) Determine the infrared absorption spectrum of Xylitol, previously dried, as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

pH <2.54> Dissolve 5.0 g of Xylitol in 10 mL of freshly boiled and cooled water: the pH of this solution is between 5.0 and 7.0.

Melting point <2.60> 93.0 – 95.0°C

Purity (1) Clarity and color of solution—Dissolve 5 g of Xylitol in 10 mL of water: the solution is clear and colorless.

(2) Chloride <1.03>—Perform the test with 2.0 g of Xylitol. Prepare the control solution with 0.30 mL of 0.01 mol/L hydrochloric acid VS (not more than 0.005%).

(3) Sulfate <1.14>—Perform the test with 4.0 g of Xylitol. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.006%).

(4) Heavy metals <1.07>—Proceed with 4.0 g of Xylitol according to Method 1, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 5 ppm).

(5) Nickel—Dissolve 0.5 g of Xylitol in 5 mL of water, add 3 drops of dimethylglyoxime TS and 3 drops of ammonia TS, and allow to stand for 5 minutes: no red color is produced.

(6) Arsenic <1.11>—Prepare the test solution with 1.5 g of Xylitol according to Method 1, and perform the test (not more than 1.3 ppm).

(7) Sugars—Dissolve 5.0 g of Xylitol in 15 mL of water, add 4.0 mL of dilute hydrochloric acid, and heat in a water bath for 3 hours under a reflux condenser. After cooling, neutralize with sodium hydroxide TS (indicator: 2 drops of methyl orange TS). Then add water to make 50 mL, transfer 10 mL of this solution to a flask, add 10 mL of water and 40 mL of Fehling's TS, boil gently for 3 minutes, and allow to stand to precipitate copper (I) oxide. Remove the supernatant liquid through a glass filter (G4), and wash the precipitate with warm water until the last washing does not show alkalinity. Filter these washings through the glass filter mentioned above. Dissolve the precipitate in the flask in 20 mL of iron (III) sulfate TS, filter the solution through the glass filter mentioned above, wash with water, combine the washings with the filtrate, heat at 80°C, and titrate <2.50> with 0.02 mol/L potassium permanganate VS: not more than 1.0 mL of 0.02 mol/L potassium permanganate VS is consumed.

Loss on drying <2.41> Not more than 1.0% (1 g, in vacuum, phosphorus (V) oxide, 24 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.2 g of Xylitol, previously dried, dissolve in water to make exactly 100 mL. Pipet 10 mL of this solution into an iodine flask, add 50 mL of potassium periodate TS exactly, and heat in a water bath for 15 minutes. After cooling, add 2.5 g of potassium iodide, stopper, shake well, allow to stand for 5 minutes in a dark place, and titrate <2.50> with 0.1 mol/L sodium thiosulfate VS (indicator: 3 mL of starch TS). Perform a blank determination.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.902 mg of C₅H₁₂O₅

Containers and storage Containers—Tight containers.

Xylitol Injection

キシリトール注射液

Xylitol Injection is an aqueous solution for injection.

tion.

It contains not less than 95% and not more than 105% of the labeled amount of xylitol (C₅H₁₂O₅: 152.15).

Method of preparation Prepare as directed under Injections, with Xylitol.

No preservative may be added.

Description Xylitol Injection is a clear, colorless liquid. It has a sweet taste.

Identification Measure a volume of Xylitol Injection, equivalent to 0.1 g of Xylitol according to the labeled amount, add water to make 10 mL, and use this solution as the sample solution. Separately, dissolve 0.1 g of xylitol in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethanol (95), ammonia solution (28) and water (25:4:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly silver nitrate-ammonia TS, and dry at 105°C for 15 minutes: the spots from the sample solution and the standard solution show a blackish brown color and the same R_f value.

pH <2.54> 4.5 – 7.5

Bacterial endotoxins <4.01> Less than 0.50 EU/mL.

Extractable volume <6.05> It meets the requirement.

Assay Measure exactly a volume of Xylitol Injection, equivalent to about 5 g of xylitol (C₅H₁₂O₅) according to the labeled amount, and add water to make exactly 250 mL. Measure exactly 10 mL of this solution, and add water to make exactly 100 mL. Then, pipet 10 mL of this solution into an iodine flask, and proceed as directed in the Assay under Xylitol.

Each mL of 0.1 mol/L sodium thiosulfate VS
= 1.902 mg of C₅H₁₂O₅

Containers and storage Containers—Hermetic containers. Plastic containers for aqueous injections may be used.

Dried Yeast

乾燥酵母

Dried Yeast is dried and powdered cells of yeast belonging to *Saccharomyces*.

It contains not less than 400 mg of protein and not less than 100 μg of thiamine compounds [as thiamine chloride hydrochloride (C₁₂H₁₇ClN₄OS.HCl: 337.27)] in each 1 g.

Description Dried Yeast occurs as a light yellowish white to brown powder. It has a characteristic odor and taste.

Identification Dried Yeast, when examined under a microscope <5.01>, shows isolated cells, spheroidal or oval in shape, and 6 to 12 μm in length.

Purity (1) Rancidity—Dried Yeast is free from any unpleasant or rancid odor or taste.

(2) Starch—Add iodine TS to Dried Yeast, and examine microscopically <5.01>: no or only a few granules are tinted blackish purple.

Loss on drying <2.41> Not more than 8.0% (1 g, 100°C, 8 hours).

Total ash <5.01> Not more than 9.0% (1 g).

Assay (1) Protein—Weigh accurately about 50 mg of Dried Yeast and perform the test as directed under Nitrogen Determination <1.08>.

$$\begin{aligned} &\text{Amount (mg) of protein in 1 g of Dried Yeast} \\ &= N \times 6.25 \times (1/W) \end{aligned}$$

N: Amount (mg) of nitrogen (N)

W: Amount (g) of sample

(2) Thiamine—Weigh accurately about 1 g of Dried Yeast, add 1 mL of dilute hydrochloric acid and 80 mL of water, and heat in a water bath at 80°C to 85°C for 30 minutes with occasional shaking. After cooling, add water to make exactly 100 mL, and centrifuge for 10 minutes. Pipet 4 mL of the supernatant liquid, add exactly 5 mL of acetic acid-sodium acetate TS and exactly 1 mL of enzyme TS, and allow to stand at 45°C to 50°C for 3 hours. Place exactly 2 mL of this solution onto a chromatographic column prepared by pouring 2.5 mL of a weakly acidic CM-bridged cellulose cation exchanger (H type) (40 to 110 μm in particle diameter) into a chromatographic tube about 1 cm in inside diameter and about 17 cm in length, and elute at the flow rate of about 0.5 mL per minute. Wash the upper part of the column with a small amount of water, and wash the column with two 10-mL portions of water at the flow rate of about 1 mL per minute. Elute the column with two 2.5-mL portions of diluted phosphoric acid (1 in 50) at the flow rate of about 0.5 mL per minute, and combine the eluate. To the eluate add exactly 1 mL of the internal standard solution and 0.01 g of sodium 1-octanesulfonate, and after dissolving, use this solution as the sample solution. Separately, weigh accurately about 15 mg of Thiamine Chloride Hydrochloride Reference Standard (previously determine the water <2.48> in the same manner as Thiamine Chloride Hydrochloride), dissolve in 0.001 mol/L hydrochloric acid TS to make exactly 100 mL. Pipet 1 mL of this solution, and add the mobile phase to make exactly 100 mL. Pipet 1 mL of this solution, add exactly 1 mL of the internal standard solution and 3 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 200 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of thiamine to that of the internal standard.

$$\begin{aligned} &\text{Amount (}\mu\text{g) of thiamine in 1 g of Dried Yeast} \\ &= (W_S \times W_T) \times (Q_T/Q_S) \times 12.5 \end{aligned}$$

W_S: Amount (mg) of Thiamine Chloride Hydrochloride Reference Standard, calculated on the anhydrous basis

W_T: Amount (g) of the sample

Internal standard solution—Dissolve 0.01 g of phenacetin in acetonitrile to make 100 mL, and to 1 mL of this solution add diluted acetonitrile (1 in 5) to make 100 mL.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column about 4 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 2.7 g of potassium dihydrogenphosphate in 1000 mL of water, and adjust the pH to 3.5 with diluted phosphoric acid (1 in 10). Dissolve 1.6 g of sodium 1-octanesulfonate in 800 mL of this solution, and add 200 mL of acetonitrile.

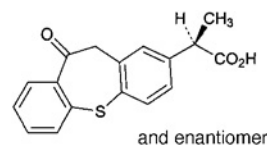
Flow rate: Adjust the flow rate so that the retention time of thiamine is about 8 minutes.

Selection of column: Proceed with 200 μL of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of thiamine and the internal standard in this order with the resolution between these peaks being not less than 8.

Containers and storage Containers—Tight containers.

Zaltoprofen

ザルトプロフェン



$C_{17}H_{14}O_3S$: 298.36
(2*RS*)-2-(10-*Ox*o-10,11-dihydrodibenzo[*b,f*]thiepin-2-yl)propanoic acid
[74711-43-6]

Zaltoprofen, when dried, contains not less than 99.0% and not more than 101.0% of $C_{17}H_{14}O_3S$.

Description Zaltoprofen occurs as white to light yellow, crystals or crystalline powder.

It is freely soluble in acetone, soluble in methanol and in ethanol (99.5), and practically insoluble in water.

It is gradually decomposed by light.

A solution of Zaltoprofen in acetone (1 in 10) shows no optical rotation.

Identification (1) To 0.2 g of Zaltoprofen add 0.5 g of sodium hydroxide, heat gradually to melt, and then carbonize. After cooling, add 5 mL of diluted hydrochloric acid (1 in 2): the gas evolved darkens moistened lead (II) acetate paper.

(2) Determine the absorption spectrum of a solution of Zaltoprofen in ethanol (99.5) (1 in 100,000) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wavelengths.

(3) Determine the infrared absorption spectrum of Zaltoprofen as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare the spectrum with the Reference Spectrum: both spectra exhibit similar intensities of absorption at the same wave numbers.

Melting point <2.60> 135 – 139°C

Purity (1) Heavy metals <1.07>—Proceed with 2.0 g of Zaltoprofen according to Method 4, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Zaltoprofen according to Method 3, using 10 mL of a solution of magnesium nitrate hexahydrate in ethanol (95) (2 in 25), and perform the test (not more than 2 ppm).

(3) Related substances—Dissolve 50 mg of Zaltoprofen in 50 mL of the mobile phase, and use this solution as the sample solution. Pipet 1 mL of the sample solution, and add the mobile phase to make exactly 50 mL. Pipet 1 mL of this solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine each peak area by the automatic integration method: the area of the peak other than zaltoprofen and other than the peak having the relative retention time of about 0.7 with respect to zaltoprofen from the sample solution is not larger than the peak area of zaltoprofen from the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (300:200:1)

Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.

Time span of measurement: About 15 times as long as the retention time of zaltoprofen beginning after the solvent peak.

System suitability—

Test for required detectability: To exactly 2 mL of the standard solution add the mobile phase to make exactly 20 mL. Confirm that the peak area of zaltoprofen obtained with 20 μ L of this solution is equivalent to 8 to 12% of that with 20 μ L of the standard solution.

System performance: Dissolve 25 mg of zaltoprofen and 50 mg of isopropyl benzoate in 100 mL of ethanol (99.5). Pipet 1 mL of this solution, and add the mobile phase to make exactly 50 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, zaltoprofen and isopropyl benzoate are eluted in this order with the resolution between these peaks being not less than 6.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of zaltoprofen is not more than 2.0%.

Loss on drying <2.41> Not more than 0.5% (1 g, 105°C, 4 hours).

Residue on ignition <2.44> Not more than 0.1% (1 g).

Assay Weigh accurately about 0.5 g of Zaltoprofen, dissolve in 50 mL of methanol, and titrate <2.50> with 0.1 mol/L sodium hydroxide VS (potentiometric titration). Perform a

blank determination in the same manner, and make any necessary correction.

Each mL of 0.1 mol/L sodium hydroxide VS
= 29.84 mg of C₁₇H₁₄O₃S

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Zaltoprofen Tablets

ザルトプロフェン錠

Zaltoprofen Tablets contain not less than 95.0% and not more than 105.0% of the labeled amount of zaltoprofen (C₁₇H₁₄O₃S: 298.36).

Method of preparation Prepare as directed under Tablets, with Zaltoprofen.

Identification Powder a suitable amount of Zaltoprofen Tablets. To a portion of the powder, equivalent to 80 mg of Zaltoprofen, add 30 mL of ethanol (99.5), shake well, and centrifuge. To 1 mL of the supernatant liquid add ethanol (99.5) to make 20 mL. To 2 mL of this solution add ethanol (99.5) to make 25 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima between 227 nm and 231 nm and between 329 nm and 333 nm, and a shoulder between 241 nm and 245 nm.

Uniformity of dosage units <6.02> Perform the test according to the following method: it meets the requirement of the Content uniformity test.

To 1 tablet of Zaltoprofen Tablets add 4 mL of water, and shake to disintegrate. Add a suitable amount of ethanol (95), shake, then add ethanol (95) to make exactly *V* mL so that each mL contains about 4 mg of zaltoprofen (C₁₇H₁₄O₃S), and centrifuge. Pipet 2 mL of the supernatant liquid, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Proceed as directed in the Assay.

$$\begin{aligned} &\text{Amount (mg) of zaltoprofen (C}_{17}\text{H}_{14}\text{O}_3\text{S)} \\ &= W_S \times (Q_T/Q_S) \times (V/20) \end{aligned}$$

W_S: Amount (mg) of zaltoprofen for assay

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000).

Dissolution <6.10> Perform the test according to the following method: it meets the requirement.

Perform the test with 1 tablet of Zaltoprofen Tablets at 50 revolutions per minute according to the Paddle method, using 900 mL of 2nd fluid for dissolution test as the dissolution medium. Withdraw not less than 20 mL of the dissolved solution 30 minutes after start of the test, and filter through a membrane filter with pore size of not more than 0.45 μ m. Discard the first 10 mL of the filtrate, pipet *V* mL of the subsequent filtrate, add 2nd fluid for dissolution test to make exactly *V'* mL so that each mL contains about 44 μ g of zaltoprofen (C₁₇H₁₄O₃S) per mL according to the labeled amount, and use this solution as the sample solution. Separately, weigh accurately about 22 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, dissolve in 20

mL of ethanol (99.5), and add 2nd fluid for dissolution test to make exactly 100 mL. Pipet 4 mL of this solution, add 2nd fluid for dissolution test to make exactly 20 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution at 340 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>, using 2nd fluid for dissolution test as the control. The dissolution rate in 30 minutes is not less than 75%.

Dissolution rate (%) with respect to the labeled amount of zaltoprofen ($C_{17}H_{14}O_3S$)

$$= W_S \times (A_T/A_S) \times (V'/V) \times (1/C) \times 180$$

W_S : Amount (mg) of zaltoprofen for assay

C: Labeled amount (mg) of zaltoprofen for assay in 1 tablet

Assay To 10 tablets of Zaltoprofen Tablets add 40 mL of water, shake to disintegrate, then add a suitable amount of ethanol (95), shake, add ethanol (95) to make exactly 200 mL, and centrifuge. Pipet an amount of the supernatant liquid, equivalent to about 8 mg of zaltoprofen ($C_{17}H_{14}O_3S$), add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 80 mg of zaltoprofen for assay, previously dried at 105°C for 4 hours, add 4 mL of water and ethanol (95) to make exactly 20 mL. Pipet 2 mL of this solution, add exactly 10 mL of the internal standard solution and ethanol (95) to make 50 mL, and use this solution as the standard solution. Perform the test with 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the ratios, Q_T and Q_S , of the peak area of zaltoprofen to that of the internal standard.

$$\begin{aligned} &\text{Amount (mg) of zaltoprofen (C}_{17}\text{H}_{14}\text{O}_3\text{S)} \\ &= W_S \times (Q_T/Q_S) \times (1/10) \end{aligned}$$

W_S : Amount (mg) of zaltoprofen for assay

Internal standard solution—A solution of benzyl benzoate in acetonitrile (1 in 1000)

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 25°C.

Mobile phase: A mixture of acetonitrile, water and acetic acid (100) (300:200:1).

Flow rate: Adjust the flow rate so that the retention time of zaltoprofen is about 4 minutes.

System suitability—

System performance: When the procedure is run with 5 μ L of the standard solution under the above operating conditions, zaltoprofen and the internal standard are eluted in this order with the resolution between these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the ratio of the

peak area of zaltoprofen to that of the internal standard is not more than 1.0%.

Containers and storage Containers—Tight containers.

Zinc Chloride

塩化亜鉛

ZnCl₂: 136.32

Zinc Chloride contains not less than 97.0% of ZnCl₂.

Description Zinc Chloride occurs as white, crystalline powder, rods, or masses. It is odorless.

It is very soluble in water, and freely soluble in ethanol (95), and its solution may sometimes be slightly turbid. The solution becomes clear on addition of a small amount of hydrochloric acid.

The pH of an aqueous solution of Zinc Chloride (1 in 2) is between 3.3 and 5.3.

It is deliquescent.

Identification An aqueous solution of Zinc Chloride (1 in 30) responds to the Qualitative Tests <1.09> for zinc salt and chloride.

Purity (1) Clarity and color of solution—Dissolve 1.0 g of Zinc Chloride in 10 mL of water and 2 drops of hydrochloric acid: the solution has no color, and is clear.

(2) Sulfate <1.14>—Perform the test with 2.0 g of Zinc Chloride. Prepare the control solution with 0.40 mL of 0.005 mol/L sulfuric acid VS (not more than 0.010%).

(3) Ammonium—Dissolve 0.5 g of Zinc Chloride in 5 mL of water, and warm with 10 mL of a solution of sodium hydroxide (1 in 6): the evolving gas does not change moistened red litmus paper to blue.

(4) Heavy metals—Dissolve 0.5 g of Zinc Chloride in 5 mL of water in a Nessler tube, shake thoroughly with 15 mL of potassium cyanide TS, add 1 drop of sodium sulfide TS, allow to stand for 5 minutes, and immediately observe from the top downward against a white background: the solution has no more color than the following control solution.

Control solution: To 2.5 mL of Standard Lead Solution add 3 mL of water and 15 mL of potassium cyanide TS, shake thoroughly, and add 1 drop of sodium sulfide TS (not more than 50 ppm).

(5) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Chloride in 120 mL of water, add ammonium sulfide TS to complete precipitation, add water to make 200 mL, shake thoroughly, and filter through dry filter paper. Discard the first 20 mL of the filtrate, take the following 100 mL of the filtrate, evaporate with 3 drops of sulfuric acid to dryness, and heat the residue strongly at 600°C to constant mass: the mass is not more than 10.0 mg.

(6) Arsenic <1.11>—Prepare the test solution with 0.40 g of Zinc Chloride according to Method 1, and perform the test (not more than 5 ppm).

(7) Oxychloride—Shake gently 0.25 g of Zinc Chloride with 5 mL of water and 5 mL of ethanol (95), and add 0.3 mL of 1 mol/L hydrochloric acid VS: the solution is clear.

Assay Weigh accurately about 0.3 g of Zinc Chloride, add

0.4 mL of dilute hydrochloric acid and water to make exactly 200 mL. Measure exactly 20 mL of the solution, add 80 mL of water, 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 1.363 mg of ZnCl₂

Containers and storage Containers—Tight containers.

Zinc Oxide

酸化亜鉛

ZnO: 81.41

Zinc Oxide, when ignited, contains not less than 99.0% of ZnO.

Description Zinc Oxide occurs as a white, amorphous powder. It is odorless and tasteless.

It is practically insoluble in water, in ethanol (95), in acetic acid (100) and in diethyl ether.

It dissolves in dilute hydrochloric acid and in sodium hydroxide TS.

It gradually absorbs carbon dioxide from air.

Identification (1) Heat Zinc Oxide strongly: a yellow color develops on strong heating, and disappears on cooling.

(2) A solution of Zinc Oxide in dilute hydrochloric acid (1 in 10) responds to the Qualitative Tests <1.09> for zinc salt.

Purity (1) Carbonate, and clarity and color of solution—Mix 2.0 g of Zinc Oxide with 10 mL of water, add 30 mL of dilute sulfuric acid, and heat on a water bath with stirring: no effervescence occurs, and the solution obtained is clear and colorless.

(2) Alkalinity—To 1.0 g of Zinc Oxide add 10 mL of water, and boil for 2 minutes. Cool, filter through a glass filter (G3), and to the filtrate add 2 drops of phenolphthalein TS and 0.20 mL of 0.1 mol/L hydrochloric acid VS: no color develops.

(3) Sulfate <1.14>—Shake 0.5 g of Zinc Oxide with 40 mL of water, and filter. Take 20 mL of the filtrate, add 1 mL of dilute hydrochloric acid and water to make 50 mL, and perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.096%).

(4) Iron—Dissolve 1.0 g of Zinc Oxide in 50 mL of diluted hydrochloric acid (1 in 2), dissolve 0.1 g of ammonium peroxodisulfate in this solution, and extract with 20 mL of 4-methyl-2-pentanone. Add 30 mL of acetic acid-sodium acetate buffer solution for Iron Limit Test, pH 4.5, to the 4-methyl-2-pentanone layer, extract again, and use the layer of the buffer solution as the test solution. Separately, perform the test in the same manner with 1.0 mL of Standard Iron Solution, and use the layer so obtained as the control solution. Add 2 mL each of L-ascorbic acid solution for Iron Limit Test (1 in 100) to the test solution and the control solution, respectively, mix, allow to stand for 30 minutes, add 5 mL of

an ethanol (95) solution of α,α' -dipyridyl (1 in 200) and water to make 50 mL. After allowing to stand for 30 minutes, compare the color of the both liquids against a white back: the color of the liquid from the test solution is not stronger than that from the control solution (not more than 10 ppm).

(5) Lead—To 2.0 g of Zinc Oxide add 20 mL of water, then add 5 mL of acetic acid (100) with stirring, and heat on a water bath until solution is complete. Cool, and add 5 drops of potassium chromate TS: no turbidity is produced.

(6) Arsenic <1.11>—Dissolve 0.5 g of Zinc Oxide in 5 mL of dilute hydrochloric acid, use this solution as the test solution, and perform the test (not more than 4 ppm).

Loss on ignition <2.43> Not more than 1.0% (1 g, 850°C, 1 hour).

Assay Weigh accurately about 0.8 g of Zinc Oxide, previously ignited at 850°C for 1 hour, dissolve in 2 mL of water and 3 mL of hydrochloric acid, and add water to exactly 100 mL. Pipet 10 mL of this solution, add 80 mL of water, then add a solution of sodium hydroxide (1 in 50) until a slight precipitate is produced. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.071 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Oil

チンク油

Zinc Oxide Oil contains not less than 45.0% and not more than 55.0% of zinc oxide (ZnO: 81.41).

Method of preparation

Zinc Oxide	500 g
Fixed oil	a sufficient quantity
To make 1000 g	

Mix the above ingredients. An appropriate quantity of Castor Oil or polysorbate 20 may be used partially in place of fixed oil.

Description Zinc Oxide Oil is a white to whitish, slimy substance, separating a part of its ingredients when stored for a prolonged period.

Identification Mix thoroughly, and place 0.5 g of Zinc Oxide Oil in a crucible, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Assay Weigh accurately about 0.8 g of Zinc Oxide Oil, mixed well, place in a crucible, heat gradually raising the temper-

ature until the mass is thoroughly charred, and then ignite until the residue becomes yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL. Pipet 20 mL of this solution, add 80 mL of water, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.071 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Ointment

亜鉛華軟膏

Zinc Oxide Ointment contains not less than 18.5% and not more than 21.5% of zinc oxide (ZnO: 81.41).

Method of preparation

Zinc Oxide	200 g
Liquid Paraffin	30 g
White Ointment	a sufficient quantity
To make 1000 g	

Prepare as directed under Ointments, with the above ingredients.

Description Zinc Oxide Ointment is white in color.

Identification Place 1 g of Zinc Oxide Ointment in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite it strongly: a yellow color is produced, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

Purity Calcium, magnesium and other foreign inorganic matters—Place 2.0 g of Zinc Oxide Ointment in a crucible, melt by warming, and heat gradually raising the temperature, until the mass is thoroughly charred. Ignite the mass strongly until the residue becomes uniformly yellow, and cool. Add 6 mL of dilute hydrochloric acid, and heat on a water bath for 5 to 10 minutes: the solution is colorless and clear. Filter the solution, add 10 mL of water to the filtrate, and add ammonia TS until the precipitate first formed redissolves. Add 2 mL each of ammonium oxalate TS and disodium hydrogenphosphate TS to this solution: the solution remains unchanged or becomes very slightly turbid within 5 minutes.

Assay Weigh accurately about 2 g of Zinc Oxide Ointment, place in a crucible, melt by warming, heat gradually raising the temperature until the mass is thoroughly charred, and then ignite until the residue becomes uniformly yellow, and cool. Dissolve the residue in 1 mL of water and 1.5 mL of hydrochloric acid, and add water to make exactly 100 mL.

Add 80 mL of water to exactly 20 mL of this solution, and add a solution of sodium hydroxide (1 in 50) until a small amount of precipitates begins to form in the solution. Add 5 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.05 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 4.071 mg of ZnO

Containers and storage Containers—Tight containers.

Zinc Oxide Starch Powder

亜鉛華デンプン

Method of preparation

Zinc Oxide	500 g
Starch	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Description Zinc Oxide Starch Powder occurs as a white powder.

Identification (1) Place 1 g of Zinc Oxide Starch Powder in a crucible, heat gradually, raising the temperature until it is charred, and then ignite strongly: a yellow color develops, and disappears on cooling. To the residue add 10 mL of water and 5 mL of dilute hydrochloric acid, shake well, and filter. To the filtrate add 2 to 3 drops of potassium hexacyanoferrate (II) TS: a white precipitate is formed (zinc oxide).

(2) Shake well 1 g of Oxide Starch Powder with 10 mL of water and 5 mL of dilute hydrochloric acid, and filter. Boil the residue on a filter paper with 10 mL of water, cool, and add 1 drop of iodine TS: a dark blue-purple color is produced (starch).

Containers and storage Containers—Tight containers.

Zinc Sulfate Hydrate

硫酸亜鉛水和物

ZnSO₄·7H₂O: 287.58

Zinc Sulfate Hydrate contains not less than 99.0% and not more than 102.0% of ZnSO₄·7H₂O.

Description Zinc Sulfate Hydrate occurs as colorless crystals or a white, crystalline powder. It is odorless, and has an astringent, characteristic taste.

It is very soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

The pH of a solution of Zinc Sulfate Hydrate (1 in 20) is between 3.5 and 6.0.

It effloresces in dry air.

Identification A solution of Zinc Sulfate Hydrate (1 in 20) responds to the Qualitative Tests <1.09> for zinc salt and for sulfate.

Purity (1) Acidity—Dissolve 0.25 g of Zinc Sulfate Hydrate in 5 mL of water, and add 1 drop of methyl orange TS: no red color develops.

(2) Heavy metals <1.07>—Dissolve 1.0 g of Zinc Sulfate Hydrate in 10 mL of water contained in a Nessler tube. Add 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS, and allow the mixture to stand for 5 minutes. Observe vertically against a white background, the color of the solution is not more intense than the following control solution.

Control solution: To 1.0 mL of Standard Lead Solution add 10 mL of water and 20 mL of potassium cyanide TS, and mix well. Add 2 drops of sodium sulfide TS (not more than 10 ppm).

(3) Alkali earth metals and alkali metals—Dissolve 2.0 g of Zinc Sulfate Hydrate in 150 mL of water, add a suitable amount of ammonium sulfide TS to complete the precipitation, and add water to make exactly 200 mL. Shake well, and filter through a dry filter paper. Discard the first 20 mL of the filtrate, take exactly 100 mL of the subsequent filtrate, evaporate to dryness, and ignite as directed under Residue on Ignition <2.44>: the mass of the residue is not more than 5.0 mg.

(4) Arsenic <1.11>—Prepare the test solution with 1.0 g of Zinc Sulfate Hydrate according to Method 1, and perform the test (not more than 2 ppm).

Assay Weigh accurately about 0.3 g of Zinc Sulfate Hydrate, and dissolve in water to make exactly 100 mL. Measure exactly 25 mL of this solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.876 mg of ZnSO₄·7H₂O

Containers and storage Containers—Tight containers.

Zinc Sulfate Ophthalmic Solution

硫酸亜鉛点眼液

Zinc Sulfate Ophthalmic Solution contains not less than 0.27 w/v% and not more than 0.33 w/v% of zinc sulfate hydrate (ZnSO₄·7H₂O: 287.58).

Method of preparation

Zinc Sulfate Hydrate	3 g
Boric Acid	20 g
Sodium Chloride	5 g
Fennel Oil	2 mL
Purified Water	a sufficient quantity

To make 1000 mL

Prepare as directed under Ophthalmic Solution, with the above ingredients.

Description Zinc Sulfate Ophthalmic Solution is a clear, colorless liquid.

Identification (1) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <1.09> for zinc salt.

(2) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <1.09> for borate.

(3) Zinc Sulfate Ophthalmic Solution responds to the Qualitative Tests <1.09> for chloride.

Assay Pipet accurately 25 mL of Zinc Sulfate Ophthalmic Solution, add 100 mL of water and 2 mL of ammonia-ammonium chloride buffer solution, pH 10.7, and titrate <2.50> with 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS (indicator: 0.04 g of eriochrome black T-sodium chloride indicator).

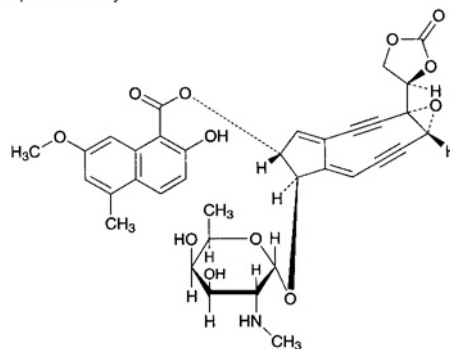
Each mL of 0.01 mol/L disodium dihydrogen ethylenediamine tetraacetate VS
= 2.876 mg of ZnSO₄·7H₂O

Containers and storage Containers—Tight containers.

Zinostatin Stimalamer

ジノスタチン スチマラマー

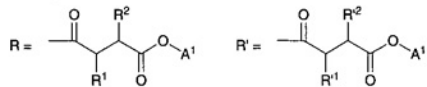
Chromophore moiety



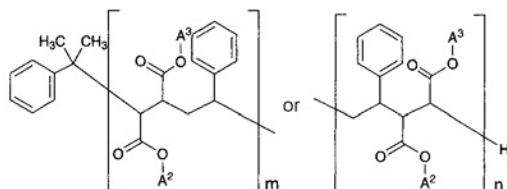
(4*S*,6*R*,11*R*,12*R*)-11-[α-D-2,6-Dideoxy-2-(methylamino)-galactopyranosyloxy]-4-[(4*R*)-2-oxo-1,3-dioxolan-4-yl]-5-oxatricyclo[8.3.0.0^{4,6}]trideca-1(13),9-diene-2,7-diyn-12-yl 2-hydroxy-7-methoxy-5-methylnaphthalene-1-carboxylate
[123760-07-6]

Apoprotein moiety bonded to styrene-maleic acid alternate copolymer

R-Ala-Ala-Pro-Thr-Ala-Thr-Val-Thr-Pro-Ser-Ser-Gly-Leu-Ser-Asp-Gly-Thr-Val-
 R^1
 Val-Lys-Val-Ala-Gly-Ala-Gly-Leu-Gln-Ala-Gly-Thr-Ala-Tyr-Asp-Val-Gly-Gln-
 Cys-Ala-Trp-Val-Asp-Thr-Gly-Val-Leu-Ala-Cys-Asn-Pro-Ala-Asp-Phe-Ser-
 Ser-Val-Thr-Ala-Asp-Ala-Asp-Gly-Ser-Ala-Ser-Thr-Ser-Leu-Thr-Val-Arg-
 Arg-Ser-Phe-Glu-Gly-Phe-Leu-Phe-Asp-Gly-Thr-Arg-Trp-Gly-Thr-Val-Asp-
 Cys-Thr-Thr-Ala-Ala-Cys-Gln-Val-Gly-Leu-Ser-Asp-Ala-Ala-Gly-Asn-Gly-
 Pro-Glu-Gly-Val-Ala-Ile-Ser-Phe-Asn



R^1 and R^2 , and R^1 and R^2 are different each other as follows, respectively.



$A^1 = H$ or NH_4

$A^2, A^3 = H, NH_4$ or C_6H_5 (no C_6H_5 appears at the same time at A^2 and A^3)

Average $m+n$ —about 5.5

Zinostatin Stimalamer consists 1 molecule of zinostatin, consisting of chromophore and apoprotein (polypeptide consisting of 113 amino acid residues) and 2 molecules of partially butyl-esterified styrene-maleic acid alternate copolymer, and has average molecular mass of about 15,000. The alternate copolymer is bound an amido bond to α -amino group of alanine of N-terminal and to ϵ -amino group of lysine 20 of the apoprotein.

It contains not less than 900 μg (potency) and not more than 1080 μg (potency) per mg, calculated on the anhydrous basis. The potency of Zinostatin Stimalamer is expressed as mass (potency) of zinostatin stimalamer.

Description Zinostatin Stimalamer occurs as a pale yellow powder.

It is freely soluble in water, and practically insoluble in ethanol (95) and in diethyl ether.

Identification (1) Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of sodium hydroxide TS, and add a drop of copper (II) sulfate TS: a purple color develops.

(2) Dissolve 1 mg of Zinostatin Stimalamer in 1 mL of 0.05 mol/L phosphate buffer solution, pH 7.0, add 0.5 mL of a solution of trichloroacetic acid (1 in 5), and shake: a white precipitate is formed.

(3) Determine the absorption spectra of solutions of Zinostatin Stimalamer and Zinostatin Stimalamer Reference Standard in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 2500) as directed under Ultraviolet-visible Spectrophotometry <2.24>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wavelengths.

(4) Determine the infrared absorption spectra of Zinostatin Stimalamer and Zinostatin Stimalamer Reference Standard as directed in the potassium bromide disk method under Infrared Spectrophotometry <2.25>, and compare these spectra: both spectra exhibit similar intensities of absorption at the same wave numbers.

Absorbance <2.24> $E_{1\text{ cm}}^{1\%}$ (268 nm): 15.5 – 18.5 (4 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer

solution, pH 7.0, 10 mL).

Optical rotation <2.49> $[\alpha]_D^{20}$: –30.0 – –38.0° (20 mg calculated on the anhydrous basis, 0.05 mol/L phosphate buffer solution, pH 7.0, 5 mL, 100 mm).

pH <2.54> Dissolve 10 mg of Zinostatin Stimalamer in 1 mL of water: the pH of the solution is between 4.5 and 5.5.

Purity (1) Clarity and color of solution—Being specified separately.

(2) Heavy metals <1.07>—Weigh accurately 40 mg of Zinostatin Stimalamer, place in a crucible, carbonize and incinerate according to Method 2, add 2 mL of hydrochloric acid, and evaporate on a water bath to dryness. After cooling, weigh the residue W_T g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), add 1 mL of water, 85 μL of diluted ammonia TS (1 in 2) and 0.1 mL of dilute acetic acid, and add water so that the mass is $W_T + 2.0$ g. Adjust the pH of this solution to 3.2 to 3.4 with diluted ammonia TS (1 in 20) or diluted hydrochloric acid (1 in 50), add water so that the mass is $W_T + 2.5$ g, and use this solution as the test solution. Separately, prepare the blank solution in the same manner without the sample. Separately, take 2 mL of nitric acid, 5 drops of sulfuric acid and 2 mL of hydrochloric acid, and evaporate to dryness according to Method 2. After cooling, weigh the residue W_S g. Then, moisten the residue with 0.1 mL of diluted hydrochloric acid (1 in 5), and proceed in the same manner as directed in the preparation of the test solution. After adjusting the pH of the solution so obtained to 3.2 to 3.4, add 80 μL of Standard Lead Solution, and add water so that the mass is $W_S + 2.5$ g, and use this solution as the control solution. Add 10 μL each of diluted sodium sulfide TS (1 in 6) to the test solution, the blank solution and the control solution, mix, and allow to stand for 5 minutes. Determine the absorbances, A_T , A_O and A_S of the test solution, the blank solution and the control solution at 400 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: $A_T - A_O$ is not larger than $A_S - A_O$ (not more than 20 ppm).

(3) Related substances—Being specified separately.

(4) Inorganic salts of manufacturing process origin—Being specified separately.

Water <2.48> Not more than 12.0% (10 mg, coulometric titration).

Assay Perform the test according to the Cylinder-plate method as directed under Microbial Assay for Antibiotics <4.02> according to the following conditions. Perform the procedures of (iii), (iv) and (v) without exposure to direct or indirect sunlight.

(i) Test organism—*Micrococcus luteus* ATCC 9341

(ii) Culture medium—Use the medium i in 3) Medium for other organisms under (1) Agar media for seed and base layer. Adjust the pH of the medium so that it will be 7.9 to 8.1 after sterilization.

(iii) Standard solutions—Weigh accurately an amount of Zinostatin Stimalamer Reference Standard equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration standard solution. Pipet 5 mL of the high concentration standard solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration standard

solution.

(iv) Sample solutions—Weigh accurately an amount of Zinostatin Stimalamer equivalent to about 20 mg (potency), dissolve in 0.1 mol/L phosphate buffer solution, pH 8.0 to make exactly 50 mL, and use this solution as the high concentration sample solution. Pipet 5 mL of the high concentration sample solution, add 0.1 mol/L phosphate buffer solution, pH 8.0, to make exactly 20 mL, and use this solution as the low concentration sample solution.

(v) Procedure—Allow to stand at 3 to 5°C for 2 hours before incubation.

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and not exceeding -20°C .

Crude Drugs

Acacia

Gummi Arabicum

アラビアゴム

Acacia is the secretions obtained from the stems and branches of *Acacia senegal* Willdenow or other species of the same genus (*Leguminosae*).

Description Colorless or light yellow-brown, translucent or somewhat opaque spheroidal tears, or angular fragments with numerous fissures on the surface; very brittle; the fractured surface glassy and occasionally iridescent.

Odorless; tasteless, but produces a mucilaginous sensation on the tongue.

Pulverized Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95).

Identification To 1 g of powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the *R_f* value, respectively.

Purity (1) Insoluble residue—To 5.0 g of pulverized Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter (G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the

standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the *R_f* value corresponding to glucose from the standard solution does not appear from the sample solution.

Loss on drying <5.01> Not more than 17.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Powdered Acacia

Gummi Arabicum Pulveratum

アラビアゴム末

Powdered Acacia is the powder of Acacia.

Description Powdered Acacia occurs as a white to light yellowish white powder. It is odorless, tasteless, but produces a mucilaginous sensation on the tongue.

Under a microscope <5.01>, Powdered Acacia, immersed in olive oil or liquid paraffin, reveals colorless, angular fragments or nearly globular grains. Usually starch grains or vegetable tissues are not observed or very trace, if any.

Powdered Acacia (1.0 g) dissolves almost completely in 2.0 mL of water, and the solution is acid.

It is practically insoluble in ethanol (95).

Identification To 1 g of Powdered Acacia add 25 mL of water and 1 mL of sulfuric acid, and heat under a reflux condenser in a boiling water bath for 60 minutes. After cooling, add gently 2.0 g of anhydrous sodium carbonate. To 1 mL of this solution add 9 mL of methanol, mix well, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 10 mg of D-galactose in 1 mL water, add methanol to make 10 mL, and use this solution as the standard solution (1). Proceed with L-arabinose and with L-rhamnose monohydrate in the same manner as for the preparation of the standard solution (1), and use so obtained solutions as the standard solution (2) and the standard solution (3), respectively. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and water (9:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: the three spots from the sample solution are the same with the spots of D-galactose, L-arabinose and L-rhamnose from the standard solution in the color tone and the *R_f* value, respectively.

Purity (1) Insoluble residue—To 5.0 g of Powdered Acacia add 100 mL of water and 10 mL of dilute hydrochloric acid, and dissolve by gentle boiling for 15 minutes with swirling. Filter the warm mixture through a tared glass filter

(G3), wash the residue thoroughly with hot water, and dry at 105°C for 5 hours: the mass of the residue does not exceed 10.0 mg.

(2) Tannin-bearing gums—To 10 mL of a solution of Powdered Acacia (1 in 50) add 3 drops of iron (III) chloride TS: no dark green color is produced.

(3) Glucose—Dissolve 10 mg of glucose in 1 mL of water, add methanol to make 10 mL, and use this solution as the standard solution. Proceed with the sample solution obtained in the Identification and the standard solution obtained here as directed in the Identification: any spot at the R_f value corresponding to glucose from the standard solution does not appear from the sample solution.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Achyranthes Root

Achyranthis Radix

ゴシツ

Achyranthes Root is the root of *Achyranthes fauriei* Leveillé et Vaniot or *Achyranthes bidentata* Blume (*Amaranthaceae*).

Description Main root or main root with some lateral roots, with or without short remains of rhizome at the crown; main root, long cylindrical and sometimes somewhat tortuous, 15–90 cm in length, 0.3–0.7 cm in diameter; externally grayish yellow to yellow-brown, with numerous longitudinal wrinkles, and with scattering scars of lateral roots. Fractured surface is flat; grayish white to light brown on the circumference, and with yellowish white xylem in the center. Hard and brittle, or flexible.

Odor, slight; taste, slightly sweet, and mucilaginous.

Under a microscope <5.01>, a transverse section reveals a rather distinct cambium separating the cortex from the xylem; small protoxylem located at the center of the xylem, and surrounded by numerous vascular bundles arranged on several concentric circles; parenchyma cells containing sand crystals of calcium oxalate; starch grains absent.

Identification Shake vigorously 0.5 g of pulverized *Achyranthes* Root with 10 mL of water: a lasting fine foam is produced.

Purity (1) Stem—The amount of stems contained in *Achyranthes* Root does not exceed 5.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized *Achyranthes* Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized *Achyranthes* Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than stems contained in *Achyranthes* Root does not exceed 1.0%.

Loss on drying <5.01> Not more than 17.0% (6 hours).

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Agar

Agar

カンテン

Agar is the solid residue obtained by freezing dehydration of a mucilage derived from *Gelidium amansii* Lamouroux, other species of the same genus (*Gelidiaceae*), or other red algae (*Rhodophyta*).

Description White, translucent rectangular column, string or flakes. Rectangular column about 26 cm in length, 4 cm square in cross section; a string of about 35 cm in length and about 3 mm in width; flakes about 3 mm in length; externally, with wrinkles and somewhat lustrous, light and pliable.

Odorless; tasteless and mucilaginous.

It is practically insoluble in organic solvents.

A boiling solution of Agar (1 in 100) is neutral.

Identification (1) To a fragment of Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to make up the water lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

Purity (1) Sulfuric acid—Dissolve 1.0 g of Agar in 100 mL of water by boiling: the solution is not acidic.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution does not decolorize immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Measure exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg.

(4) Water absorption—To 5.0 g of Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

Loss on drying <5.01> Not more than 22.0% (6 hours).

Total ash <5.01> Not more than 4.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Powdered Agar

Agar Pulveratum

カンテン末

Powdered Agar is the powder of Agar.

Description Powdered Agar appears as a white powder, is odorless, and is tasteless and mucilaginous.

Under a microscope <5.01>, Powdered Agar, immersed in olive oil or liquid paraffin, reveals angular granules with striations or nearly spheroidal granules 5 to 60 μm in diameter.

It becomes transparent in chloral hydrate TS.

It is practically insoluble in organic solvents.

A boiling solution of Powdered Agar (1 in 100) is neutral.

Identification (1) To a part of Powdered Agar add dropwise iodine TS: a dark blue to reddish purple color develops.

(2) Dissolve 1 g of Powdered Agar in 65 mL of water by boiling for 10 minutes with constant stirring, and add a sufficient amount of hot water to maintain the original volume lost by evaporation: the solution is clear. Cool the solution between 30°C and 39°C: the solution forms a firm, resilient gel, which does not melt below 85°C.

Purity (1) Sulfuric acid—Dissolve 1.0 g of Powdered Agar in 100 mL of water by boiling: the solution is not acid.

(2) Sulfurous acid and starch—To 5 mL of the solution obtained in (1) add 2 drops of iodine TS: the solution is not decolorized immediately, and does not show a blue color.

(3) Insoluble matter—To 7.5 g of Powdered Agar add 500 mL of water, boil for 15 minutes, and add water to make exactly 500 mL. Take exactly 100 mL of the solution, add 100 mL of hot water, heat to boiling, filter while hot through a tared glass filter (G3), wash the residue with a small amount of hot water, and dry the residue at 105°C for 3 hours: the mass of the residue is not more than 15.0 mg.

(4) Water absorption—To 5.0 g of Powdered Agar add water to make 100 mL, shake well, allow to stand at 25°C for 24 hours, and filter through moistened glass wool in a 100-mL graduated cylinder: the volume of the filtrate is not more than 75 mL.

Loss on drying <5.01> Not more than 22.0% (6 hours).

Total ash <5.01> Not more than 4.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Akebia Stem

Akebiae Caulis

モクツウ

Akebia Stem is the climbing stem of *Akebia quinata* Decaisne or *Akebia trifoliata* Koidzumi (*Lardizabalaceae*), usually cut transversely.

Description Circular or ellipsoidal sections 0.2–0.3 cm in

thickness, and 1–3 cm in diameter; phloem on both fractured surfaces is dark grayish brown; xylem reveals light brown vessel portions and grayish white medullary rays lined alternately and radially; pith light grayish yellow, and distinct; flank grayish brown, and with circular or transversely elongated elliptical lenticels.

Almost odorless; slightly acrid taste.

Under a microscope <5.01>, a transverse section reveals ring layers mainly consisting of fiber bundles with crystal cells and stone cell groups and surrounding the outside of the phloem in arc shape. Medullary rays of the phloem consisting of sclerenchymatous cells containing solitary crystals; portion near cambium is distinct; cells around the pith remarkably thick-walled; xylem medullary rays and parenchymatous cells around the pith contain solitary crystals of calcium oxalate and starch grains less than 8 μm in diameter.

Identification To 0.5 g of pulverized Akebia Stem add 10 mL of water, boil, allow to cool, and shake vigorously: lasting fine foams are produced.

Total ash <5.01> Not more than 10.0%.

Alisma Rhizome

Alismatis Rhizoma

タクシャ

Alisma Rhizome is the tuber of *Alisma orientale* Juzepczuk (*Alismataceae*), from which periderm has been usually removed.

Description Spherical or conical tubers, 3–8 cm in length, 3–5 cm in diameter, sometimes a 2- to 4-branched irregular tuber; externally light grayish brown to light yellow-brown, and slightly annulate; many remains of root appearing as small warty protrusions; fractured surface nearly dense, the outer portion grayish brown, and the inner part white to light yellow-brown in color; rather light in texture and difficult to break.

Slight odor and taste.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of pulverized Alisma Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Alisma Rhizome according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Powdered Alisma Rhizome

Alismatis Rhizoma Pulveratum

タクシャ末

Powdered Alisma Rhizome is the powder of Alisma

Rhizome.

Description Powdered Alisma Rhizome occurs as a light grayish brown powder, and has a slight odor and taste.

Under a microscope <5.01>, Powdered Alisma Rhizome reveals mainly starch grains, fragments of parenchyma containing them, parenchyma cells containing yellow contents, and fragments of vascular bundles. Starch grains, spheroidal to ellipsoidal simple grains, 3 – 15 μm in diameter.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Powdered Alisma Rhizome according to Method 3, and perform the test. Prepare the control solution with 2.0 mL of Standard Lead Solution (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Alisma Rhizome according to Method 3, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Aloe

Aloe

アロエ

Aloe is the dried juice of the leaves mainly of *Aloe ferox* Miller, or of hybrids of the species with *Aloe africana* Miller or *Aloe spicata* Baker (*Liliaceae*).

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Description Aloe occurs as blackish brown to dark brown, irregular masses; sometimes the external surface covered with a yellow powder; the fractured surface smooth and glassy.

Odor, characteristic; taste, extremely bitter.

Identification (1) Dissolve 0.5 g of pulverized Aloe in 50 mL of water by warming. After cooling, add 0.5 g of siliceous earth, and filter. Perform the following tests using the filtrate as the sample solution.

(i) Dissolve 0.2 g of sodium tetraborate decahydrate in 5 mL of the sample solution by warming in a water bath. Add a few drops of this solution into 30 mL of water, and shake: a green fluorescence is produced.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.

(2) To 0.2 g of pulverized Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution and a red

fluorescent spot from the standard solution show the same color tone and the same R_f value.

Purity (1) Resin—Warm 0.5 g of pulverized Aloe with 10 mL of diethyl ether on a water bath, and filter. Wash the residue and the filter paper with 3 mL of diethyl ether. Combine the filtrate and the washing, and evaporate the diethyl ether solution: the mass of the residue is not more than 5.0 mg.

(2) Ethanol-insoluble substances—Boil 1.0 g of pulverized Aloe with 50 mL of ethanol (95) on a water bath for 30 minutes under a reflux condenser. Filter the warm mixture through a tared glass filter (G4), and wash the residue on the filter with ethanol (95) until the last washing becomes colorless. Dry the residue at 105°C for 5 hours, and weigh: the mass of the residue is not more than 0.10 g.

Loss on drying <5.01> Not more than 12.0%.

Total ash <5.01> Not more than 2.0%.

Extract content <5.01> Water-soluble extract: not less than 40.0%.

Component determination Weigh accurately about 0.1 g of pulverized Aloe, add 40 mL of methanol, and heat under a reflex condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of barbaloin for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the peak areas of barbaloin, A_T and A_S , of both solutions.

$$\text{Amount (mg) of barbaloin} = W_S \times (A_T/A_S) \times (1/2)$$

W_S : Amount (mg) of barbaloin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (74:26:1).

Flow rate: Adjust the flow rate so that the retention time of barbaloin is about 12 minutes.

System suitability—

System performance: Dissolve 10 mg of barbaloin for component determination add 40 mg of oxalic acid dihydrate, in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add 1 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make exactly 10 mL. When the procedure is run with 5 μL of this solution under the above operating conditions except the wavelength of 300

nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloin is not more than 1.5%.

Powdered Aloe

Aloe Pulverata

アロエ末

Powdered Aloe is the powder of Aloe.

It contains not less than 4.0% of barbaloin, calculated on the basis of dried material.

Description Powdered Aloe occurs as a dark brown to yellowish dark brown powder. It has a characteristic odor and an extremely bitter taste.

Under a microscope <5.01>, Powdered Aloe, immersed in olive oil or liquid paraffin, reveals greenish yellow to reddish brown, angular or rather irregular fragments.

Identification (1) Dissolve 0.5 g of Powdered Aloe in 50 mL of water by warming. After cooling, add 0.5 g of siliceous earth, and filter. Perform the following tests with the filtrate as the sample solution.

(i) Dissolve 0.2 g of sodium tetraborate decahydrate in 5 mL of the sample solution by warming in a water bath. Add a few drops of this solution into 30 mL of water, and shake: a green fluorescence is produced.

(ii) Shake 2 mL of the sample solution with 2 mL of nitric acid: a yellow-brown color which changes gradually to green is produced. Then warm this colored solution in a water bath: the color of the solution changes to red-brown.

(2) To 0.2 g of Powdered Aloe add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of barbaloin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (20:5:2:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among several spots from the sample solution has the same color tone and the same *R_f* value with the red fluorescent spot from the standard solution.

Purity (1) Resin—Warm 0.5 of Powdered Aloe with 10 mL of diethyl ether on a water bath, and filter. Wash the residue and the filter paper with 3 mL of diethyl ether. Combine the filtrate and the washing, and evaporate the diethyl ether: the mass of the residue does not exceed 5.0 mg.

(2) Ethanol-insoluble substances—Boil 1.0 g of Powdered Aloe with 50 mL of ethanol (95) on a water bath for 30 minutes under a reflux condenser. Filter the warm mixture through a tared glass filter (G4), and wash the residue on the filter with ethanol (95) until the last washing becomes colorless. Dry the residue at 105°C for 5 hours, and weigh: the

mass of the residue is not more than 0.10 g.

Loss on drying <5.01> Not more than 12.0%.

Total ash <5.01> Not more than 2.0%.

Extract content <5.01> Water-soluble extract: not less than 40.0%.

Component determination Weigh accurately about 0.1 g of Powdered Aloe, add 40 mL of methanol, and heat under a reflux condenser on a water bath for 30 minutes. After cooling, filter, and add methanol to the filtrate to make exactly 50 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of barbaloin for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas of barbaloin, *A_T* and *A_S*, of both solutions.

$$\text{Amount (mg) of barbaloin} = W_S \times (A_T/A_S) \times (1/2)$$

W_S: Amount (mg) of barbaloin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 360 nm).

Column: A stainless steel column about 6 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (74:26:1).

Flow rate: Adjust the flow rate so that the retention time of barbaloin is about 12 minutes.

System suitability—

System performance: To about 10 mg of barbaloin for component determination add 40 mg of oxalic acid dihydrate, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add 1 mL of a solution of ethenzamide in methanol (1 in 2000) and methanol to make exactly 10 mL. When the procedure is run with 5 μ L of this solution under the above operating conditions except the wavelength of 300 nm, barbaloin and ethenzamide are eluted in this order with the resolution between these peaks being not less than 2.0.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of barbaloin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Alpinia Officinarum Rhizome

Alpiniae officinari Rhizoma

リヨウキョウ

Alpinia Officinarum Rhizome is the rhizome of *Alpinia officinarum* Hance (*Zingiberaceae*).

Description *Alpinia Officinarum* Rhizome is a slightly curved and cylindrical rhizome, sometimes branched; 2 to 8 cm in length, 6 to 15 mm in diameter; externally red-brown to dark brown with fine striped lines, grayish white nodes and several traces of rootlet; hard to break; fracture surface, light brown in color and thickness of cortex is approximately the same as that of stele.

Odor, characteristic; taste, extremely pungent.

Under a microscope <5.01>, transverse section reveals epidermal cells often containing resin-like substances; cortex, endodermis and stele present beneath the epidermis; cortex and stele divided by endodermis; vascular bundles surrounded by fibers, scattered throughout the cortex and stele, cortex and stele composed of parenchyma interspersed with oil cells; parenchymatous cells containing solitary crystals of calcium oxalate and starch grains, starch grains generally simple (sometimes 2- to 8-compound), ovate, oblong or narrowly ovate, 10 – 40 μm in diameter and with an eccentric navel.

Identification To 0.5 g of pulverized *Alpinia Officinarum* Rhizome add 5 mL of acetone, shake for 5 minutes, and filter. Perform the test with the filtrate as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the filtrate on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane, ethyl acetate and acetic acid (100) (12:8:1) to a distance of about 10 cm, and air-dry the plate: two yellow-brown spots appear at around *R_f* 0.4 – 0.5.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 7.5%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-extract: not less than 14.0%.

Amomum Seed

Amomi Semen

シユクシャ

Amomum Seed is the seed mass of *Amomum xanthioides* Wallich (*Zingiberaceae*).

Description Approximately spherical or ellipsoidal mass, 1 – 1.5 cm in length, 0.8 – 1 cm in diameter; externally grayish brown to dark brown, and with white powder in those dried by spreading lime over the seeds; the seed mass is divided into three loculi by thin membranes, and each loculus contains 10 to 20 seeds joining by aril; each seed is polygonal

and spherical, 0.3 – 0.5 cm in length, about 0.3 cm in diameter, externally dark brown, with numerous, fine protrusions; hard tissue; under a magnifying glass, a longitudinal section along the raphe reveals oblong section, with deeply indented hilum and with slightly indented chalaza; white perisperm covering light yellow endosperm and long embryo. Characteristic aroma when cracked, and taste acid.

Total ash <5.01> Not more than 9.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Essential oil content <5.01> Perform the test with 30.0 g of pulverized Amomum Seed: the volume of essential oil is not less than 0.6 mL.

Powdered Amomum Seed

Amomi Semen Pulveratum

シユクシャ末

Powdered Amomum Seed is the powder of Amomum Seed.

Description Powdered Amomum seed occurs as a grayish brown powder, and has a characteristic aroma and an acid taste.

Under a microscope <5.01>, Powdered Amomum Seed reveals fragments of wavy perisperm cells filled with starch grains and containing in each cell a calcium oxalate crystal; yellow and long epidermal cells of seed coat and fragments of thin-walled tissue perpendicular to them; fragments of groups of brown, thick-walled polygonal stone cells.

Total ash <5.01> Not more than 9.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Essential oil content <5.01> Perform the test with 30.0 g of Powdered Amomum Seed: the volume of essential oil is not less than 0.4 mL.

Containers and storage Containers—Tight containers.

Anemarrhena Rhizome

Anemarrhenae Rhizoma

チモ

Anemarrhena Rhizome is the rhizome of *Anemarrhena asphodeloides* Bunge (*Liliaceae*).

Description Rather flat and cord-like rhizome, 3 – 15 cm in length, 0.5 – 1.5 cm in diameter, slightly bent and branched; externally yellow-brown to brown; on the upper surface, a longitudinal furrow and hair-like remains or scars of leaf sheath forming fine ring-nodes; on the lower surface, scars of root appearing as numerous round spot-like hollows; light and easily broken. Under a magnifying glass, a light yellow-brown transverse section reveals an extremely narrow cortex; stele porous, with many irregularly scattered vascular bundles.

Odor, slight; taste, slightly sweet and mucous, followed by bitterness.

Identification (1) Shake vigorously 0.5 g of pulverized Anemarrhena Rhizome with 10 mL of water in a test tube: a lasting fine foam is produced. Filter the mixture, and to 2 mL of the filtrate add 1 drop of iron (III) chloride TS: a dark green precipitate is produced.

(2) Warm 0.5 g of pulverized Anemarrhena Rhizome with 2 mL of acetic anhydride on a water bath for 2 minutes while shaking, then filter, and to the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

Purity Foreign matter <5.01>—The amount of fiber, originating from the dead leaves, and other foreign matters contained in Anemarrhena Rhizome is not more than 3.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Angelica Dahurica Root

ビャクシ

Angelica Dahurica Root is the root of *Angelica dahurica* Bentham et Hooker (*Umbelliferae*).

Description Main root from which many long roots are branched out and nearly fusiform and conical in whole shape, 10 – 25 cm in length; externally grayish brown to dark brown, with longitudinal wrinkles, and with numerous scars of rootlets laterally elongated and protruded. A few remains of leaf sheath at the crown and ring-nodes closely protruded near the crown. In a transverse section, the outer region is grayish white in color, and the central region is sometimes dark brown in color.

Odor, characteristic; taste, slightly bitter.

Identification To 0.2 g of pulverized Angelica Dahurica Root add 5 mL of ethanol (95), allow to stand for 5 minutes with shaking, and filter. Examine the filtrate under ultraviolet light (main wavelength: 365 nm): a blue to blue-purple fluorescence develops.

Purity (1) Leaf sheath—The amount of leaf sheath contained in Angelica Dahurica Root does not exceed 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than leaf sheath contained in Angelica Dahurica Root is not more than 1.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 25.0%.

Apricot Kernel

Armeniaca Semen

キョウニン

Apricot Kernel is the seed of *Prunus armeniaca*

Linné or *Prunus armeniaca* Linné var. *ansu* Maximowicz (*Rosaceae*).

Description Flattened, somewhat asymmetric ovoid seed, 1.1 – 1.8 cm in length, 0.8 – 1.3 cm in width, 0.4 – 0.7 cm in thickness; sharp at one end and rounded at the other end where chalaza situated; seed coat brown and its surface being powdery with rubbing easily detachable stone cells of epidermis; numerous vascular bundles running from chalaza throughout the seed coat, appearing as thin vertical furrows; seed coat and thin semitransparent white albumen easily separate from cotyledon when soaked in boiling water; cotyledon, white in color.

Almost odorless; taste, bitter and oily.

Under a microscope <5.01>, surface of epidermis reveals stone cells on veins protruded by vascular bundles, forming angular circle to ellipse and approximately uniform in shape, with uniformly thickened walls, and 60 – 90 μm in diameter; in lateral view, stone cell appearing obtusely triangular and its wall extremely thickened at the apex.

Identification To 1.0 g of ground Apricot Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 105°C for 10 minutes: one spot among the spots from the sample solution and a brown to dark green spot from the standard solution show the same color tone and the same Rf value.

Purity (1) Rancidity—Grind Apricot Kernel with hot water: no unpleasant odor of rancid oil is perceptible.

(2) Foreign matter <5.01>—Apricot Kernel does not contain fragments of endocarp and other foreign matter.

Apricot Kernel Water

キョウニン水

Apricot Kernel Water contains not less than 0.09 w/v% and not more than 0.11 w/v% of hydrogen cyanide (HCN: 27.03).

Method of preparation Prepare by one of the following methods.

(1) To Apricot Kernels, previously crushed and pressed to remove fixed oils as much as possible, add a suitable amount of Water or Purified Water, and carry out steam distillation. Determine the amount of hydrogen cyanide in the distillate by the method as directed in the Assay, and carry on the distillation until the content of hydrogen cyanide in the distillate is about 0.14 w/v%. To the distillate add Ethanol in about 1/3 of the volume of the distillate, and dilute with a mixture of Purified Water and Ethanol (3:1) until the content of hydrogen cyanide meets the specification.

(2) Dissolve 7.5 mL of freshly prepared mandelonitrile in 1000 mL of a mixture of Purified Water and Ethanol (3:1), mix well, and filter. Determine the amount of hydrogen cyanide in the solution as directed in the Assay, and, if the amount is more than that specified above, dilute the solution to the specified concentration by the addition of the mixture of Purified Water and Ethanol (3:1).

Description Apricot Kernel Water is a clear, colorless or pale yellow liquid. It has an odor of benzaldehyde and a characteristic taste.

pH: 3.5 – 5.0

Identification To 2 mL of Apricot Kernel Water add 1 mL of ammonia TS, and allow to stand for 10 minutes: a slight turbidity is produced. Allow to stand for 20 minutes: the turbidity is intensified.

Specific gravity <2.56> d_{20}^{20} : 0.968 – 0.978

Purity (1) Sulfate <1.14>—Add a few drops of 0.1 mol/L sodium hydroxide VS to 5.0 mL of Apricot Kernel Water to make slightly alkaline, evaporate on a water bath to dryness, and ignite between 450°C and 550°C. Dissolve the residue in 1.0 mL of dilute hydrochloric acid, and add water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution with 0.50 mL of 0.005 mol/L sulfuric acid VS (not more than 0.005%).

(2) Heavy metals <1.07>—Evaporate 50 mL of Apricot Kernel Water on a water bath to dryness, ignite between 450°C and 550°C, dissolve the residue in 5 mL of dilute acetic acid with warming, add water to make exactly 50 mL, and filter. Remove the first 10 mL of the filtrate, dilute the subsequent 20 mL to 50 mL with water, and perform the test using this solution as the test solution. Prepare the control solution as follows: to 2.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 1 ppm).

(3) Free hydrogen cyanide—To 10 mL of Apricot Kernel Water add 0.8 mL of 0.1 mol/L silver nitrate VS and 2 to 3 drops of nitric acid at 15°C, filter, and add 0.1 mol/L silver nitrate VS to the filtrate: no change occurs.

(4) Residue on evaporation—Evaporate 5.0 mL of Apricot Kernel Water to dryness, and dry the residue at 105°C for 1 hour: the mass of the residue is not more than 1.0 mg.

Assay Measure exactly 25 mL of Apricot Kernel Water, add 100 mL of water, 2 mL of potassium iodide TS and 1 mL of ammonia TS, and titrate <2.50> with 0.1 mol/L silver nitrate VS until a yellow turbidity persists.

Each mL of 0.1 mol/L silver nitrate VS
= 5.405 mg of HCN

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Areca

Arecae Semen

ピンロウジ

Areca is the seed of *Areca catechu* Linné (*Palmae*).

Description Rounded-conical or flattened nearly spherical seed 1.5 – 3.5 cm high and 1.5 – 3 cm in diameter; hilum at the center of its base and usually forming a dent; externally grayish red-brown to grayish yellow-brown, with a network of pale lines; hard in texture; cross section dense in texture, exhibiting a marbled appearance of grayish brown seed coat alternating with white albumen; center of the seed often hollow.

Odor, slight; taste, astringent and slightly bitter.

Identification Weigh 3 g of pulverized Areca in a glass-stoppered centrifuge tube, and add 30 mL of diethyl ether and 5 mL of sodium hydroxide TS, stopper tightly, shake for 5 minutes, centrifuge, and separate the diethyl ether layer. Evaporate the diethyl ether on a water bath, dissolve the residue in 1.5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of arecoline hydrobromide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and acetic acid (100) (10:6:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iodine TS on the plate: one spot among the spots from the sample solution and a red-brown spot from the standard solution show the same color tone and the same R_f value.

Purity (1) Pericarp—The amount of pericarp contained in Areca is not more than 2.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the pericarp contained in Areca does not exceed 1.0%.

Total ash <5.01> Not more than 2.5%.

Artemisia Capillaris Flower

Artemisiae Capillaris Flos

インチンコウ

Artemisia Capillaris Flower is the capitulum of *Artemisia capillaris* Thunberg (*Compositae*).

Description Capitulum of ovoid to spherical, capitula, about 1.5 – 2 mm in length, about 2 mm in diameter, with linear leaves, peduncles, and thin stem. Outer surface of capitulum, light green to light yellowish brown in color; peduncle, greenish brown to dark brown in color. Under a magnifying glass, the capitulum; involucre scale, in 3 – 4 succubous rows, outer scale of ovate with obtuse, inner scale of elliptical, 1.5 mm in length, longer than outer one, with keel midrib and thin membranous margin. Floret; tubular, marginal flower of female, disk flower of hermaphrodite. Achene of obovoid, 0.8 mm in length. Light in texture.

Odor, characteristic, slight; taste, slightly acrid, which gives slightly numbing sensation to the tongue.

Identification To 0.5 g of pulverized Artemisia Capillaris Flower add 10 mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test

with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone and *n*-hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a principal spot with a blue fluorescence appears at the *R_f* value of about 0.5.

Purity Stem—*Artemisia Capillaris* Flower does not contain any stem less than 2 mm in diameter.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 9.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

Asiasarum Root

Asiasari Radix

サイシン

Asiasarum Root is the rhizome and root of *Asiasarum sieboldii* F. Maekawa or *Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa (*Aristolochiaceae*).

Description Asiasarum Root is a nearly cylindrical rhizome with numerous thin and long roots, externally light brown to dark brown. The root, about 15 cm in length, about 0.1 cm in diameter, with shallow longitudinal wrinkles on the surface, and brittle. The rhizome, 2–4 cm in length, 0.2–0.3 cm in diameter, often branched, with longitudinal wrinkles on the surface; internode short; each node has several scars of petiole and peduncle, and several thin and long roots.

Odor, characteristic; taste, acrid, with some sensation of numbness on the tongue.

Purity (1) Terrestrial part—Any terrestrial parts are not found.

(2) Foreign matter <5.01>—The amount of foreign matter other than terrestrial part contained in Asiasarum Root does not exceed 1.0%.

(3) Aristolochic acid I—To exactly 2.0 g of pulverized Asiasarum Root add exactly 50 mL of diluted methanol (3 in 4), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve exactly 1.0 mg of aristolochic acid I for crude drugs purity test in diluted methanol (3 in 4) to make exactly 100 mL. Pipet 1 mL of this solution, add diluted methanol (3 in 4) to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01>, according to the following conditions: the sample solution shows no peak at the retention time corresponding to aristolochic acid I from the standard solution. If the sample solution shows such a peak, repeat the test under different conditions to confirm that the peak in question is not aristolochic acid I.

Operating conditions—

Detector: An ultraviolet or visible absorption photometer

(wavelength: 400 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution prepared by dissolving 7.8 g of sodium dihydrogen phosphate dihydrate and 2 mL of phosphoric acid in water to make 1000 mL and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of aristolochic acid I is about 15 minutes.

System suitability—

Test for required detectability: Measure exactly 1 mL of the standard solution, and add diluted methanol (3 in 4) to make exactly 10 mL. Confirm that the ratio, S/N, of the signal (S) and noise (N) of aristolochic acid I obtained from 20 μ L of this solution is not less than 3. In this case, S means the peak height on the chromatogram not including noise obtained by drawing an average line of the detector output, and N is 1/2 of the difference between the maximum and minimum output signals of the baseline around the peak in the range of 20 times the width at half-height of the peak.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of aristolochic acid I is not more than 5.0%.

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Essential oil content <5.01> Perform the test with 30.0 g of pulverized Asiasarum Root: the volume of essential oil is not less than 0.6 mL.

Asparagus Tuber

Asparagi Tuber

テンモンドウ

Asparagus Tuber is the tuber of *Asparagus cochinchinensis* Merrill (*Liliaceae*), from which most of the cork layer is removed, usually, after being steamed.

Description Asparagus Tuber is a fusiform to cylindrical tuber, 5 to 15 cm in length, 5 to 20 mm in diameter; externally light yellow-brown to light brown, translucent and often with longitudinal wrinkles; flexible, or hard and easily broken in texture; fractured surface, grayish yellow, glossy and horny.

Odor, characteristic; taste, sweet at first, followed by a slightly bitter aftertaste.

Under a microscope <5.01>, a transverse section of Asparagus Tuber reveals stone cells and bundles of them on outer layer of cortex; mucilaginous cells containing raphides of calcium oxalate in the parenchyma cells of cortex and central cylinder; no starch grains.

Identification To 1 g of coarsely cut Asparagus Tuber add 5

mL of a mixture of 1-butanol and water (40:7), shake for 30 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (10:6:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 2 minutes: the spot of a red-brown at first then changes to brown color appears at around Rf 0.4.

Loss on drying <5.01> Not more than 18.0% (6 hours).

Total ash <5.01> Not more than 3.0%.

Astragalus Root

Astragali Radix

オウギ

Astragalus Root is the root of *Astragalus membranaceus* Bunge, *Astragalus mongholicus* Bunge (*Leguminosae*).

Description Nearly cylindrical root, 30 – 100 cm in length, 0.7 – 2 cm in diameter, with small bases of lateral root dispersed on the surface, twisted near the crown; externally light grayish yellow to light yellow-brown, and covered with irregular, dispersed longitudinal wrinkles and horizontal lenticel-like patterns; difficult to break; fractured surface fibrous. Under a magnifying glass, a transverse section reveals an outer layer composed of periderm; cortex light yellowish white, xylem light yellow, and zone near the cambium somewhat brown in color; thickness of cortex from about one-third to one-half of the diameter of xylem; white medullary ray from xylem to cortex in thin root, but often appearing as radiating cracks in thick root; usually pith unobservable.

Odor, slight; taste, sweet.

Purity (1) Root of *Hedysarum* species and others—Under a microscope <5.01>, a vertical section of Astragalus Root reveals no crystal fiber containing solitary crystals of calcium oxalate outside the fiber bundle.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Astragalus Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Astragalus Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Atractylodes Lancea Rhizome

Atractylodis Lanceae Rhizoma

ソウジュツ

Atractylodes Lancea Rhizome is the rhizome of *Atractylodes lancea* De Candolle or of *Atractylodes chinensis* Koidzumi (*Compositae*).

Description Irregularly curved, cylindrical rhizome, 3 – 10 cm in length, 1 – 2.5 cm in diameter; externally dark grayish brown to dark yellow-brown; a transverse section nearly orbicular, with light brown to red-brown secretes as fine points.

Often white cotton-like crystals produced on its surface.

Odor, characteristic; taste, slightly bitter.

Under a microscope <5.01>, a transverse section usually reveals periderm with stone cells; parenchyma of cortex, usually without any fiber bundle; oil sacs, containing light brown to yellow-brown substances, located at the end region of medullary rays; xylem exhibits vessels surrounded by fiber bundles and arranged radially on the region adjoining the cambium; pith and medullary rays exhibit the same oil sacs as in the cortex; parenchyma cells contain spherocrystals of inulin and fine needle crystals of calcium oxalate.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Atractylodes Lancea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Atractylodes Lancea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Atractylodes rhizome—Macerate 0.5 g of pulverized Atractylodes Lancea Rhizome with 5 mL of ethanol (95) by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: no red to red-purple color develops within 1 minute.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Atractylodes Lancea Rhizome: the volume of essential oil is not less than 0.7 mL.

Powdered Atractylodes Lancea Rhizome

Atractylodis Lanceae Rhizoma Pulveratum

ソウジュツ末

Powdered Atractylodes Lancea Rhizome is the powder of Atractylodes Lancea Rhizome.

Description Powdered Atractylodes Lancea Rhizome occurs as a yellow-brown powder. It has a characteristic odor,

and a slightly bitter taste.

Under a microscope <5.01>, Powdered Atractylodes Lancea Rhizome reveals mainly parenchyma cells, spherocrystals of inulin, fragments of parenchyma cells containing fine needle crystals of calcium oxalate as their contents; and further fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels, and small yellow-brown secreted masses or oil drops; starch grains absent.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Atractylodes Lancea Rhizome according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Atractylodes Lancea Rhizome according to Method 4, and perform the test (not more than 5 ppm).

(3) Powdered atractylodes rhizome—To 0.5 g of Powdered Atractylodes Lancea Rhizome add 5 mL of ethanol (95), macerate by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: no red to red-purple color develops within 1 minute.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered Atractylodes Lancea Rhizome: the volume of essential oil is not less than 0.5 mL.

Containers and storage Containers—Tight containers.

Atractylodes Rhizome

Atractylodis Rhizoma

ビャクジュツ

Atractylodes Rhizome is the rhizome of *Atractylodes japonica* Koidzumi ex Kitamura (Wa-byakujutsu), or is the rhizome of *Atractylodes ovata* De Candolle (Kara-byakujutsu) (*Compositae*).

Description (1) Wa-byakujutsu—Periderm-removed rhizome is irregular masses or irregularly curved cylinder, 3–8 cm in length, 2–3 cm in diameter; externally light grayish yellow to light yellowish white, with scattered grayish brown parts. The rhizome covered with periderm is externally grayish brown, often with node-like protuberances and coarse wrinkles. Difficult to break, and the fractured surface is fibrous. A transverse section, with fine dots of light yellow-brown to brown secrete.

Odor, characteristic; taste, somewhat bitter.

Under a microscope <5.01>, a transverse section reveals periderm with stone cell layers; fiber bundles in the parenchyma of the cortex, often adjoined to the outside of the phloem; oil sacs containing light brown to brown substances, situated at the outer end of medullary rays; in the xylem, radially lined vessels, surrounding large pith, and distinct fiber bundle surrounding the vessels; in pith and in medullary rays, oil sacs similar to those in cortex, and in parenchyma,

crystals of inulin and small needle crystals of calcium oxalate.

(2) Kara-byakujutsu—Irregularly enlarged mass, 4–8 cm in length, 2–5 cm in diameter; externally grayish yellow to dark brown, having sporadic, knob-like small protrusions. Difficult to break; fractured surface has a light brown to dark brown xylem remarkably fibrous.

Odor, characteristic; taste, somewhat sweet, but followed by slight bitterness.

Under a microscope <5.01>, a transverse section usually reveals periderm with stone cells, absence of fibers in the cortex; oil sacs containing yellow-brown contents in phloem ray and also at the outer end of it; xylem with radially lined vessels surrounding large pith, and distinct fiber bundle surrounding the vessels; pith and medullary ray exhibit oil sacs as in cortex; parenchyma contains crystals of inulin and small needle crystals of calcium oxalate.

Identification Macerate 0.5 g of pulverized Atractylodes Rhizome with 5 mL of ethanol (95) by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: a red to red-purple color develops and persists.

Purity Atractylodes lancea rhizome—To 2.0 g of pulverized Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears between R_f 0.3 and 0.6.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Atractylodes Rhizome: the volume of essential oil is not less than 0.5 mL.

Powdered Atractylodes Rhizome

Atractylodis Rhizoma Pulveratum

ビャクジュツ末

Powdered Atractylodes Rhizome is the powder of Atractylodes Rhizome.

Description Powdered Atractylodes Rhizome occurs as a light brown to yellow-brown powder, and has a characteristic odor and a slightly bitter or slightly sweet taste, followed by a slightly bitter aftertaste.

Under a microscope <5.01>, Powdered Atractylodes Rhizome reveals mainly parenchyma cells, crystals of inulin and fragments of parenchyma cells containing small needle crystals of calcium oxalate; fragments of light yellow thick-walled fibers, stone cells and cork cells; a few fragments of reticulate and scalariform vessels; small yellow-brown secrete masses or oil droplets; starch grains absent.

Identification Macerate 0.5 g of Powdered Atractylodes Rhizome with 5 mL of ethanol (95) by warming in a water bath for 2 minutes, and filter. To 2 mL of the filtrate add 0.5 mL of vanillin-hydrochloric acid TS, and shake immediately: a red to red-purple color develops and persists.

Purity Atractylodes lancea rhizome—To 2.0 g of Powdered Atractylodes Rhizome add exactly 5 mL of hexane, shake for 5 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 100°C for 5 minutes: no green to grayish green spot appears at the Rf value of between 0.3 and 0.6.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered Atractylodes Rhizome: the volume of essential oil is not less than 0.4 mL.

Containers and storage <5.01> Containers—Tight containers.

Bear Bile

Fel Ursi

ユウタン

Bear Bile is the dried bile of *Ursus arctos* Linné or allied animals (*Ursidae*).

Description Indefinite small masses; externally yellow-brown to dark yellow-brown; easily broken; fractured surface has a glassy luster, and is not wet; usually in a gall sac, occasionally taken out, the gall sac consists of a fibrous and strong membrane, 9 – 15 cm in length and 7 – 9 cm in width; externally dark brown and translucent.

Odor, slight and characteristic; taste, extremely bitter.

Identification Warm 0.3 g of pulverized Bear Bile with 50 mL of petroleum ether under a reflux condenser on a water bath for about 1 hour, and filter. To 20 mg of the residue add 0.5 mL of hydrochloric acid, 2 mL of acetic anhydride and 2 mL of chloroform, shake the mixture vigorously for 2 minutes, and filter. To the filtrate add carefully 0.5 mL of sulfuric acid: a red color develops at the zone of contact, then changes to reddish brown, and the upper layer acquires a somewhat red color. Shake gently to mix the two layers together, and allow to stand: a persistent reddish brown color is produced.

Bearberry Leaf

Uvae Ursi Folium

ウワウルシ

Bearberry Leaf is the leaf of *Arctostaphylos uva-ursi* (Linné) Sprengel (*Ericaceae*).

It contains not less than 7.0% of arbutin.

Description Obovate to spatulate leaves, 1 – 3 cm in length, 0.5 – 1.5 cm in width; upper surface yellow-green to dark green; lower surface light yellow-green; margin entire; apex obtuse or round, sometimes retuse; base cuneate; petiole very short; lamina thick with characteristic reticular vein, and easily broken.

Odor, slight; taste, slightly bitter and astringent.

Under a microscope <5.01>, the transverse section reveals thick cuticula; parenchyma cells of palisade tissue and sponge tissue being similar in form; in the vascular bundle, medullary ray consisting of 2 to 7 rows of one-cell line, appearing as bones of Japanese fan; polygonal solitary crystals and clustered crystals of calcium oxalate present sparsely in cells on both outer and inner sides of the vascular bundle, but no crystals in mesophyll.

Identification (1) Macerate 0.5 g of pulverized Bearberry Leaf with 10 mL of boiling water, shake the mixture for a few minutes, allow to cool, and filter. Place 1 drop of the filtrate on filter paper, and add 1 drop of iron (III) chloride TS: a dark purple color appears.

(2) To 0.2 g of pulverized Bearberry Leaf add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of arbutin for thin-layer chromatography in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl formate, formic acid and water (8:1:1) to a distance of about 15 cm, and air-dry the plate. Spray evenly diluted sulfuric acid (1 in 2) upon the plate, and heat at 105°C for 10 minutes: one spot among several spots from the sample solution and that from the standard solution show a yellow-brown to blackish brown color and the same Rf value.

Purity (1) Twig—The amount of twigs contained in Bearberry Leaf does not exceed 4.5%.

(2) Foreign matter <5.01>—The amount of foreign matter other than twigs contained in Bearberry Leaf does not exceed 2.0%.

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Component determination Weigh accurately about 0.5 g of pulverized Bearberry Leaf in a glass-stoppered centrifuge tube, add 40 mL of water, shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of water, and proceed in the same manner. To the

combined extracts add water to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 40 mg of arbutin for component determination, previously dried for 12 hours (in vacuum, silica gel), dissolve in water to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of arbutin in each solution.

$$\text{Amount (mg) of arbutin} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of arbutin for component determination

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 280 nm).

Column: A stainless steel column 4–6 mm in inside diameter and 15–25 cm in length, packed with octadecylsilanized silica gel (5–10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, methanol and 0.1 mol/L hydrochloric acid TS (94:5:1).

Flow rate: Adjust the flow rate so that the retention time of arbutin is about 6 minutes.

Selection of column: Dissolve 0.05 g each of arbutin for component determination, hydroquinone and gallic acid in water to make 100 mL. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of arbutin, hydroquinone and gallic acid in this order, and clearly dividing each peak.

System repeatability: Repeat the test five times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of arbutin is not more than 1.5%.

Belladonna Extract

Extractum Belladonnae

ベラドンナエキス

Belladonna Extract contains not less than 0.85% and not more than 1.05% of hyoscyamine ($C_{17}H_{23}NO_3$; 289.37).

Method of preparation To 1000 g of a coarse powder of Belladonna Root add 4000 mL of 35 vol% ethanol, and digest for 3 days. Press the mixture, add 2000 mL of 35 vol% ethanol to the residue, and digest again for 2 days. Combine all the extracts, and allow to stand for 2 days. Filter, and prepare the viscous extract as directed under Extracts. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Belladonna Extract has a dark brown color, a characteristic odor and a bitter taste.

Identification Mix 0.5 g of Belladonna Extract with 30 mL of ammonia TS in a flask, transfer the mixture to a separator, then add 40 mL of ethyl acetate, and shake the mixture.

Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification under Belladonna Root.

Assay Weigh accurately about 0.4 g of Belladonna Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Proceed as directed under Belladonna Root.

$$\begin{aligned} \text{Amount (mg) of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_3) \\ = W_S \times (Q_T/Q_S) \times (1/5) \times 0.8551 \end{aligned}$$

W_S : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Containers and storage Containers—Tight containers. Storage—Light-resistant, and in a cold place.

Belladonna Root

Belladonnae Radix

ベラドンナコン

Belladonna Root is the root of *Atropa belladonna* Linné (*Solanaceae*).

Belladonna Root, when dried, contains not less than 0.4% of hyoscyamine ($C_{17}H_{23}NO_3$; 289.37).

Description Cylindrical root, usually 10–30 cm in length, 0.5–4 cm in diameter; often cut crosswise or lengthwise; externally grayish brown to grayish yellow-brown, with longitudinal wrinkles; periderm often removed; fractured surface is light yellow to light yellow-brown in color and is powdery.

Almost odorless; taste, bitter.

Identification Place 2.0 g of pulverized Belladonna Root in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution

and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: the principal spot from the sample solution is the same in color tone and *R_f* value with a yellow-red spot from the standard solution.

Purity (1) Stem and crown—The amount of stems and crowns contained in Belladonna Root does not exceed 10.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than stems and crowns contained in Belladonna Root does not exceed 2.0%.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 4.0%.

Assay Weigh accurately about 0.7 g of pulverized Belladonna Root, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate Reference Standard (previously determine the loss on drying <2.41> in the same manner as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as the standard stock solution. Pipet 5 mL of the standard stock solution, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratios, Q_T and Q_S , of the peak area of hyoscyamine (atropine), to that of the internal standard in each solution.

$$\begin{aligned} &\text{Amount (mg) of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_3) \\ &= W_S \times (Q_T / Q_S) \times (1/5) \times 0.8551 \end{aligned}$$

WS: Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogen

phosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL, and mix this solution with acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of atropine is about 14 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of atropine and the internal standard in this order with the resolution between these peaks being not less than 4.

Benincasa Seed

Benincasae Semen

トウガシ

Benincasa seed is the seed of *Benincasa cerifera* Savi (1) or *Benincasa cerifera* Savi forma *emarginata* K. Kimura et Sugiyama (2) (*Cucurbitaceae*).

Description (1) Flattened, ovate to orbicular – ovate seed, 10 – 13 mm in length, 6 – 7 mm in width, about 2 mm in thickness; slightly acute at base; hilum and germ pore form two protrusions; externally light grayish yellow to light yellowish brown; prominent band along with marginal edge of seed; under a magnifying glass, surface of the seed is with fine wrinkles and minute hollows.

(2) Flattened, ovate to ellipsoidal seed, 9 – 12 mm in length, 5 – 6 mm in width, about 2 mm in thickness; hilum and germ pore form two protrusions as in (1); externally light grayish yellow, smooth, no prominent band along with marginal edge of seed.

Both (1) and (2) odorless; bland taste and slightly oily.

Under a microscope <5.01>, a transverse section of (1) reveals the outermost layer of seed coat composed of a single-layered and palisade like epidermis, the epidermis obvious at prominent band along with marginal edge of seed; a transverse section of (2) reveals the outermost layer composed of a single-layered epidermis coated with cuticle, often detached; hypodermis of (1) and (2) composed of slightly sclerified parenchyma beneath epidermis; inside of the parenchyma several layers of stone cells lie; the innermost layer of seed coat composed of parenchyma several cells thick; perisperm coated with cuticle, composed of parenchyma several cells thick; endosperm composed of a row of compressed cells; cotyledon contains oil drops and aleurone grains, occasionally starch grains.

Identification To about 0.5 g of pulverized Benincasa Seed add 10 mL of a mixture of methanol and water (4:1), shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:6:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): two blue-white spots appear around *R_f* 0.4, and the spot having the smaller *R_f* value shows more intense fluorescence.

Purity Foreign matter <5.01>—It contains not more than

2.0%.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 3.0%.

Benzoin

Benzoinum

アンソッコウ

Benzoin is the resin obtained from *Styrax benzoin* Dryander or other species of the same genus (*Styracaceae*).

Description Benzoin occurs as grayish brown to dark red-brown blocks varying in size; the fractured surface exhibiting whitish to light yellow-red grains in the matrix; hard and brittle at ordinary temperature but softened by heat.

Odor, characteristic and aromatic; taste, slightly pungent and acrid.

Identification (1) Heat a fragment of Benzoin in a test tube: it evolves an irritating vapor, and a crystalline sublimate is produced.

(2) Digest 0.5 g of Benzoin with 10 mL of diethyl ether, decant 1 mL of the diethyl ether into a porcelain dish, and add 2 to 3 drops of sulfuric acid: a deep red-brown to deep red-purple color develops.

Purity Ethanol-insoluble substances—Boil gently 1.0 g of Benzoin with 30 mL of ethanol (95) on a water bath for 15 minutes under a reflux condenser. After cooling, collect the insoluble substances through a tared glass filter (G3), and wash with three 5-mL portions of ethanol (95). Dry the residue at 105°C for 4 hours: the mass of the residue does not exceed 0.30 g.

Total ash <5.01> Not more than 2.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Bitter Cardamon

Alpiniae Fructus

ヤクチ

Bitter Cardamon is the fruit of *Alpinia oxyphylla* Miq. (Zingiberaceae).

Description Spherical to fusiform fruit, with both ends somewhat pointed; 1–2 cm in length, 0.7–1 cm in width; externally brown to dark brown, with numerous longitudinal, knob-like protruding lines; pericarp 0.3–0.5 mm in thickness, closely adhering to the seed mass, and difficult to separate; inside divided vertically into three loculi by thin membranes, each loculus containing 5 to 8 seeds adhering by aril; seeds irregularly polygonal, about 3.5 mm in diameter,

brown to dark brown in color, and hard in texture.

Odor, characteristic; taste, slightly bitter.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Bitter Cardamon: the volume of essential oil is not less than 0.4 mL.

Bitter Orange Peel

Aurantii Pericarpium

トウヒ

Bitter Orange Peel is the pericarp of the ripe fruit of *Citrus aurantium* Linné or *Citrus aurantium* Linné var. *daidai* Makino (*Rutaceae*).

Description Usually quartered sections of a sphere, sometimes warped or flattened, 4–8 cm in length, 2.5–4.5 cm in width and 0.5–0.8 cm in thickness; the outer surface is dark red-brown to grayish yellow-brown, with numerous small dents associated with oil sacs; the inner surface is white to light grayish yellow-red, with irregular indented reticulation left by vascular bundles; light and brittle in texture.

Odor, characteristic aroma; taste, bitter, somewhat mucilaginous and slightly pungent.

Identification To 1.0 g of Bitter Orange Peel add 10 mL of ethanol (95), allow to stand for 30 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a grayish green spot from the standard solution show the same color tone and the same *R_f* value.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 5.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Essential oil content <5.01> Perform the test with 50 g of pulverized Bitter Orange Peel provided that 1 mL of silicon resin is previously added to the test sample in the flask: the volume of essential oil is not less than 0.2 mL.

Bitter Tincture

Tinctura Amara

苦味チンキ

Method of preparation

Bitter Orange Peel, in coarse powder	50 g
Swertia Herb, in coarse powder	5 g
Zanthoxylum Fruit, in coarse powder	5 g
70 vol% Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Tinctures, with the above ingredients. An appropriate quantity of Ethanol and Purified Water may be used in place of 70 vol% Ethanol.

Description Bitter Tincture is a yellow-brown liquid. It has a characteristic aroma and a bitter taste.

Specific gravity d_{20}^{20} : about 0.90

Identification (1) To 1 mL of Bitter Tincture add 5 mL of methanol, then add 0.1 g of magnesium in ribbon form and 1 mL of hydrochloric acid, and allow to stand: the solution is red-purple in color.

(2) Use Bitter Tincture as the sample solution. Separately, to 5.0 g of pulverized Bitter Orange Peel add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and use the filtrate as the standard solution (1). Proceed with 0.5 g each of pulverized Swertia Herb and Zanthoxylum Fruit in the same manner, and use the solutions so obtained as the standard solution (2) and the standard solution (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1), (2) and (3) on the plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine the plate under ultraviolet light (broad spectrum wavelength): three of the several spots from the sample solution show the same color tone and *Rf* value as those of the upper spot of the two bright blue to purple spots among the several spots from the standard solution (1), appearing close to each other at an *Rf* value of about 0.4, and a bright red spot from the standard solution (2), appearing at an *Rf* value of about 0.35, and a bright grayish red to red spot from the standard solution (3), appearing at an *Rf* value of about 0.7.

Alcohol number <1.01> Not less than 6.9 (Method 2).

Containers and storage Containers—Tight containers.

Bupleurum Root

Bupleuri Radix

サイコ

Bupleurum Root is the root of *Bupleurum falcatum* Linné (*Umbelliferae*).

It contains not less than 0.35% of the total saponin (saikosaponin a and saikosaponin d), calculated on the basis of dried material.

Description Single or branched root of long cone or column shape, 10–20 cm in length, 0.5–1.5 cm in diameter; occasionally with remains of stem on the crown; externally light brown to brown and sometimes with deep wrinkles; easily broken, and fractured surface somewhat fibrous.

Odor, characteristic, and taste, slightly bitter.

Under a microscope <5.01>, a transverse section reveals the thickness of cortex reaching 1/3 ~ 1/2 of the radius, tangentially extended clefts in cortex; and cortex scattered with a good many intercellular schizogenous oil canals 15–35 μ m in diameter; in xylem, vessels lined radially or stepwise, and fiber groups scattered; in the pith at the crown, the same oil canals as in the cortex; parenchyma cells containing starch grains and oil droplets. Starch grains composed of simple grains, 2–10 μ m in diameter, or compound grains.

Identification (1) Shake vigorously 0.5 g of pulverized Bupleurum Root with 10 mL of water: lasting fine foams are produced.

(2) To 2.0 g of pulverized Bupleurum Root add 10 mL of methanol, boil gently under a reflux condenser on a water bath for 15 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of saikosaponin a for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol and water (30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a mixture of sulfuric acid and ethanol (95) (1:1) on the plate, and warm at 50 °C for 5 minutes: one spot among the several spots from the sample solution and the blue spot from the standard solution show the same *Rf* value, and the color tone is blue to blue-purple.

Purity (1) Stem and leaf—The amount of the stems and leaves contained in Bupleurum Root does not exceed 10.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Bupleurum Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Bupleurum Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than stems and leaves contained in Bupleurum Root does not exceed 1.0%.

Loss on drying <5.01> Not more than 12.5% (6 hours).

Component determination Weigh accurately about 1 g of pulverized Bupleurum Root, transfer in a glass-stoppered centrifuge tube, add 20 mL of diluted methanol (9 in 10), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Perform the same procedure with the precipitate using two 15-mL portions of diluted methanol (9 in 10), combine whole supernatant liquids, and add diluted methanol (9 in 10) to make exactly 50 mL. Pipet 5 mL of this solution, add 2.5 mL of dilute sodium hydroxide TS, heat in a water bath at 50°C for 1 hour, and add 7.5 mL of phosphate buffer solution for component determination of bupleurum root. Allow this solution to flow through a chromatographic column [about 10 mm inside diameter containing 0.36 g of octadecylsilanized silica gel for pretreatment (55 to 105 μm in particle diameter), conditioned with 10 mL of methanol then 10 mL of water just before use]. Wash the column with 10 mL of diluted methanol (7 in 20), then flow with methanol to get exactly 10 mL of effluent solution, and use this as the sample solution. Separately, weigh accurately each about 10 mg of saikosaponin a for component determination and saikosaponin d for component determination, previously dried in a desiccator (silica gel) for 24 hours, dissolve in methanol to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TA} and A_{SA} , of saikosaponin a and A_{TD} and A_{SD} , of saikosaponin d. Calculate the amount of saikosaponin a and saikosaponin d by the following equation.

Amount (mg) of saikosaponin a = $W_{\text{SA}} \times (A_{\text{TA}}/A_{\text{SA}}) \times (1/2)$

W_{SA} : Amount (mg) of saikosaponin a for component determination

Amount (mg) of saikosaponin d = $W_{\text{SD}} \times (A_{\text{TD}}/A_{\text{SD}}) \times (1/2)$

W_{SD} : Amount (mg) of saikosaponin d for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 206 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of saikosaponin a is about 8 minutes.

System suitability—

System performance: When the procedure is run with 20 μL of the standard solution under the above operating conditions, saikosaponin a and saikosaponin d are eluted in this order, and the numbers of theoretical plates and the symmetry factors of their peaks are not less than 4000 and not more than 1.4, respectively.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviations of the peak area of saikosaponin a and saikosaponin d are not more than 1.5%, respectively.

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 11.0%.

Burdock Fruit

Arctii Fructus

ゴボウシ

Burdock Fruit is the fruit of *Arctium lappa* Linné (*Compositae*).

Description Burdock Fruit is slightly curved, long obovate achene, 5 to 7 mm in length, 2.0 to 3.2 mm in width, 0.8 to 1.5 mm in thickness; externally grayish brown to brown, with black spots; hollow about 1 mm in diameter at one broad end; flat, indistinct, longitudinal ridge at the other narrow end. 100 fruits weighing 1.0 to 1.5 g.

Practically odorless; taste, bitter and oily.

Under a microscope <5.01>, transverse section reveals an exocarp of single-layered epidermal tissue, mesocarp of slightly sclerified parenchyma, and endocarp of a single layer of stone cells; seed coat composed of radially elongated, sclerified epidermis, and parenchyma several cells thick; parenchymatous cells of the mesocarp contain a brown substance; stone cells of endocarp contain solitary, discrete crystals of calcium oxalate; cotyledons with starch grains, oil drops, aleurone grains, and minute crystals of calcium oxalate.

Identification To 0.5 g of pulverized Burdock Fruit add 20 mL of methanol, shake for 10 minutes, filter, and use filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate and water (15:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: a red-purple spot appears at around R_f 0.4.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-extract: not less than 15.0%.

Calumba

Calumbae Radix

コロンボ

Calumba is the cross-sectioned root of *Jateorhiza columba* Miers (*Menispermaceae*).

Description Disk-like slices, 0.5 – 2 cm in thickness, 3 – 8 cm in diameter; mostly with concave center and slightly waned; side surface grayish brown in color, with irregular

wrinkles; cut surface light yellow and powdery, with pale and dark radiating stripes; cortex rather yellowish; cambium and its neighborhood light grayish brown, warty protrusions in the center; hard in texture, but brittle.

Odor characteristic; taste, bitter.

Identification To 3 g of pulverized Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and, after cooling, add carefully chlorine TS to make two layers: a light red to red color develops at the zone of contact.

Total ash <5.01> Not more than 7.5%.

Powdered Calumba

Calumbae Radix Pulverata

コロンボ末

Powdered Calumba is the powder of Calumba.

Description Powdered Calumba occurs as a grayish yellow powder, and has a characteristic odor and a bitter taste.

Under a microscope <5.01>, Powdered Calumba reveals numerous starch grains, fragments of parenchyma cells containing them; fragments of cork cells, stone cells, fibers, substitute fibers, vessels, tracheids, and also solitary crystals of calcium oxalate; starch grains consisting of solitary grains or 2- to 3-compound grains; hilum, unevenly scattered, usually 25 – 50 μm , but up to 90 μm in diameter.

Identification To 3 g of Powdered Calumba add 30 mL of water, allow to stand for 5 minutes with occasional shaking, and filter. To 2 mL of the filtrate add gently 1 mL of sulfuric acid, and after cooling, add carefully chlorine TS to make two layers: a light red to red color develops at the zone of contact.

Total ash <5.01> Not more than 7.5%.

Capsicum

Capsici Fructus

トウガラシ

Capsicum is the fruit of *Capsicum annuum* Linné (*Solanaceae*).

Capsicum contains not less than 0.10% of total capsaicins (capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

Description Elongated conical to fusiform fruit, often bent, 3 – 10 cm in length, about 0.8 cm in width; outer surface lustrous and dark red to dark yellow-red; interior of pericarp hollow and usually divided into two loculi, containing numerous seeds nearly circular and compressed, light yellow-red, about 0.5 cm in diameter; usually with remains of calyx and peduncle.

Odor, slight and characteristic; taste, hot and acrid.

Identification To 2.0 g of pulverized Capsicum add 5 mL of

ethanol (95), warm on a water bath for 5 minutes, cool, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and methanol (19:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a blue spot from the standard solution show the same color tone and the same *R_f* value.

Purity Foreign matter <5.01>—The amount of foreign matter contained in Capsicum does not exceed 1.0%.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 1.2%.

Component determination Weigh accurately about 0.5 g of medium powder of Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of capsaicin for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD} , of capsaicin and dihydrocapsaicin (the relative retention time to capsaicin is about 1.3) in the sample solution, and the peak area, A_{S} , of capsaicin in the standard solution.

$$\begin{aligned} & \text{Amount (mg) of total capsaicins} \\ & = W_{\text{S}} \times \{(A_{\text{TC}} + A_{\text{TD}}) / A_{\text{S}}\} \times 0.08 \end{aligned}$$

W_{S} : Amount (mg) of capsaicin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of capsaicin for component determination and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the

procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of capsaicin is not more than 1.5%.

Powdered Capsicum

Capsici Fructus Pulveratus

トウガラシ末

Powdered Capsicum is the powder of Capsicum.

Powdered Capsicum contains not less than 0.10% of total capsaicins (capsaicin and dihydrocapsaicin), calculated on the basis of dried material.

Description Powdered Capsicum occurs as a yellow-red powder. It has a slight, characteristic odor and a hot, acrid taste.

Under a microscope <5.01>, Powdered Capsicum reveals fragments of parenchyma containing oil droplets and yellow-red chromoplasts; fragments of outer pericarp with thick cuticle; fragments of stone cells from inner surface of pericarp, with wavy curved side walls; fragments of thin vessels; fragments of seed coat with thick wall, and fragments of parenchyma consisting of small cells of endosperm containing fixed oil and aleuron grains.

Identification To 2.0 g of Powdered Capsicum add 5 mL of ethanol (95), warm on a water bath for 5 minutes, cool, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of capsaicin for thin-layer chromatography in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of diethyl ether and methanol (19:1) to a distance of about 12 cm, and air-dry the plate. Spray evenly 2,6-dibromo-N-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and blue spot from the standard solution show the same in color tone and *R_f* value.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 1.2%.

Component determination Weigh accurately about 0.5 g of medium powder of Powdered Capsicum in a glass-stoppered centrifuge tube, add 30 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of methanol, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure again, combine the extracts, add methanol to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of capsaicin for component determination, previously dried in a desicca-

tor (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, and dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD} , of capsaicin and dihydrocapsaicin (the relative retention time to capsaicin is about 1.3) in the sample solution, and the peak area, A_{S} , of capsaicin in the standard solution.

$$\begin{aligned} & \text{Amount (mg) of total capsaicins} \\ & = W_{\text{S}} \times \{(A_{\text{TC}} + A_{\text{TD}})/A_{\text{S}}\} \times 0.08 \end{aligned}$$

W_{S} : Amount (mg) of capsaicin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength : 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of capsaicin for component determination and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of capsaicin is not more than 1.5%.

Capsicum Tincture

トウガラシチンキ

Capsicum Tincture contains not less than 0.010 w/v% of total capsaicins (capsaicin and dihydrocapsaicin).

Method of preparation

Capsicum, in medium cutting	100 g
Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Tinctures, with the above ingredients.

Description Capsicum Tincture is a yellow-red liquid. It has a burning, pungent taste.

Specific gravity d_{20}^{20} : about 0.82

Identification Proceed as directed in the Identification un-

der Capsicum, using Capsicum Tincture as the sample solution. Spot 20 μL each of the sample solution and the standard solution.

Alcohol number <1.01> Not less than 9.7 (Method 2).

Component determination Pipet 2 mL of Capsicum Tincture, add methanol to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of capsaicin for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide, 40°C) for 5 hours, dissolve in methanol to make exactly 50 mL. Pipet 2 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{TC} and A_{TD} , of capsaicin and dihydrocapsaicin (the relative retention time to capsaicin is about 1.3) in the sample solution, and the peak area, A_{S} , of capsaicin in the standard solution.

$$\text{Amount (mg) of total capsaicins} \\ = W_{\text{S}} \times \{(A_{\text{TC}} + A_{\text{TD}}) / A_{\text{S}}\} \times 0.032$$

W_{S} : Amount (mg) of capsaicin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 281 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with phenylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of capsaicin is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of capsaicin for component determination and 4-hydroxy-3-methoxybenzyl nonylic acid amide in methanol to make 50 mL. When the procedure is run with 20 μL of this solution under the above operating conditions, 4-hydroxy-3-methoxybenzyl nonylic acid amide and capsaicin are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of capsaicin is not more than 1.5%.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Capsicum and Salicylic Acid Spirit

トウガラシ・サリチル酸精

Method of preparation

Capsicum Tincture	40 mL
Salicylic Acid	50 g
Liquefied Phenol	20 mL
Castor Oil	100 mL
aromatic substance	a suitable quantity
Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients.

Description Capsicum and Salicylic Acid Spirit is a light brown-yellow liquid.

Specific gravity d_{20}^{20} : about 0.84

Identification (1) Shake 10 mL of Capsicum and Salicylic Acid Spirit with 15 mL of sodium hydrogen carbonate TS and 10 mL of diethyl ether, and separate the water layer. To 1 mL of the solution add hydrochloric acid-potassium chloride buffer solution, pH 2.0, to make 200 mL, and to 5 mL of this solution add 5 mL of a solution of iron (III) nitrate enneahydrate (1 in 200): a red-purple color is produced (salicylic acid).

(2) To 0.5 mL of Capsicum and Salicylic Acid Spirit add 20 mL of water and 5 mL of dilute hydrochloric acid, extract with 20 mL of diethyl ether, wash the diethyl ether extract with two 5-mL portions of sodium hydrogen carbonate TS, and then extract with 20 mL of dilute sodium hydroxide TS. To 1 mL of the extract add 1 mL of sodium nitrite TS and 1 mL of dilute hydrochloric acid, shake, and allow to stand for 10 minutes. Add 3 mL of sodium hydroxide TS: a yellow color is produced (phenol).

(3) To 0.2 mL of Capsicum and Salicylic Acid Spirit add 5 mL of dilute hydrochloric acid, extract with 5 mL of chloroform, and use the extract as the sample solution. Dissolve 0.01 g of salicylic acid and 0.02 g of phenol in 5 mL and 25 mL of chloroform, respectively, and use both solutions as the standard solution (1) and the standard solution (2). Perform the test with the sample solution and standard solutions (1) and (2) as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of these solutions on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of chloroform, acetone and acetic acid (100) (45:5:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): two spots from the sample solution exhibit the same R_f values as those from standard solution (1) and standard solution (2). Spray evenly iron (III) chloride TS upon the plate: the spot from standard solution (1) and the corresponding spot from the sample solution reveal a purple color.

Alcohol number <1.01> Not less than 8.1 (Method 2). Prepare the sample solution as follows: Pipet 5 mL of Capsicum and Salicylic Acid Spirit at $15 \pm 2^\circ\text{C}$ into a glass-stoppered, conical flask containing exactly 45 mL of water while shaking

vigorously, allow to stand, and filter the lower layer. Discard the first 15 mL of the filtrate. Pipet 25 mL of the subsequent filtrate, add exactly 10 mL of the internal standard solution, and add water to make exactly 100 mL.

Containers and storage Containers—Tight containers.

Cardamon

Cardamomi Fructus

シヨウズク

Cardamon is the fruit of *Elettaria cardamomum* Maton (*Zingiberaceae*). The capsules are removed from the seeds before use.

Description Nearly ellipsoidal, 1–2 cm in length, 0.5–1 cm in diameter; externally, light yellow with three blunt ridges and many longitudinal lines; 0.1–0.2-cm beak at one end; pericarp thin, light and fibrous; interior longitudinally divided into three loculi by thin membranes, each loculus containing 3 to 7 seeds joining by aril; seed irregularly angular ovoid, 0.3–0.4 cm in length, dark brown to blackish brown; the dorsal side convex, the ventral side longitudinally grooved; external surface coarsely tuberculated.

Seed has a characteristic aroma, and pungent, slightly bitter taste; pericarp, odorless and tasteless.

Total ash <5.01> Not more than 6.0% (seed).

Acid-insoluble ash <5.01> Not more than 4.0% (seed).

Essential oil content <5.01> Perform the test with 30.0 g of the pulverized seeds of Cardamon: the volume of essential oil is not less than 1.0 mL.

Cassia Seed

Cassiae Semen

ケツメイシ

Cassia Seed is the seed of *Cassia obtusifolia* Linné or *Cassia tora* Linné (*Leguminosae*).

Description Short cylindrical seed, 3–6 mm in length, 2–3.5 mm in diameter; acuminate at one end and flat at the other; externally green-brown to brown and lustrous, with light yellow-brown longitudinal lines or bands on both sides; hard in texture; cross section round or obtuse polygonal; under a magnifying glass, albumen enclosing a bent, dark-colored cotyledon.

When ground, characteristic odor and taste.

Identification Place 0.1 g of pulverized Cassia Seed, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both internal diameter and height on it, then cover with moistened filter paper, and heat gently the slide glass over a small flame. Take off the filter paper when a yellow color has developed on the upper surface of it, and place 1 drop of potassium hydroxide TS on the surface of the filter paper where a sublimate is present: a red color appears.

Purity Foreign matter <5.01>—The amount of foreign matter contained in Cassia Seed does not exceed 1.0%.

Total ash <5.01> Not more than 5.0%.

Catalpa Fruit

Catalpae Fructus

キササゲ

Catalpa Fruit is the fruit of *Catalpa ovata* G. Don or *Catalpa bungei* C. A. Meyer (*Bignoniaceae*).

Description Slender stick-like fruit, 30–40 cm in length and about 0.5 cm in diameter; externally, dark brown; inner part contains numerous seeds; seed compressed or semitubular, about 3 cm in length and about 0.3 cm in width, externally grayish brown; hairs, about 1 cm in length, attached to both ends of seed; pericarp, thin and brittle.

Almost odorless; taste, slightly astringent.

Identification To 1.0 g of pulverized Catalpa Fruit add 20 mL of water, warm on a water bath for 5 minutes, and filter immediately. Transfer the filtrate to a separator, and extract with two 20-mL portions of 1-butanol. Combine the extracts, evaporate to dryness under reduced pressure on a water bath, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Separately, dissolve 1 mg of parahydroxybenzoic acid in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (20:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultra-violet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value. Prescribe that the moving distance of the spot corresponding to parahydroxybenzoic acid from the sample solution is 1: a dark purple spot develops at the relative moving distance of about 0.3.

Purity Peduncle—The amount of peduncles contained in Catalpa Fruit does not exceed 5.0%.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.0%.

Chrysanthemum Flower

Chrysanthemi Flos

キクカ

Chrysanthemum Flower is the capitulum of 1) *Chrysanthemum morifolium* Ramatulle or 2) *Chrysanthemum*

themum indicum Linné (*Compositae*).

Description 1) Chrysanthemum Flower is capitulum, 15 to 40 mm in diameter; involucre consisting of 3 to 4 rows of involucre scales; the outer involucre scale linear to lanceolate, inner involucre scale narrow ovate to ovate; ligulate flowers are numerous, white to yellow; tubular flowers in small number, light yellow-brown; tubular flowers occasionally degenerate; outer surface of involucre green-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

2) Chrysanthemum Flower is capitulum, 3 to 10 mm in diameter; involucre consisting of 3 to 5 rows of involucre scales; the outer involucre scale linear to lanceolate, inner involucre scale narrow ovate to ovate; ligulate flower is single, yellow to light yellow-brown; tubular flowers in numerous, light yellow-brown; outer surface of involucre yellow-brown to brown; light in texture and easy to break.

Odor, characteristic; taste, slightly bitter.

Identification To 1 g of pulverized Chrysanthemum Flower add 20 mL of methanol, shake for 10 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this as the sample solution. Separately, dissolve 1 mg of luteolin for thin-layer chromatography in 1 mL of methanol, and use this as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 2-butanone, water and formic acid (25:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one of several spots obtained from the sample solution has the same color tone and the same *R_f* value with the dark green spot obtained from the standard solution.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 8.5%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Cimicifuga Rhizome

Cimicifugae Rhizoma

シヨウマ

Cimicifuga Rhizome is the rhizome of *Cimicifuga simplex* Wormskjold, *Cimicifuga dahurica* (Turcz.) Maximowicz, *Cimicifuga foetida* Linné or *Cimicifuga heracleifolia* Komarov (*Ranunculaceae*).

Description Knotted, irregularly shaped rhizome, 6 – 18 cm in length, 1 – 2.5 cm in diameter; externally dark brown to blackish brown, with many remains of roots, often with scars of terrestrial stems; the center of the scar dented, and the circumference being pale in color and showing a radial pattern; fractured surface fibrous; pith dark brown in color and often hollow; light and hard in texture.

Almost odorless; taste, bitter and slightly astringent.

Purity Rhizome of *Astilbe thunbergii* Miquel—Under a microscope <5.01>, powdered Cimicifuga Rhizome does not contain crystal druses in the parenchyma.

Total ash <5.01> Not more than 9.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 18.0%.

Cinnamon Bark

Cinnamomi Cortex

ケイヒ

Cinnamon Bark is the bark of the trunk of *Cinnamomum cassia* Blume (*Lauraceae*), or such bark from which a part of the periderm has been removed.

Description Usually semi-tubular or tubularly rolled pieces of bark, 0.1 – 0.5 cm in thickness, 5 – 50 cm in length, 1.5 – 5 cm in diameter; the outer surface dark red-brown, and the inner surface red-brown and smooth; brittle; the fractured surface is slightly fibrous, red-brown, exhibiting a light brown, thin layer.

Characteristic aroma; taste, sweet and pungent at first, later rather mucilaginous and slightly astringent.

Under a microscope <5.01>, a transverse section of Cinnamon Bark reveals a primary cortex and a secondary cortex divided by an almost continuous ring consisting of stone cells; nearly round bundles of fibers in the outer region of the ring; wall of each stone cell often thickened in a U-shape; secondary cortex lacking stone cells, and with a small number of sclerenchymatous fibers coarsely scattered; parenchyma scattered with oil cells, mucilage cells and cells containing starch grains; medullary rays with cells containing fine needles of calcium oxalate.

Identification To 2.0 g of pulverized Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot develops at the *R_f* value of about 0.4. Spray evenly 2,4-dinitrophenylhydrazine TS upon the spot: a yellow-orange color develops.

Purity Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 15.5% (6 hours).

Total ash <5.01> Not more than 6.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.

Powdered Cinnamon Bark

Cinnamomi Cortex Pulveratus

ケイヒ末

Powdered Cinnamon Bark is the powder of Cinnamon Bark.

Description Powdered Cinnamon Bark is red-brown to brown in color. It has a characteristic aroma and a sweet, pungent taste with a slightly mucilaginous and astringent aftertaste.

Under a microscope <5.01>, Powdered Cinnamon Bark reveals starch grains, fragments of parenchyma cells containing them; fragments of fibers, oil cells containing yellow-brown oil droplets, stone cells, cork stone cells, cork tissue, and fine crystals of calcium oxalate. Starch grains are simple and compound grains 6 to 15 μm in diameter.

Identification To 2.0 g of Powdered Cinnamon Bark add 10 mL of diethyl ether, shake for 3 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot develops at the R_f value of about 0.4. Spray 2,4-dinitrophenylhydrazine TS upon the spot: a yellow orange color develops.

Purity (1) Petiole—Under a microscope <5.01>, Powdered Cinnamon Bark does not reveal epidermal cells, hairs, cells containing chlorophyll granules, and fragments of vascular bundle.

(2) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered Cinnamon Bark provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.35 mL.

Containers and storage Containers—Tight containers.

Cinnamon Oil

Oleum Cinnamomi

ケイヒ油

Cinnamon Oil is the essential oil distilled with steam from the leaves and twigs or bark of *Cinnamomum cassia* Blume or from the bark of *Cinnamomum zeylanicum* Nees (*Lauraceae*).

It contains not less than 60 vol% of the total aldehydes.

Description Cinnamon Oil is a yellow to brown liquid. It

has a characteristic, aromatic odor and a sweet, pungent taste.

It is clearly miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

It is weakly acidic. Upon aging or long exposure to air, it darkens and becomes viscous.

Specific gravity d_{20}^{20} : 1.010 – 1.065

Identification Shake 4 drops of Cinnamon Oil with 4 drops of nitric acid: the mixture forms white to light yellow crystals at a temperature below 5°C.

Purity (1) Rosin—Mix 1.0 mL of Cinnamon Oil with 5 mL of ethanol (95), then add 3 mL of freshly prepared, saturated ethanol solution of lead (II) acetate trihydrate: no precipitate is produced.

(2) Heavy metals <1.07>—Proceed with 1.0 mL of Cinnamon Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Pipet 5.0 mL of Cinnamon Oil into a cassia flask, add 70 mL of sodium hydrogensulfite TS, and heat the mixture in a water bath with frequent shaking to dissolve completely. To this solution add sodium hydrogensulfite TS to raise the lower level of the oily layer within the graduate portion of the neck. Allow to stand for 2 hours, and measure the volume (mL) of the separated oily layer.

Total aldehydes (vol%)

= [5.0 – (volume of separated oily layer)] × 20

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Citrus Unshiu Peel

Aurantii Bobilis Pericarpium

チンピ

Citrus Unshiu Peel is the pericarp of the ripe fruit of *Citrus unshiu* Markovich or *Citrus reticulata* Blanco (*Rutaceae*).

Description Irregular pieces of pericarp, about 2 mm in thickness; externally yellow-red to dark yellow-brown, with numerous small dents associated with oil sacs; internally white to light grayish yellow-brown; light and brittle in texture.

Odor, characteristic aroma; taste, bitter and slightly pungent.

Identification To 0.5 g of pulverized Citrus Unshiu Peel add 10 mL of methanol, warm on a water bath for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon-form and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

Purity Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Citrus Unshiu Peel provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Clematis Root

Clematidis Radix

イレイセン

Clematis Root is the root and rhizome of *Clematis chinensis* Osbeck, *Clematis manshurica* Ruprecht, or *Clematis hexapetala* Pallas (*Ranunculaceae*).

Description Clematis Root consists of short rhizome and numerous slender roots. The root, 10 to 20 cm in length, 1 to 2 mm in diameter, externally brown to blackish brown, with fine longitudinal wrinkles, brittle. The cortex easily separable from central cylinder; root, grayish white to light brown in the transverse section, light grayish yellow to yellow in the central cylinder; under a magnifying glass, central cylinder almost round, slight 2 to 4 sinuses on xylem. The rhizome, 2 to 4 cm in length, 5 to 20 mm in diameter, externally light grayish brown to grayish brown; cortex peeled off and fibrous, often with rising node; apex having the residue of lignified stem.

Odor, slight; practically tasteless.

Under a microscope, <5.01> transverse section of root reveals a uni-layered epidermis in the outermost layer; with exodermis lying just inside of the epidermis; cortex and stele divided by endodermis; cortex composed of parenchymatous tissue; xylem with 2–4 small concavities where phloem is present; parenchymatous cells contain both simple and 2- to 8-compound starch grains.

Identification (1) To 0.5 g of pulverized Clematis Root add 10 mL of water, and boil for 2 to 3 minutes. After cooling, shake vigorously: lasting fine foams appear.

(2) To 0.5 g of pulverized Clematis Root add 3 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To the filtrate add 1 mL of sulfuric acid gently: a brown color appears at the zone of contact.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 8.5%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

Clove

Caryophylli Flos

チョウジ

Clove is the flowering bud of *Syzygium aromaticum* Merrill et Perry (*Eugenia caryophyllata* Thunberg)

(*Myrtaceae*).

Description Dark brown to dark red buds, 1–1.8 cm in length, consisting of slightly compressed and four-sided receptacle, crowned by 4 thick sepals and 4 nearly spherical, membranous, imbricated petals, enclosing numerous stamens and a single style.

Odor, strong and characteristic; taste, pungent, followed by a slight numbness of the tongue.

Identification Mix 0.1 mL of the mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity (1) Stem—The amount of the stem contained in Clove does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the stem contained in Clove does not exceed 1.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Essential oil content <5.01> Perform the test with 10.0 g of pulverized Clove: the volume of essential oil is not less than 1.6 mL.

Powdered Clove

Caryophylli Flos Pulveratus

チョウジ末

Powdered Clove is the powder of Clove.

Description Powdered Clove occurs as a dark brown powder. It has a strong, characteristic odor and a pungent taste, followed by slight numbness of the tongue.

Under a microscope <5.01>, Powdered Clove reveals epidermal tissue with stomata, collenchyma, parenchyma with oil sacs, and spongy parenchyma or its fragments; furthermore, a few fusiform thick-walled fibers, spiral vessels 6–10 μm in diameter, anther and pollen grains, and rosette aggregates of calcium oxalate 10–15 μm in diameter. Epidermis of anther shows characteristically reticulated walls; pollen grains tetrahedral 10–20 μm in diameter; rosette aggregates of calcium oxalate arranged in crystal cell rows, or contained in collenchyma cells and parenchyma cells.

Identification Mix 0.1 mL of a mixture of essential oil and xylene, obtained in the Essential oil content, with 2 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green to blue color develops.

Purity Foreign matter <5.01>—Under a microscope, Powdered Clove does not contain stone cells or starch grains.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Essential oil content <5.01> Perform the test with 10.0 g of Powdered Clove: the volume of essential oil is not less than 1.3 mL.

Containers and storage Containers—Tight containers.

Clove Oil

Oleum Caryophylli

チヨウジ油

Clove Oil is the volatile oil distilled with steam from the flower buds or leaves of *Syzygium aromaticum* Merrill et Perry (*Eugenia caryophyllata* Thunberg) (*Myrtaceae*).

It contains not less than 80.0 vol% of total eugenol.

Description Clove Oil is a colorless or light yellow-brown, clear liquid. It has a characteristic aroma and a burning taste.

It is miscible with ethanol (95) and with diethyl ether.

It is slightly soluble in water.

It acquires a brown color upon aging or by air.

Identification (1) To 5 drops of Clove Oil add 10 mL of calcium hydroxide TS, and shake vigorously: the oil forms a flocculent mass, and a white to light yellow color develops.

(2) Dissolve 2 drops of Clove Oil in 4 mL of ethanol (95), and add 1 to 2 drops of iron (III) chloride TS: a green color is produced.

Refractive index <2.45> n_D^{20} : 1.527 – 1.537

Optical rotation <2.49> α_D^{20} : 0 – –1.5° (100 mm).

Specific gravity <1.13> d_{20}^{20} : 1.040 – 1.068

Purity (1) Clarity of solution—Dissolve 1.0 mL of Clove Oil in 2.0 mL of diluted ethanol (7 in 10): the solution is clear.

(2) Water-soluble phenols—To 1.0 mL of Clove Oil add 20 mL of boiling water, shake vigorously, filter the aqueous layer after cooling, and add 1 to 2 drops of iron (III) chloride TS: a yellow-green, but no blue or violet, color develops.

(3) Heavy metals <1.07>—Proceed with 1.0 mL of Clove Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Take 10.0 mL of Clove Oil in a Cassia flask, add 70 mL of sodium hydroxide TS, shake for 5 minutes and warm for 10 minutes in a water bath with occasional shaking, add sodium hydroxide TS to the volume after cooling, and allow to stand for 18 hours. Measure the volume (mL) of the separated oily layer.

$$\begin{aligned} & \text{Total eugenol (vol\%)} \\ & = [10 - (\text{volume of separated oily layer})] \times 10 \end{aligned}$$

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Cnidium Monnieri Fruit

Cnidii Monnieri Fructus

ジャシヨウシ

Cnidium Monnieri Fruit is the fruit of *Cnidium monnieri* Cusson (*Umbelliferae*).

Description Elliptical cremocarp, often each mericarp separated; 2 – 3 mm in length, 1 – 2 mm in width; externally light brown to brown, each mericarp usually with five winged longitudinal ridges; inner surface of mericarp almost flat.

Odor, characteristic; it gives characteristic aroma, later a slight sensation of numbness on chewing.

Under a microscope <5.01>, a transverse section reveals one oil canal between longitudinal ridges, usually two oil canals in the inner part of mericarp facing to gynophore; longitudinal ridges composed of slightly lignified parenchymatous cells, with vascular bundles in the base; epidermal cells and parenchymatous cells of longitudinal ridges contain solitary crystals of calcium oxalate; parenchymatous cells of albumen contain oil drops and aleurone grains, and occasionally starch grains.

Identification To 1 g of pulverized *Cnidium Monnieri* Fruit add 10 mL of ethyl acetate, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of osthole for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and the *R_f* value with the blue-white fluorescent spot from the standard solution.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 17.0%.

Acid-insoluble ash <5.01> Not more than 6.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.0%.

Cnidium Rhizome

Cnidii Rhizoma

センキュウ

Cnidium Rhizome is the rhizome of *Cnidium officinale* Makino (*Umbelliferae*), usually passed through hot water.

Description Irregular massive rhizome, occasionally cut lengthwise; 5 – 10 cm in length, and 3 – 5 cm in diameter; externally grayish brown to dark brown, with gathered nodes, and with knobbed protrusions on the node; margin of the vertical section irregularly branched; internally grayish white to grayish brown, translucent and occasionally with hollows; dense and hard in texture.

Odor, characteristic; taste, slightly bitter.

Under a microscope <5.01>, a transverse section reveals cortex and pith with scattered oil canals; in the xylem, thick-walled and lignified xylem fibers appear in groups of various sizes; starch grains usually gelatinized, but rarely remaining as grains of 5 – 25 μ m in diameter; crystals of calcium oxalate

not observable.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Powdered Cnidium Rhizome

Cnidii Rhizoma Pulveratum

センキュウ末

Powdered Cnidium Rhizome is the powder of Cnidium Rhizome.

Description Powdered Cnidium Rhizome occurs as a gray to light grayish brown powder. It has a characteristic odor and a slightly bitter taste.

Under a microscope <5.01>, Powdered Cnidium Rhizome reveals colorless and gelatinized starch masses, and fragments of parenchyma containing them; fragments of scalariform and reticulate vessels 15 – 30 μm in diameter; fragments of thick-walled and lignified xylem fibers 20 – 60 μm in diameter; fragments of yellow brown cork tissue; fragments of secretory tissue.

Purity Foreign matter <5.01>—Under a microscope, Powdered Cnidium Rhizome does not contain a large quantity of starch grains, stone cells, crystals of calcium oxalate or other foreign matter.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Coix Seed

Coicis Semen

ヨクイニン

Coix Seed is the seed of *Coix lachryma-jobi* Linné var. *ma-yuen* Stapf (*Gramineae*), from which the seed coat has been removed.

Description Ovoid or broad ovoid seed, about 6 mm in length, and about 5 mm in width; with a slightly hollowed apex and base; dorsal side distended; ventral side longitudinally and deeply furrowed in the center; dorsal side mostly white in color and powdery; in the furrow on the ventral surface, attached brown, membranous pericarp and seed coat. Under a magnifying glass, the cross section reveals light yellow scutellum in the hollow of the ventral side. Hard in texture.

Odor, slight; taste, slightly sweet; adheres to the teeth on chewing.

Identification To a cross-section of Coix Seed add iodine TS dropwise: a dark red-brown color develops in the endosperm, and a dark gray color develops in the scutellum.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 3.0%.

Powdered Coix Seed

Coicis Semen Pulveratum

ヨクイニン末

Powdered Coix Seed is the powder of Coix Seed.

Description Powdered Coix Seed occurs as a brownish, grayish white to grayish yellow-white powder, and has a slight odor and a slightly sweet taste.

Under a microscope <5.01>, Powdered Coix Seed reveals starch grains, and fragments of endosperm containing them; fragments of tissue accompanied with epidermal cells of pericarp composed of yellowish and oblong cells, and fragments of parenchyma cells containing fixed oil, aleuron grains and starch grains; a very few fragments of spiral vessels. Starch grains are simple and 2-compound grains, simple grain nearly equidiameter to obtuse polygon, 10 – 20 μm in diameter, and have a stellate cleft-like hilum in the center. Spherical starch grains, coexisting with aleuron grains, are spherical simple grains, 3 – 7 μm in diameter.

Identification Place a small amount of Powdered Coix Seed on a slide glass, add dropwise iodine TS, and examine under a microscope <5.01>: nearly equidiameter and obtuse polygonal simple starch grains, usually 10 – 15 μm in diameter, and compound starch grains have a reddish brown color. Small spheroidal starch grains, coexisting with fixed oil and with aleuron grains in parenchymatous cells, have a blue-purple color.

Purity Foreign matter—Under a microscope <5.01>, Powdered Coix Seed reveals no fragments of tissue having silicified cell wall, no stone cells, no fragments of other thick-walled and lignified cells, no fragments of reticulate, scalariform and pitted vessels, no fragments of fibers and hairs, and no large starch grains, more than 10 μm in diameter, appearing blue-purple upon addition of iodine TS.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 3.0%.

Containers and storage Containers—Tight containers.

Condurango

Condurango Cortex

コンズランゴ

Condurango is the bark of the trunk of *Marsdenia condurango* Reichenbach fil. (*Asclepiadaceae*).

Description Tubular or semi-tubular pieces of bark, 0.1 – 0.6 cm in thickness, 4 – 15 cm in length; outer surface grayish brown to dark brown, nearly smooth and with numerous lenticels, or more or less scaly and rough; inner surface light grayish brown and longitudinally striate; fractured surface fibrous on the outer region and generally granular in the inner

region.

Odor, slight; taste, bitter.

Under a microscope <5.01>, a transverse section reveals a cork layer composed of several layers of thin-walled cells; primary cortex with numerous stone cell groups; secondary cortex with phloem fiber bundles scattered inside the starch sheath consisting of one-cellular layer; articulate latex tubes scattered in both cortices; parenchyma cells containing starch grains or rosette aggregates of calcium oxalate; starch grain 3 – 20 μm in diameter.

Identification Digest 1 g of pulverized Condurango in 5 mL of water, and filter: the clear filtrate becomes turbid on heating, but becomes clear again upon cooling.

Purity Foreign matter <5.01>—The xylem and other foreign matter contained in Condurango do not exceed 2.0%.

Total ash <5.01> Not more than 12.0%.

Condurango Fluidextract

コンズランゴ流エキス

Method of preparation Take medium powder of Condurango, and prepare the fluidextract as directed under Fluidextracts using a suitable quantity of a mixture of Purified Water, Ethanol and Glycerin (5:3:2) as the first solvent, and a suitable quantity of a mixture of Purified Water and Ethanol (3:1) as the second solvent.

Description Condurango Fluidextract is a brown liquid. It has a characteristic odor and a bitter taste.

Identification Mix 1 mL of Condurango Fluidextract with 5 mL of water, filter, if necessary, and heat the clear solution: turbidity is produced. However, it becomes almost clear upon cooling.

Containers and storage Containers—Tight containers.

Coptis Rhizome

Coptidis Rhizoma

オウレン

Coptis Rhizome is the rhizome of *Coptis japonica* Makino, *Coptis chinensis* Franchet, *Coptis deltoidea* C.Y. Cheng et Hsiao or *Coptis teeta* Wallich (*Ranunculaceae*), from which the roots have been removed practically.

It contains not less than 4.2% of berberine [as berberine chloride ($\text{C}_{20}\text{H}_{18}\text{ClNO}_4$: 371.81)], calculated on the basis of dried material.

Description Irregular, cylindrical rhizome, 2 to 4 cm, rarely up to 10 cm in length, 0.2 – 0.7 cm in diameter, slightly curved and often branched; externally grayish yellow-brown, with ring nodes, and with numerous remains of rootlets; generally remains of petiole at one end; fractured surface rather fibrous; cork layer light grayish brown, cortex and pith are yellow-brown to reddish yellow-brown, xylem is yellow to

reddish yellow in color.

Odor, slight; taste, extremely bitter and lasting; it colors the saliva yellow on chewing.

Under a microscope <5.01>, a transverse section of Coptis Rhizome reveals a cork layer composed of thin-walled cork cells; cortex parenchyma usually exhibiting groups of stone cells near the cork layer and yellow phloem fibers near the cambium; xylem consisting chiefly of vessels, tracheae and wood fibers; medullary ray distinct; pith large; in pith, stone cells or stone cells with thick and lignified cells are sometimes recognized; parenchyma cells contain minute starch grains.

Identification (1) To 0.5 g of pulverized Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of pulverized Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution with yellow to yellow-green fluorescence show the same color tone and the same R_f value.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of pulverized Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (previously determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of berberine in each solution.

$$\begin{aligned} & \text{Amount (mg) of berberine [as berberine chloride} \\ & \text{(\text{C}_{20}\text{H}_{18}\text{ClNO}_4)]} \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Berberine Chloride Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer

(wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride Reference Standard and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of berberine is not more than 1.5%.

Powdered Coptis Rhizome

Coptidis Rhizoma Pulveratum

オウレン末

Powdered Coptis Rhizome is the powder of Coptis Rhizome.

It contains not less than 4.2% of berberine [as berberine chloride ($\text{C}_{20}\text{H}_{18}\text{ClNO}_4$: 371.81)], calculated on the basis of dried material.

Description Powdered Coptis Rhizome occurs as a yellow-brown to grayish yellow-brown powder. It has a slight odor and an extremely bitter, lasting taste, and colors the saliva yellow on chewing.

Under a microscope <5.01>, almost all elements are yellow in color; it reveals mainly fragments of vessels, tracheids and xylem fibers; parenchyma cells containing starch grains; polygonal cork cells. Usually, round to obtuse polygonal stone cells and their groups, and phloem fibers, 10 - 20 μm in diameter, and fragments of their bundles. Sometimes, polygonal and elongated epidermal cells, originated from the petiole, having characteristically thickened membranes. Starch grains are single grains 1 - 7 μm in diameter.

Identification (1) To 0.5 g of Powdered Coptis Rhizome add 10 mL of water, allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid and 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) To 0.5 g of Powdered Coptis Rhizome add 20 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol,

water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot from the standard solution with yellow to yellow-green fluorescence show the same color tone and the same R_f value.

Purity (1) Phellodendron bark—Under a microscope <5.01>, crystal cell rows or mucilage masses are not observable. Stir 0.5 g of Powdered Coptis Rhizome with 2 mL of water: the solution does not become gelatinous.

(2) Curcuma—Place Powdered Coptis Rhizome on a filter paper, drop diethyl ether on it, and allow to stand. Remove the powder from the filter paper, and drop 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <5.01>, Powdered Coptis Rhizome does not contain gelatinized starch or secretory cells containing yellow-red resin.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Coptis Rhizome, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (previously determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of berberine in each solution.

$$\begin{aligned} &\text{Amount (mg) of berberine [as berberine chloride} \\ &(\text{C}_{20}\text{H}_{18}\text{ClNO}_4)] \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Berberine Chloride Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride Reference Standard and palmatine chloride in 10 mL of methanol. Proceed with 20 μL of this solution under

the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of berberine is not more than 1.5%.

Cornus Fruit

Corni Fructus

サンシュユ

Cornus Fruit is the sarcocarp of the pseudocarp of *Cornus officinalis* Siebold et Zuccarini (*Cornaceae*).

Description Flattened oblong, 1.5–2 cm in length, about 1 cm in width; externally dark red-purple to dark purple, lustrous, and with coarse wrinkles; a crack-like scar formed by removal of true fruit; a scar of calyx at one end, and a scar of peduncle at the other; soft in texture.

Odor, slight; taste, acid and slightly sweet.

Identification To 1 g of coarse cuttings of Cornus Fruit add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spots from the sample solution is the same with a red-purple spot from the standard solution in color tone and *R_f* value.

Purity (1) Foreign matter <5.01>—The amount of its peduncles and other foreign matter contained in Cornus Fruit does not exceed 2.0%.

(2) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Corydalis Tuber

Corydalis Tuber

エンゴサク

Corydalis Tuber is the tuber of *Corydalis turtchaninowii* Basser forma *yanhusuo* Y. H. Chou et C. C. Hsu (*Papaveraceae*).

It contains not less than 0.08% of dehydrocorydaline (as dehydrocorydaline nitrate), calculated on the basis of dried material.

Description Nearly flattened spherical, 1–2 cm in diameter, and with stem scar at one end; externally grayish yellow to grayish brown; hard in texture; fractured surface is yellow and smooth or grayish yellow-green in color and granular.

Almost odorless; taste, bitter.

Identification To 0.5 g of pulverized Corydalis Tuber add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes with occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: immediately, an orange-yellow precipitate is produced.

Loss on drying <5.01> Not more than 15.0%.

Total ash <5.01> Not more than 3.0%.

Component determination Weigh accurately about 1 g of powdered Corydalis Tuber, add 30 mL of a mixture of methanol and dilute hydrochloric acid (3:1), heat under a reflux condenser on a water bath for 30 minutes, and filter after cooling. To the residue add 15 mL of a mixture of methanol and dilute hydrochloric acid (3:1), and repeat the above procedure. Combine the filtrates so obtained, add a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of dehydrocorydaline nitrate for component determination, previously dried in a desiccator (silica gel) for not less than 1 hour, dissolve in a mixture of methanol and dilute hydrochloric acid (3:1) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 5 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of dehydrocorydaline in each solution.

Amount (mg) of dehydrocorydaline [as dehydrocorydaline nitrate $C_{22}H_{24}N_2O_7$]
 $= W_S \times (A_T/A_S) \times (1/4)$

W_S : Amount (mg) of dehydrocorydaline nitrate for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40 °C.

Mobile phase: Dissolve 17.91 g of disodium hydrogen phosphate dodecahydrate in 970 mL of water, and adjust to pH 2.2 with phosphoric acid. In this solution add 14.05 g of sodium perchlorate, dissolve, and add water to make exactly 1000 mL. To this solution add 450 mL of acetonitrile, then dissolve 0.20 g of sodium lauryl sulfate.

Flow rate: Adjust the flow rate so that the retention time of dehydrocorydaline is about 24 minutes.

System suitability—

System performance: Dissolve 1 mg each of dehydrocorydaline nitrate for component determination and berberine chloride in 20 mL of a mixture of water and acetonitrile (20:9). When the procedure is run with 5 μ L of

this solution under the above operating conditions, berberine and dehydrocorydaline are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 5 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of dehydrocorydaline is not more than 1.5%.

Cyperus Rhizome

Cyperi Rhizoma

コウブシ

Cyperus Rhizome is the rhizome of *Cyperus rotundus* Linné (*Cyperaceae*).

Description Fusiform rhizome, 1.5 – 2.5 cm in length, 0.5 – 1 cm in diameter; externally grayish brown to grayish blackish brown, with 5 to 8 irregular ring nodes, and with hair-like fiber bundles on each node; hard in texture. The transverse section red-brown to light yellow in color, with waxy luster; thickness of cortex approximately equal to or slightly smaller than the diameter of stele. Under a magnifying glass, a transverse section reveals fiber bundles as brown spots lined in rings along circumference; here and there in the cortex, vascular bundles appear as red-brown spots, and numerous secretory cells scattered as minute yellow-brown spots; in the stele, numerous vascular bundles scattered as spots or lines.

Characteristic odor and taste.

Total ash <5.01> Not more than 3.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized *Cyperus Rhizome*, provided that 1 mL of silicon resin is previously added on the sample in the flask: the volume of essential oil is not less than 0.3 mL.

Powdered Cyperus Rhizome

Cyperi Rhizoma Pulveratum

コウブシ末

Powdered *Cyperus Rhizome* is the powder of *Cyperus Rhizome*.

Description Powdered *Cyperus Rhizome* occurs as a light red-brown powder, and has a characteristic odor and taste.

Under a microscope <5.01>, Powdered *Cyperus Rhizome* reveals fragments of polygonal parenchyma cells, scalariform vessels, and seta-like fibers; a large quantity of starch, mostly gelatinized; an extremely small number of stone cells.

Purity Foreign matter—Under a microscope <5.01>, Powdered *Cyperus Rhizome* does not show extremely lignified cells, except stone cells, or crystals.

Total ash <5.01> Not more than 3.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered *Cyperus Rhizome* provided that 1 mL of silicon re-

sin is previously added on the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage Containers—Tight containers.

Digenea

Digenea

マケリ

Digenea is the whole algae of *Digenea simplex* C. Agardh (*Rhodomelaceae*).

Description Rounded, string-like algae, 2 – 3 mm in diameter; externally, dark red-purple to dark grayish red or grayish brown; a few branched rods irregularly forked, covered with short hairy twigs; calcified weeds and other small algae often attached.

Odor, seaweed-like; taste, disagreeable and slightly salty.

Identification To 5 g of *Digenea* add 50 mL of water, macerate between 50°C and 60°C for 1 hour, and filter while warm. Add 50 mL of water to the residue, macerate again between 50°C and 60°C for 1 hour, and filter while warm. Evaporate all the filtrate on a water bath to about 25 mL, and use this solution as the sample solution. Separately, dissolve 0.05 g of kainic acid in 10 mL of water, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the upper layer of a mixture of water, 1-butanol and acetic acid (100) (5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a water-saturated solution of ninhydrin in 1-butanol (1 in 500) upon the plate, and heat at 90°C for 10 minutes: the spots obtained from the sample solution and the standard solution show a light yellow color and the same *R_f* values.

Purity Foreign matter <5.01>—The amount of other algae in *Digenea* does not exceed 20.0%.

Loss on drying <5.01> Not more than 22.0%.

Acid-insoluble ash <5.01> Not more than 8.0%.

Daiokanzoto Extract

大黃甘草湯エキス

Daiokanzoto Extract contains not less than 3.5 mg and not more than 10.5 mg of sennoside A (C₄₂H₃₈O₂₀: 862.74) and not less than 9 mg and not more than 27 mg (for preparation prescribed 1 g of *Glycyrrhiza*) or not less than 18 mg and not more than 54 mg (for preparation prescribed 2 g of *Glycyrrhiza*) of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 4 g of *Rhubarb* and 1 g of *Glycyrrhiza*, or with 4 g of *Rhubarb* and 2 g of *Glycyrrhiza*.

Description Daiokanzoto Extract occurs as a brown powder. It has a characteristic odor and an astringent first then slightly sweet taste.

Identification (1) Rhubarb—To 1.0 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of rhein for thin-layer chromatography in 1 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the orange fluorescent spot from the standard solution.

(2) Glycyrrhiza—To 0.5 g of Daiokanzoto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Daiokanzoto Extract as directed in (4) in Extracts under the General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Daiokanzoto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 7.0% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%.

Assay (1) Sennoside A—Weigh accurately about 0.2 g of Daiokanzoto Extract, add 20 mL of ethyl acetate and 10 mL of water, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 20 mL of ethyl acetate, shake for 10 minutes, centrifuge, and remove the upper layer. To the water layer add 10 mL of methanol, shake for 30 minutes, centrifuge, and take the supernatant liquid. To the residue add 20 mL of diluted methanol (1 in 2), shake for 5 minutes, centrifuge, and take the supernatant liquid. Combine these supernatant liquids, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 5 mg of Sennoside A Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 200 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography

<2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of sennoside A.

$$\begin{aligned} \text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) \\ = W_S \times (A_T/A_S) \times (1/4) \end{aligned}$$

W_S : Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (2460:540:1).

Flow rate: 1.0 mL/min (the retention time of sennoside A is about 14 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of sennoside A are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

(2) Glycyrrhizic acid—Use the sample solution obtained in the Assay (1) as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid.

$$\begin{aligned} \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7)

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and

not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Dioscorea Rhizome

Dioscoreae Rhizoma

サンヤク

Dioscorea Rhizome is the rhizome (rhizophore) of *Dioscorea japonica* Thunberg or *Dioscorea batatas* Decaisne (*Dioscoreaceae*), from which the periderm has been removed.

Description Cylindrical or irregular cylindrical rhizome, 5 – 15 cm in length, 1 – 4 cm in diameter, occasionally longitudinally split or transversely cut; externally whitish to yellowish white; fractured surface, whitish, smooth and powdery; hard in texture but breakable.

Practically odorless and tasteless.

Identification (1) To the cut surface of *Dioscorea* Rhizome add dilute iodine TS dropwise: a dark blue color develops.

(2) To 0.2 g of pulverized *Dioscorea* Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add 0.5 mL of sulfuric acid carefully to make two layers: a red-brown to purple-brown color appears at the zone of contact.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Powdered Dioscorea Rhizome

Dioscoreae Rhizoma Pulveratum

サンヤク末

Powdered *Dioscorea* Rhizome is the powder of *Dioscorea* Rhizome.

Description Powdered *Dioscorea* Rhizome occurs as nearly yellowish white to white; odorless and tasteless.

Under a microscope <5.01>, *Dioscorea* rhizome powder reveals starch grains; fragments of parenchyma cells containing starch grains; raphides of calcium oxalate, 100 to 200 μ m in length and its containing mucilage cells; ring and scalariform vessels, 15 to 35 μ m in diameter; starch grain isosceles deltoid or oblong, solitary, 18 to 35 μ m, hilum and striation being distinct.

Identification To 0.2 g of Powdered *Dioscorea* Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 to 3 minutes, and filter. To the filtrate add 0.5 mL of acetic anhydride, shake, and add carefully 0.5 mL of sulfuric acid to

make two layers: a red-brown to purple-brown color develops at the zone of contact.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Dolichos Seed

Dolichi Semen

ヘンズ

Dolichos Seed is the seed of *Dolichos lablab* Linné (*Leguminosae*).

Description Flattened ellipsoidal to flattened orbicular-ovate seed, 9 – 14 mm in length, 6 – 10 mm in width, 4 – 7 mm in thickness; externally light yellowish white to light yellow, smooth and somewhat lustrous; caruncle white, like a half-moon, protrudent at one side; hard in texture.

Almost odorless; taste, slightly sweet and acid.

Under a microscope <5.01>, a transverse section reveals the outermost layer of seed coat composed of a single layer of palisade like epidermal cells coated with cuticle; beneath epidermis a single layer of sclerenchymatous and sandglass like cells; inside of the layer mentioned above parenchyma lie, the innermost portion of the parenchyma decayed; cotyledons occur inside of the seed coat; the outermost layer of cotyledon composed of a single layer of epidermal cells, inner part of cotyledon mainly parenchyma, containing aleurone grains and oil drops, and occasionally starch grains.

Identification Put about 3 g of pulverized *Dolichos* Seed in a centrifuge tube, add 30 mL of methanol, shake for 10 minutes, centrifuge, and take the supernatant liquid. Evaporate the solvent of the supernatant liquid, add 30 mL of water and 50 mL of ethyl acetate to the residue, shake, and take the ethyl acetate layer. To the ethyl acetate add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the solvent of the filtrate, add 1 mL of ethyl acetate to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and acetic acid (100) (100:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a blue-white fluorescent spot appears around *R_f* 0.4.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 4.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 9.0%.

Eleutherococcus Senticosus Rhizome

Eleutherococci senticosi Rhizoma

シゴカ

Eleutherococcus Senticosus Rhizome is the rhizomes of *Eleutherococcus senticosus* (Ruprecht et Maximowicz) Maximowicz (*Acanthopanax senticosus* (Ruprecht et Maximowicz) Harms) (*Araliaceae*), often with root.

Description Slightly curved subcolumnar rhizome, 15–30 cm in length, 1–2.5 cm in diameter; externally grayish brown and slightly rough; transversely cut surface light brown, cortex thin, xylem thick with a pith in center; extremely hard in texture.

Odor, slightly characteristics; tasteless or slightly sweet, astringency.

Under a microscope <5.01>, a transverse section reveals the outermost layer consisting of a cork layer 3–7 cells thick; oil canals scattered in parenchyma; fiber bundles lined stepwise in phloem; phloem and xylem separated clearly by cambium; xylem composed of vessels, xylem fibers and xylem parenchyma; ray composed of 2–6 rows of cells; pith composed of parenchyma; parenchyma of cortex and ray contain aggregate crystals of calcium oxalate; occasionally starch grains in ray, parenchyma of cortex and xylem.

Identification To about 0.5 g of pulverized Eleutherococcus Senticosus Rhizome add 20 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of eleutheroside B for liquid chromatography in diluted methanol (1 in 2) to make 20 mL. To 2 mL of this solution add diluted methanol (1 in 2) to make 20 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions: the peak correspond to eleutheroside B shows the same retention time with that obtained with the standard solution.

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 265 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of eleutheroside B is about 10 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of eleutheroside B are not less than 5000 and not more than 1.5, respectively.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 2.5%.

Ephedra Herb

Ephdrae Herba

マオウ

Ephedra Herb is the terrestrial stem of *Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C.A. Meyer or *Ephedra equisetina* Bunge (*Ephedraceae*).

Ephedra Herb, when dried, contains not less than 0.7% of total alkaloids [as ephedrine (C₁₀H₁₅NO: 165.23) and pseudoephedrine (C₁₀H₁₅NO: 165.23)].

Description Thin cylindrical or ellipsoidal cylinder, 0.1–0.2 cm in diameter; 3–5 cm in length of internode; light green to yellow-green; numerous parallel vertical furrows on the surface; scaly leaves at the node portion; leaves, 0.2–0.4 cm in length, light brown to brown in color, usually being opposite at every node, adhering at the base to form a tubular sheath around the stem. Under a magnifying glass, the transverse section of the stem appears as circle and ellipse, the outer portion grayish green to yellow-green in color, and the center filled with a red-purple substance or hollow. When fractured at internode, the outer part is fibrous and easily split vertically.

Odor, slight; taste, astringent and slightly bitter, giving a slight sensation of numbness on the tongue.

Identification To about 0.5 g of pulverized Ephedra Herb add 10 mL of methanol, shake for 2 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly a solution of ninhydrin in ethanol (95) (1 in 50), and heat the plate at 105°C for 5 minutes: a red-purple spot appears near R_f 0.35.

Purity (1) Woody stem—The amount of the woody stems contained in Ephedra Herb does not exceed 5.0%.

(2) Foreign matter <5.01>—Ephedra Herb does not contain stems of *Equisetaceae* or *Gramineae* plants, or any other foreign matter.

Total ash <5.01> Not more than 11.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of medium powder of Ephedra Herb, previously dried in a desiccator (silica gel) for 24 hours, in a glass-stoppered centrifuge tube, add 20 mL of diluted methanol (1 in 2), shake for 30 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 20-mL portion of diluted

methanol (1 in 2). Combine all the extracts, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 50 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 20 mL. Pipet 2 mL of the solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP} , of ephedrine and pseudoephedrine (the relative retention time to ephedrine is about 0.9) in the sample solution, and the peak area, A_S , of ephedrine in the standard solution.

Amount (mg) of total alkaloids (ephedrine and pseudoephedrine)

$$= W_S \times \{(A_{TE} + A_{TP})/A_S\} \times (1/10) \times 0.819$$

W_S : Amount (mg) of ephedrine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 45°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 128), acetonitrile and phosphoric acid (640:360:1).

Flow rate: Adjust the flow rate so that the retention time of ephedrine is about 14 minutes.

Selection of column: Dissolve 1 mg of ephedrine hydrochloride for assay and 4 mg of Atropine Sulfate in diluted methanol (1 in 2) to make 100 mL. Perform the test with 10 μ L of this solution under the above operating conditions. Use a column giving elution of ephedrine and atropine in this order, clearly separating each peak.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

Epimedium Herb

Epimedii Herba

インヨウカク

Epimedium Herb is the terrestrial part of *Epimedium pubescens* Maximowicz, *Epimedium brevicornum* Maximowicz, *Epimedium wushanense* T. S. Ying, *Epimedium sagittatum* Maximowicz, *Epimedium koreanum* Nakai, *Epimedium grandiflorum* Morren var. *thunbergianum* Nakai or *Epimedium sempervirens* Nakai (*Berberidaceae*).

Description Epimedium Herb is composed of a stem and a ternate to triternate compound leaf; leaflet ovate to broadly

ovate or ovate-lanceolate, 3–20 cm in length, 2–8 cm in width, petiolule 15–70 mm in length, apex of leaflet acuminate, needle hair on margin 0.1–0.2 cm in length, base of leaflet cordate to deeply cordate, lateral leaflet asymmetry; upper surface green to greenish brown, sometimes lustrous, lower surface light green, often pilose, especially on vein densely pilose, papery or coriaceous; petiole and stem cylindrical, light yellowish brown to slightly purplish and light greenish brown, easily broken.

Odor, slight; taste, slightly bitter.

Under a microscope <5.01>, a transverse section of the leaf reveals 3–6 vascular bundles in midvein; mesophyll composed of upper epidermis, single-layered palisade, spongy tissue and lower epidermis; leaf margins orbicular or oblong, sclerenchymatous; multi-cellular hairs on epidermis; 8–20 vascular bundles in petiole and 6–15 vascular bundles in petiolule. Under a microscope <5.01>, a transverse section of the stem reveals a single to several-layered hypodermis, cortex of 4–10 layers of sclerenchymatous cells, vascular bundle 13–30 in number, oblong to obovate.

Identification To 2 g of pulverized Epimedium Herb add 20 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of icariin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and R_f value with the dark purple spot from the standard solution.

Loss on drying <5.01> Not more than 12.5% (6 hours).

Total ash <5.01> Not more than 8.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 17.0%.

Eucommia Bark

Eucommiae Cortex

トチュウ

Eucommia Bark is the bark of *Eucommia ulmoides* Oliver (*Eucommiaceae*).

Description Eucommia Bark is a semi-tubular or plate-like bark, 2 to 6 mm in thickness; externally pale grayish brown to grayish brown, and rough in texture, sometimes reddish-brown due to the cork layer falling off; internally dark violet, smooth and covered with a linear pattern that runs longitudinally, silk-like threads of gutta-percha (a thermoplastic rubber-like substance) appearing when broken.

Odor faint but distinctive; taste slightly sweet.

Under a microscope <5.01>, transverse section reveals

parenchymatous cells containing gutta-percha; phloem with stone-cell and fiber layers; rays in rows of 2 – 3 cells; calcium oxalate crystals absent.

Identification Put 1 g of pulverized Eucommia Bark in a glass-stoppered centrifuge tube, add 10 mL of water and 20 mL of diethyl ether, shake for 15 minutes, and centrifuge. Take the diethyl ether layer so obtained, evaporate the diethyl ether on a water bath, and add 1 mL of ethanol (99.5) to the residue: colloidal substances appear.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 7.0%.

Evodia Fruit

Evodiae Fructus

ゴシユコ

Evodia Fruit is the fruit of *Evodia rutaecarpa* Benth or *Evodia officinalis* Dode or *Evodia bodinieri* Dode (*Rutaceae*).

Description Flattened spheroidal or globular fruit, 2 – 5 mm in diameter; externally dark brown to grayish brown, with many oil sacs appearing as hollow pits, and often with peduncle, 2 – 5 mm in length, covered densely with hairs; matured pericarp split to reveal five loculi, and each loculus containing obovoid or globular seeds of a lustrous brown to blackish brown or bluish black color.

Odor, characteristic; taste, acrid, followed by a lasting bitterness.

Identification To 1.0 g of pulverized Evodia Fruit add 20 mL of methanol, heat for 5 minutes on a water bath, cool, and filter. Evaporate the filtrate to dryness, add 3 mL of dilute acetic acid to the residue, warm for 2 minutes on a water bath, cool, and filter. Perform the following tests using the filtrate as the sample solution.

(1) Spot one drop of the sample solution on a filter paper, air-dry, spray Dragendorff's TS for spraying, and allow to stand: a yellow-red color develops.

(2) To 0.2 mL of the sample solution add 0.8 mL of dilute acetic acid. To this solution add gently 2 mL of 4-dimethylaminobenzaldehyde TS, and warm in a water bath: a purple-brown ring develops at the zone of contact.

Purity (1) Peduncle—The amount of peduncles contained in Evodia Fruit does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than peduncles contained in Evodia Fruit does not exceed 1.0%.

Total ash <5.01> Not more than 8.0%.

Fennel

Foeniculi Fructus

ウイキヨウ

Fennel is the fruit of *Foeniculum vulgare* Miller (*Umbelliferae*).

Description Cylindrical cremocarp, 3.5 – 8 mm in length, 1 – 2.5 mm in width; externally grayish yellow-green to grayish yellow; two mericarps closely attached with each other, and with five longitudinal ridges; cremocarp often with pedicel 2 – 10 mm in length.

Characteristic odor and taste.

Under a microscope <5.01>, ridges near the bentral side are far protruded than those on the dorsal side; one large oil canal between each ridge, and two oil canals on the bentral side.

Identification To 0.5 g of pulverized Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a main spot with a dark purple color appears at the *R_f* value of about 0.4.

Purity (1) Peduncle—The amount of peduncles contained in Fennel does not exceed 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the peduncle contained in Fennel does not exceed 1.0%.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Fennel: the volume of essential oil is not less than 0.7 mL.

Powdered Fennel

Foeniculi Fructus Pulveratus

ウイキヨウ末

Powdered Fennel is the powder of Fennel.

Description Powdered Fennel occurs as a greenish pale brown to greenish brown, and is a characteristic odor and taste.

Under a microscope <5.01>, Powdered Fennel reveals fragments of parenchyma cells of perisperm containing aleurone grain, fragments of parenchyma cells of endosperm containing fatty oil, fragments of sclerenchyma with characteristic single pits, fragments of oil canal within yellowish brown material, fragments of endocarp shown scalariform, spiral

vessels, epidermis, stomata.

Identification To 0.5 g of Powdered Fennel add 10 mL of hexane, allow to stand for 5 minutes with occasional shaking, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate prepared with silica gel with fluorescent indicator for thin-layer chromatography. Then develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a main spot with dark purple color appears at the *Rf* value of about 0.4.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered Fennel: the volume of essential oil is not less than 0.45 mL.

Containers and storage Containers—Tight containers.

Fennel Oil

Oleum Foeniculi

ウイキョウ油

Fennel Oil is the essential oil distilled with steam from the fruit of *Foeniculum vulgare* Miller (*Umbelliferae*) or of *Illicium verum* Hooker fil. (*Illiciaceae*).

Description Fennel Oil is a colorless to pale yellow liquid. It has a characteristic, aromatic odor and a sweet taste with a slight, bitter aftertaste.

It is miscible with ethanol (95) and with diethyl ether.

It is practically insoluble in water.

When cold, white crystals or crystalline masses may often separate from the oil.

Identification Dissolve 0.30 g of Fennel Oil in 20 mL of hexane, pipet 1 mL of this solution, add hexane to make exactly 10 mL, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (20:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a main spot with a dark purple color appears at the *Rf* value of about 0.4.

Refractive index <2.45> n_D^{20} : 1.528 – 1.560

Specific gravity <1.13> d_{20}^{20} : 0.955 – 0.995

Purity (1) Clarity of solution—To 1.0 mL of Fennel Oil add 3 mL of ethanol (95): the solution is clear. To this solution add 7 mL of ethanol (95): the solution remains clear.

(2) Heavy metals <1.07>—Proceed with 1.0 mL of Fennel Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Foeniculated Ammonia Spirit

アンモニア・ウイキョウ精

Method of preparation

Ammonia Water	170 mL
Fennel Oil	30 mL
Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Medicated Spirits, with the above ingredients. A sufficient quantity of ammonia solution (28) and Purified Water may be used in place of Ammonia Water.

Description Foeniculated Ammonia Spirit is a colorless to yellow liquid, having a characteristic odor. It has a slightly sweet, pungent taste.

Specific gravity d_{20}^{20} : about 0.85

Alcohol number <1.01> Not less than 7.8 (Method 2).

Containers and storage Containers—Tight containers.

Forsythia Fruit

Forsythiae Fructus

レンギョウ

Forsythia Fruit is the fruit of *Forsythia suspensa* Vahl or *Forsythia viridissima* Lindley (*Oleaceae*).

Description Ovoid to long ovoid capsule, 1.5 – 2.5 cm in length, 0.5 – 1 cm in width, with acute apex, and sometimes with a peduncle at the base; externally light gray to dark brown, scattered with light gray and small ridged dots, and with two longitudinal furrows; a capsule dehiscing along the longitudinal furrows has the apex bent backward; the inner surface of dehisced pericarp is yellow-brown in color, with a longitudinal partition-wall in the middle; seeds, slender and oblong, 0.5 – 0.7 cm in length, and usually with a wing.

Odor, slight; tasteless.

Identification (1) To 0.2 g of pulverized Forsythia Fruit add 2 mL of acetic anhydride, shake well, allow to stand for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid to form two layers: a red-purple color develops at the zone of contact.

(2) To 1 g of pulverized Forsythia Fruit add 10 mL of methanol, warm on a water bath for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 1 mL of hydrochloric acid, and allow to stand: a light red to yellow-red color develops.

Purity (1) Branchlet—The amount of branchlets contained in Forsythia Fruit does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than branchlets contained in Forsythia Fruit does not exceed 1.0%.

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 10.0%.

Fritillaria Bulb

Fritillariae Bulbus

バイモ

Fritillaria Bulb is the bulb of *Fritillaria verticillata* Willdenow var. *thunbergii* Baker (*Liliaceae*).

Description Fritillaria Bulb is a depressed spherical bulb, 2 to 3 cm in diameter, 1 to 2 cm in height, consisting of 2 thickened scaly leaves often separated; externally and internally white to light yellow-brown in color; inside base is in a slightly dark color; the bulb sprinkled with lime before drying is dusted with white powder; fractured surface, white in color and powdery.

Odor, slight and characteristic; taste, bitter.

Under the microscope <5.01>, a transverse section reveals the outermost layer (epidermis) to be composed of a single layer of cells; numerous vascular bundles scattered throughout the parenchyma inside of the epidermis; parenchyma filled with starch grains; starch grains are mainly simple (rarely 2 – 3 composite), 5 – 50 μm in diameter, narrowly ovate to ovate or triangular to obovate, stratiform figure obvious; epidermal cells and parenchymatous cells near the vessels contain solitary crystals of calcium oxalate.

Identification Put 2 g of pulverized Fritillaria Bulb in a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake for 20 minutes, and centrifuge. Take the upper layer, add 20 g of anhydrous sodium sulfate to the layer, shake, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (17:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: two spots of a yellow-red color appear at around *R_f* 0.4 and at around *R_f* 0.6.

Loss on drying <5.01> Not more than 16.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.0%.

Gambir

Gambir

アセンヤク

Gambir is the dried aqueous extract prepared from the leaves and young twigs of *Uncaria gambir* Roxburgh (*Rubiaceae*).

Description Brown to dark brown, brittle mass; inside light brown.

Odor, slight; taste, extremely astringent and bitter.

Identification (1) To 0.2 g of pulverized Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2) Shake 0.1 g of pulverized Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 70.0%.

Powdered Gambir

Gambir Pulveratum

アセンヤク末

Powdered Gambir is the powder of Gambir.

Description Powdered Gambir occurs as a red-brown to dark brown powder. It has a slight odor, and an extremely astringent and bitter taste.

Under a microscope <5.01>, Powdered Gambir, immersed in olive oil or liquid paraffin, consists of needle crystalline masses or yellow-brown to red-brown angular fragments, and reveals epidermal tissue and thick-walled hairs.

Identification (1) To 0.2 g of Powdered Gambir add 10 mL of water, warm in a water bath for 5 minutes with occasional shaking, and filter. Cool the filtrate, and add 2 to 3 drops of gelatin TS: a white turbidity or precipitate is produced.

(2) Shake 0.1 g of Powdered Gambir with 20 mL of dilute ethanol for 2 minutes, and filter. Mix 1 mL of the filtrate with 9 mL of dilute ethanol, and to the solution add 1 mL of vanillin-hydrochloric acid TS: a light red to red-brown color develops.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 70.0%.

Gardenia Fruit

Gardeniae Fructus

サンシシ

Gardenia Fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*).

It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

Description Nearly long ovoid to ovoid fruit, 1–5 cm in length, 1–1.5 cm in width; usually having 6, rarely 5 or 7, markedly raised ridges; calyx or its scar at one end, and sometimes peduncle at the other end; inner surface of pericarp yellow-brown, smooth and lustrous; internally divided into two loculi, containing a mass of seeds in yellow-red to dark red placenta; seed nearly circular, flat, about 0.5 cm in major axis, blackish brown or yellow-red.

Odor, slight; taste, bitter.

Identification (1) To 1.0 g of pulverized Gardenia Fruit, previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate for component determination in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of pulverized Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Loss on drying <5.01> Not more than 13.0%.

Total ash <5.01> Not more than 6.0%.

Component determination Weigh accurately about 0.5 g of pulverized Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for

component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the peak areas of geniposide, *A_T* and *A_S*, of both solutions.

$$\text{Amount (mg) of geniposide} = W_S \times (A_T/A_S) \times 2$$

W_S: Amount (mg) of geniposide for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for component determination and caffeine in methanol to make 15 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Powdered Gardenia Fruit

Gardeniae Fructus Pulveratus

サンシシ末

Powdered Gardenia Fruit is the powder of Gardenia Fruit.

It contains not less than 3.0% of geniposide, calculated on the basis of dried material.

Description Powdered Gardenia Fruit occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope <5.01>, Powdered Gardenia Fruit reveals fragments of yellow-brown epidermis consisting of polygonal epidermal cells in surface view; unicellular hairs, spiral and ring vessels, stone cells often containing crystals of calcium oxalate; fragments of thin-walled parenchyma containing yellow pigments, oil drops and rosette aggregates of calcium oxalate (the above elements from fruit receptacle and pericarp); fragments of large and thick-walled epidermis of seed coat, containing a red-brown substance; fragments of endosperm filled with aleuron grains (the above elements from seed).

Identification (1) To 1.0 g of Powdered Gardenia Fruit,

previously dried in a desiccator (silica gel) for 24 hours, add 100 mL of hot water, warm the mixture between 60°C and 70°C for 30 minutes with frequent shaking, and filter after cooling. To 1.0 mL of the filtrate add water to make 10 mL: the color of the resulting solution is yellow and is not lighter than that of the following control solution.

Control solution: Dissolve 9.8 mg of carbazochrome sodium sulfonate for component determination in water to make exactly 10 mL. Pipet 1 mL of this solution, and add water to make exactly 50 mL.

(2) To 1.0 g of Powdered Gardenia Fruit add 20 mL of methanol, warm for 3 minutes on a water bath, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and methanol (3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 10 minutes: one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same in color tone and *R_f* value.

Loss on drying <5.01> Not more than 13.0%.

Total ash <5.01> Not more than 6.0%.

Component determination Weigh accurately about 0.5 g of Powdered Gardenia Fruit, transfer into a glass-stoppered centrifuge tube, add 40 mL of diluted methanol (1 in 2), shake for 15 minutes, centrifuge, and take the supernatant liquid. To the residue add 40 mL of diluted methanol (1 in 2), and repeat the same procedure as above. Combine the extracts so obtained, and add diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 20 mL, use this solution as the sample solution. Separately, weigh accurately about 10 mg of geniposide for component determination, previously dried in a desiccator (in vacuum, phosphorus (V) oxide) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 5 mL of the solution, add methanol to make exactly 10 mL, and use this solution as the standard solution. Perform the test with exactly 10 µL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the peak areas of geniposide, *A_T* and *A_S*, of both solutions.

$$\text{Amount (mg) of geniposide} = W_S \times (A_T/A_S) \times 2$$

W_S: Amount (mg) of geniposide for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 µm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (22:3).

Flow rate: Adjust the flow rate so that the retention time of geniposide is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of geniposide for component determination and caffeine in methanol to make 15 mL. When the procedure is run with 10 µL of this solution under the above operating conditions, caffeine and geniposide are eluted in this order with the resolution between these peaks being not less than 3.5.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

Gastrodia Tuber

Gastrodiae Tuber

テンマ

Gastrodia Tuber is the steamed tuber of *Gastrodia elata* Blume (*Orchidaceae*).

Description Gastrodia Tuber is an irregularly curved and flattened cylindrical to flattened fusiform tuber, 5 to 15 cm in length, 2 to 5 cm in diameter, 1 to 2 cm in thickness; externally light yellow-brown to light yellowish white; with ring nodes, and irregular longitudinal wrinkles; hard in texture; fractured surface, dark brown to yellow-brown in color, with luster, horny and gluey.

Odor, characteristic; practically tasteless.

Under a microscope <5.01>, a transverse section reveals parenchyma cells containing needle raphides of calcium oxalate; starch grain absent.

Identification To 1 g of pulverized Gastrodia Tuber add 5 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 1 mL of methanol, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 µL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 1 minutes: a red-purple spot appears at around *R_f* 0.4.

Loss on drying <5.01> Not more than 16.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 16.0%.

Gentian

Gentiana Radix

ゲンチアナ

Gentian is the root and rhizome of *Gentiana lutea* Linné (*Gentianaceae*).

Description Nearly cylindrical pieces, 10 – 50 cm in length, 2 – 4 cm in diameter; externally dark brown; the rhizome short, with fine, transverse wrinkles, and sometimes with buds and remains of leaves at the upper edge. The root longitudinally and deeply wrinkled, and more or less twisted; fractured surface yellow-brown and not fibrous, and a cambium and its neighborhood tinged dark brown.

Odor, characteristic; taste, sweet at first, later persistently bitter.

Under a microscope <5.01>, a transverse section of the root reveals several layers of collenchyma adjoined internally to 4 to 6 layers of thin-walled cork; secondary cortex of the parenchyma with irregularly distributed phloem; xylem consisting chiefly of parenchyma, with individual or clustered vessels and tracheids, and exhibiting some sieve tubes of xylem; parenchyma of the xylem and the cortex containing oil droplets, minute needle crystals of calcium oxalate and very rarely starch grains 10 – 20 μm in diameter.

Identification (1) Place 0.1 g of pulverized Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide, and heat gently and gradually: pale yellow crystals are sublimed on the upper slide. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of pulverized Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Powdered Gentian

Gentiana Radix Pulverata

ゲンチアナ末

Powdered Gentian is the powder of Gentian.

Description Powdered Gentian occurs as a yellowish brown powder, and has a characteristic odor. It has a sweet taste at first, which later becomes persistently bitter.

Under a microscope <5.01>, Powdered Gentian reveals parenchyma cells containing oil droplets and minute needle crystals, vessels, tracheids, cork tissues, and crystals of calcium oxalate. Vessels are chiefly reticulate vessels and scalariform vessels, 20 – 80 μm in diameter. Starch grains are observed very rarely, in simple grains about 10 – 20 μm in diameter.

Identification (1) Place 0.1 g of Powdered Gentian, previously dried in a desiccator (silica gel) for 48 hours, on a slide glass, put a glass ring 10 mm in both inside diameter and in height on it, then cover with another slide glass, and heat gently and gradually: light yellow crystals are sublimed on the upper glass. The crystals are insoluble in water and in ethanol (95), and soluble in potassium hydroxide TS.

(2) To 0.5 g of Powdered Gentian add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity Foreign matter—Under a microscope <5.01>, no stone cell or fiber is observed.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Containers and storage Containers—Tight containers.

Gentian and Sodium Bicarbonate Powder

ゲンチアナ・重曹散

Method of preparation

Powdered Gentian	300 g
Sodium Bicarbonate	700 g
To make 1000 g	

Prepare as directed under Powders, with the above ingredients.

Description Gentian and Sodium Bicarbonate Powder occurs as a light yellow-brown powder, and has a bitter taste.

Identification (1) To 2 g of Gentian and Sodium Bicarbonate Powder add 10 mL of water, stir, and filter: the filtrate responds to the Qualitative Tests <1.09> (1) for bicarbonate.

(2) To 1.5 g of Gentian and Sodium Bicarbonate Powder add 10 mL of methanol, shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Containers and storage Containers—Well-closed containers.

Geranium Herb

Geranii Herba

ゲンノシヨウコ

Geranium Herb is the terrestrial part of *Geranium thunbergii* Siebold et Zuccarini (*Geraniaceae*).

Description Stem with leaves opposite; stem, slender and long, green-brown; stem and leaf covered with soft hairs; leaf divided palmately into 3 to 5 lobes, and 2–4 cm in length, grayish yellow-green to grayish brown; each lobe oblong to obovate, and its upper margin crenate.

Odor, slight; taste, astringent.

Identification Boil 0.1 g of Geranium Herb with 10 mL of water, filter, and to the filtrate add 1 drop of iron (III) chloride TS: a dark blue color develops.

Purity Foreign matter <5.01>—The amount of the root and other foreign matter contained in Geranium Herb does not exceed 2.0%.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

Powdered Geranium Herb

Geranii Herba Pulverata

ゲンノシヨウコ末

Powdered Geranium Herb is the powder of Geranium Herb.

Description Powdered Geranium Herb occurs as a grayish green to light yellow-brown powder. It has a slight odor and an astringent taste.

Under a microscope <5.01>, Powdered Geranium Herb reveals mainly fibers, spiral vessels, pitted vessels, and unicellu-

lar hairs; furthermore, multicellular glandular hairs, epidermis with stomata, fragments of palisade tissue, rosette aggregates of calcium oxalate, and starch grains. Fiber is thick-walled, with somewhat distinct pits; unicellular hair shows small point-like protrusions on the surface; palisade tissue consisting of circular parenchyma cells in surface view, each cell containing one rosette aggregate of calcium oxalate which is about 20 μm in diameter. Starch grains consisting of simple grains but rarely of 2-compound grains, ovoid to spherical, 5–30 μm in diameter, with distinct hilum.

Identification Boil 0.1 g of Powdered Geranium Herb with 10 mL of water, filter, and to the filtrate add 1 drop of iron (III) chloride TS: a dark blue color develops.

Purity Foreign matter—Under a microscope <5.01>, Powdered Geranium Herb reveals no stone cells.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 15.0%.

Ginger

Zingiberis Rhizoma

シヨウキヨウ

Ginger is the rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae*).

Description Irregularly compressed and often branched massive rhizome or a part of it; the branched parts are slightly curved ovoid or oblong-ovoid, 2–4 cm in length, and 1–2 cm in diameter; external surface grayish white to light grayish brown, and often with white powder; fractured surface is somewhat fibrous, powdery, light yellowish brown; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles and secretes scattered all over the surface as small dark brown dots.

Odor, characteristic; taste, extremely pungent.

Identification To 2 g of pulverized Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spots from the sample solution and a green spot from the standard solution show the same color tone and *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Ginger according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 8.0%

Powdered Ginger

Zingiberis Rhizoma Pulveratum

ショウキョウ末

Powdered Ginger is the powder of Ginger.

Description Powdered Ginger occurs as a light grayish brown to light grayish yellow powder. It has a characteristic odor and an extremely pungent taste.

Under a microscope <5.01>, Powdered Ginger reveals mainly starch grains and parenchyma cells containing them; also, parenchyma cells containing yellow-brown to dark brown resinous substances or single crystals of calcium oxalate; fragments of fibers with distinct pits; fragments of spiral, ring and reticulate vessels, and rarely fragments of cork tissue; starch grains composed of simple, compound or half-compound grains, spherical, ovoid or globular, with abaxial hilum, usually 20 – 30 μm in long axis.

Identification To 2 g of Powdered Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spots from the sample solution and a green spot from the standard solution show the same color tone and *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Ginger according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Ginger according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Ginger does not show stone cells, lignified parenchyma cells and other foreign matter.

Total ash <5.01> Not more than 8.0%.

Containers and storage Containers—Tight containers.

Ginseng

Ginseng Radix

ニンジン

Ginseng is the root of *Panax ginseng* C. A. Meyer (*Panax schinseng* Nees) (*Araliaceae*), from which rootlets have been removed, or the root that has been quickly passed through hot water.

It contains not less than 0.10% of ginsenoside Rg₁ (C₄₂H₇₂O₁₄: 801.01) and not less than 0.20% of ginsenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29), calculated on the basis of dried material.

Description Thin and long cylindrical to fusiform root, often branching 2 to 5 lateral roots from the middle; 5 – 20 cm in length, main root 0.5 – 3 cm in diameter; externally light yellow-brown to light grayish brown, with longitudinal wrinkles and scars of rootlets; sometimes crown somewhat constricted and with short remains of rhizome; fractured surface practically flat, light yellow-brown in color, and brown in the neighborhood of the cambium.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

Identification (1) On a section of Ginseng add dilute iodine TS dropwise: a dark blue color is produced on the surface.

(2) To 2.0 g of pulverized Ginseng add 20 mL of methanol, boil gently under a reflux condenser on a water bath for 15 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rg₁ Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mixture of chloroform, methanol and water (13:7:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 5 minutes: one of the spots from the sample solution and a red-purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Foreign matter <5.01>—The amount of stems and other foreign matter contained in Ginseng does not exceed 2.0%.

(2) Heavy metals <1.07>—Proceed with 1.0 g of pulverized Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of pulverized Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 4.2%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 14.0%.

Assay (1) Ginsenoside Rg₁—Weigh accurately about 1.0 g of pulverized Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg₁ Reference Standard (previously determine the water) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rg₁.

Amount (mg) of ginsenoside Rg₁ (C₄₂H₇₂O₁₄) = W_S × (A_T/A_S)

W_S: Amount (mg) of Ginsenoside Rg₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rg₁ is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg₁ Reference Standard and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg₁ and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg₁ is not more than 1.5%.

(2) Ginsenoside Rb₁—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb₁ Reference Standard (previously determine the water) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb₁.

Amount (mg) of ginsenoside Rb₁ (C₅₄H₉₂O₂₃) = W_S × (A_T/A_S)

W_S: Amount (mg) of Ginsenoside Rb₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rb₁ is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb₁ Reference Standard and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb₁ and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb₁ is not more than 1.5%.

Powdered Ginseng

Ginseng Radix Pulverata

ニンジン末

Powdered Ginseng is the powder of Ginseng.

It contains not less than 0.10% of ginsenoside Rg₁ (C₄₂H₇₂O₁₄: 801.01) and not less than 0.20% of ginsenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29), calculated on the basis of dried material.

Description Powdered Ginseng occurs as a light yellowish white to light yellowish-brown powder. It has characteristic odor and is a slight sweet taste followed by a slight bitterness.

Under a microscope <5.01>, Powdered Ginseng reveals round to rectangular parenchyma cells containing starch grains, occasionally gelatinized starch, vessels, secretory cell, sclerenchyma cell, big and thin-walled cork cell; crystals of calcium oxalate and starch. Vessel are reticulate vessel, 45 μm in diameter; scalariform vessel and spiral vessel, 15 to 40 μm in diameter. Secretory cell containing a mass of yellow glistened contents; rosette aggregate of calcium oxalate, 20 to 50 μm in diameter, and 1 to 5 μm in diameter, rarely 10 μm, in diameter. Starch grains are observed in simple grain and 2 to 4-compound grain, simple grain, 3 to 15 μm in diameter.

Identification To 2.0 g of Powdered Ginseng add 20 mL of methanol, boil gently under a reflux condenser on a water bath for 15 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rg₁ Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mix-

ture of chloroform, methanol and water (13:7:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 5 minutes: one of the spots from the sample solution and a red-purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 1.0 g of Powdered Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 1.0 g of Powdered Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(3) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 4.2%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Extract content <5.01> Dilute ethanol-soluble extract; not less than 14.0%.

Assay (1) Ginsenoside Rg₁—Weigh accurately about 1.0 g of Powdered Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg₁ Reference Standard (previously determine the water), and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of ginsenoside Rg₁.

Amount (mg) of ginsenoside Rg₁ (C₄₂H₇₂O₁₄) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of Ginsenoside Rg₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rg₁ is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg₁ Reference Standard and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating condi-

tions, ginsenoside Rg₁ and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg₁ is not more than 1.5%.

(2) Ginsenoside Rb₁—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb₁ Reference Standard, separately determined its water content, dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of ginsenoside Rb₁.

Amount (mg) of ginsenoside Rb₁ (C₅₄H₉₂O₂₃) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of Ginsenoside Rb₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rb₁ is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb₁ Reference Standard and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb₁ and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb₁ is not more than 1.5%.

Containers and storage Containers—Tight containers.

Glehnia Root

Glehniae Radix cum Rhizoma

ハマボウフウ

Glehnia Root is the root and rhizome of *Glehnia littoralis* Fr. Schmidt ex Miquel (*Umbelliferae*).

Description Cylindrical to long conical root or rhizome, 10–20 cm in length, 0.5–1.5 cm in diameter; externally light yellow-brown to red-brown. Rhizome short, with fine ring nodes; roots having longitudinal wrinkles and numerous, dark red-brown, warty protrusions or transversely elongated

protuberances. Brittle and easily breakable. A transverse section white and powdery, and under a magnifying glass, oil canals scattered as brown dots.

Odor, slight; taste, slightly sweet.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Glycyrrhiza

Glycyrrhizae Radix

カンゾウ

Glycyrrhiza is the root and stolon, with (unpeeled) or without (peeled) the periderm, of *Glycyrrhiza uralensis* Fisher or *Glycyrrhiza glabra* Linné (*Leguminosae*).

It contains not less than 2.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Nearly cylindrical pieces, 0.5–3.0 cm in diameter, over 1 m in length. Glycyrrhiza is externally dark brown to red-brown, longitudinally wrinkled, and often has lenticels, small buds and scaly leaves; peeled Glycyrrhiza is externally light yellow and fibrous. The transverse section reveals a rather clear border between phloem and xylem, and a radial structure which often has radiating splits; a pith in Glycyrrhiza originated from stolon, but no pith from root.

Odor, slight; taste, sweet.

Under a microscope <5.01>, the transverse section reveals several layers of yellow-brown cork layers, and 1- to 3-cellular layer of cork cortex inside the cork layer; the cortex exhibiting medullary rays and obliterated sieve portions radiated alternately; the phloem exhibiting groups of phloem fibers with thick but incompletely lignified walls and surrounded by crystal cells; peeled Glycyrrhiza some times lacks periderm and a part of phloem; the xylem exhibiting large yellow vessels and medullary rays in 3 to 10 rows radiated alternately; the vessels accompanied with xylem fibers surrounded by crystal cells, and with xylem parenchyma cells; the parenchymatous pith only in Glycyrrhiza originated from stolon. The parenchyma cells contain starch grains and often solitary crystals of calcium oxalate.

Identification To 2 g of pulverized Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizic Acid Reference Standard in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of pulverized Glycyrrhiza in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid Reference Standard (previously determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 μ L each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, *A_T* and *A_S*, of glycyrrhizic acid of each solution.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S: Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

Selection of column: Dissolve 5 mg of Glycyrrhizic Acid Reference Standard and 1 mg of propyl parahydroxybenzoate in dilute ethanol to make 20 mL. Proceed with 20 μ L of this solution under the above operating conditions. Use a column giving elution of glycyrrhizic acid and propyl parahydroxybenzoate in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Powdered Glycyrrhiza

Glycyrrhizae Radix Pulverata

カンゾウ末

Powdered Glycyrrhiza is the powder of Glycyrrhiza.

It contains not less than 2.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93), calculated on the basis of dried material.

Description Powdered Glycyrrhiza is light yellow-brown or light yellow to grayish yellow (powder of peeled Glycyrrhiza) in color. It has a slight odor and a sweet taste.

Under a microscope <5.01>, Powdered Glycyrrhiza reveals mainly yellow sclerenchymatous fiber bundles accompanied with crystal cell rows; vessels, 80 to 200 μm in diameter, with pitted, reticulate and scalariform pits, and with round perforations; parenchyma cells, containing starch grains and solitary crystals of calcium oxalate, their fragments, and cork tissues; but powder of peeled Glycyrrhiza shows no cork tissue; if any, a very few. Starch grains are simple grains, 2–20 μm in diameter; simple grains of calcium oxalate, 10–30 μm in a diameter.

Identification To 2 g of Powdered Glycyrrhiza add 10 mL of a mixture of ethanol (95) and water (7:3), heat by shaking on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of Glycyrrhizic Acid Reference Standard in 1 mL of a mixture of ethanol (95) and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same R_f value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Glycyrrhiza according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Glycyrrhiza according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Glycyrrhiza shows no stone cells.

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 25.0%.

Assay Weigh accurately about 0.5 g of Powdered Glycyrrhiza

in a glass-stoppered centrifuge tube, add 70 mL of dilute ethanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 25 mL of dilute ethanol, and proceed in the same manner. Combine all the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 25 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Pipet 20 μL each of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of glycyrrhizic acid of each solution.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: Use a column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 mL in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (3:2).

Flow rate: Adjust the flow rate so that the retention time of glycyrrhizic acid is about 10 minutes.

Selection of column: Dissolve 5 mg of Glycyrrhizic Acid Reference Standard and 1 mg of propyl parahydroxybenzoate in dilute ethanol to make 20 mL. Proceed with 20 μL of this solution under the above operating conditions. Use a column giving elution of glycyrrhizic acid and propyl parahydroxybenzoate in this order, and clearly dividing each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Glycyrrhiza Extract

カンゾウエキス

Glycyrrhiza Extract contains not less than 4.5% of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93).

Method of preparation To 1 kg of fine cuttings of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza add 5 L of Water or Purified Water, and macerate for 2 days. Filter the macerated solution through a cloth filter. Add 3 L of Water or Purified Water to the residue, macerate again for 12 hours, and filter through a cloth filter. Evaporate the combined filtrates until the whole volume becomes 3 L. After cooling, add 1 L of Ethanol, and allow to stand in a cold place for 2 days. Filter, and evaporate the filtrate to a viscous extract.

Description Glycyrrhiza Extract is a brown to blackish brown, viscous extract, and has a characteristic odor and a sweet taste.

It dissolves in water, forming a clear solution, or with a slight turbidity.

Identification To 0.8 g of Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), shake for 2 minutes, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity Insoluble matter—Dissolve 2.0 g of Glycyrrhiza Extract in 18 mL of water, and filter. To 10 mL of the filtrate add 5 mL of ethanol (95): a clear solution results.

Assay Weigh accurately about 0.15 g of Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid Reference Standard (previously determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in Assay under Glycyrrhiza.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Crude Glycyrrhiza Extract

カンゾウ粗エキス

Glycyrrhiza Extract contains not less than 6.0% of glycyrrhizic acid ($\text{C}_{42}\text{H}_{62}\text{O}_{16}$: 822.93).

Method of preparation Boil coarse powder of Glycyrrhiza or the root and stolon of *Glycyrrhiza glabra* Linné (*Leguminosae*) which meets the requirement of Glycyrrhiza with Water or Purified Water, filter the solution under pressure, and evaporate the filtrate.

Description Crude Glycyrrhiza Extract occurs as lustrous, dark yellow-red to blackish brown plates, rods or masses. It is comparatively brittle when cold, and the fractured surface is dark yellow-red, shell-like, and lustrous. It softens when warmed.

It has a characteristic odor and a sweet taste.

It dissolves in water with turbidity.

Identification To 0.6 g of Crude Glycyrrhiza Extract add 10 mL of a mixture of ethanol (95) and water (7:3), dissolve by warming if necessary, cool, centrifuge, and use the supernatant liquid as the sample solution. Proceed as directed in the Identification under Glycyrrhiza.

Purity (1) Water-insoluble substances—Boil 5.0 g of pul-

verized Crude Glycyrrhiza Extract with 100 mL of water. After cooling, filter the mixture through tared filter paper, wash with water, and dry the residue at 105°C for 5 hours: the mass of the residue is not more than 1.25 g.

(2) Foreign matter—The filtrate obtained in (1) does not have a strong bitter taste.

(3) Starch—To about 1 g of pulverized Crude Glycyrrhiza Extract add water to make 20 mL, shake the mixture thoroughly, and filter. Examine the insoluble substance on the filter paper under a microscope: the residue contains no starch grains.

Total ash <5.01> Not more than 12.0% (1 g).

Assay Weigh accurately about 0.15 g of Crude Glycyrrhiza Extract, place in a glass-stoppered centrifuge tube, add 25 mL of dilute ethanol, and heat at 50°C for 30 minutes with occasional shaking. Cool, centrifuge, and separate the supernatant liquid. To the residue add 20 mL of dilute ethanol, and proceed in the same manner. Combine the extracts, add dilute ethanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 20 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in dilute ethanol to make exactly 100 mL, and use this solution as the standard solution. Proceed as directed in Assay under Glycyrrhiza.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Containers and storage Containers—Tight containers.

Gypsum

Gypsum Fibrosum

セッコウ

Gypsum is natural hydrous calcium sulfate. It possibly corresponds to the formula $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$.

Description Gypsum occurs as lustrous, white, heavy, fibrous, crystalline masses, which easily split into needles or very fine crystalline powder.

It is odorless and tasteless.

It is slightly soluble in water.

Identification To 1 g of pulverized Gypsum add 20 mL of water, allow to stand with occasional shaking for 30 minutes, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt and to the Qualitative Tests <1.09> for sulfate.

Purity (1) Heavy metals <1.07>—Boil 4.0 g of pulverized Gypsum with 4 mL of acetic acid (100) and 96 mL of water for 10 minutes, cool, add water to make exactly 100 mL, and filter. Perform the test using 50 mL of the filtrate as the test solution. Prepare the control solution as follows: to 4.0 mL of Standard Lead Solution add 2 mL of dilute acetic acid and water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Gypsum according to Method 2, and perform

the test (not more than 5 ppm).

Containers and storage Containers—Well-closed containers.

Exsiccated Gypsum

Gypsum Exsiccatum

焼セッコウ

Exsiccated Gypsum possibly corresponds to the formula $\text{CaSO}_4 \cdot \frac{1}{2}\text{H}_2\text{O}$.

Description Exsiccated Gypsum occurs as a white to grayish white powder. It is odorless and tasteless.

It is slightly soluble in water, and practically insoluble in ethanol (95).

It absorbs moisture slowly on standing in air to lose its solidifying property.

When it is heated to yield an anhydrous compound at a temperature above 200°C, it loses its solidifying property.

Identification Shake 1 g of Exsiccated Gypsum with 20 mL of water for 5 minutes, and filter: the filtrate responds to the Qualitative Tests <1.09> (2) and (3) for calcium salt and to the Qualitative Tests <1.09> for sulfate.

Purity Alkalinity—Take 3.0 g of Exsiccated Gypsum in a glass-stoppered test tube, add 10 mL of water and 1 drop of phenolphthalein TS, and shake vigorously: no red color develops.

Solidification To 10.0 g of Exsiccated Gypsum add 10 mL of water, stir immediately for 3 minutes, and allow to stand: the period until water no longer separates, when the material is pressed with a finger, is not more than 10 minutes from the time when the water was added.

Containers and storage Containers—Tight containers.

Hemp Fruit

Cannabis Fructus

マシニン

Hemp Fruit is the fruit of *Cannabis sativa* Linné (*Moraceae*).

Description Hemp Fruit is a slightly compressed void fruit, 4 to 5 mm in length, 3 to 4 mm in diameter; externally grayish green to grayish brown; pointed at one end, a scar of gynophore at the other end, and crest lines on both sides; outer surface lustrous with white mesh-like pattern; slightly hard pericarp; seed, slightly green in color and internally has grayish white albumen; 100 fruits weighing 1.6 to 2.7 g.

Practically odorless, aromatic on chewing; taste, mild and oily.

Under a microscope <5.01>, a transverse section reveals the exocarp to be a single-layered epidermis; mesocarp composed of parenchyma, a pigment cell layer and rows of short, small cells; endocarp made up of a layer of radially elongated stone cells; seed coat comprises a tubular cell layer and spongy tis-

sue. Inside of the seed; exosperm consists of one layer of parenchymatous cells, endosperm of one to several layers of parenchymatous cells; most of the embryo composed of parenchyma, vascular bundles occurring in the center of hypocotyls and cotyledons; embryo parenchyma contains aleurone grains and oil drops.

Identification To 0.3 g of pulverized Hemp Fruit add 3 mL of methanol, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (9:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: a dark blue-purple spot appears at around *R_f* 0.6.

Purity Bract—Hemp Fruit does not contain bract.

Loss on drying <5.01> Not more than 9.0% (6 hours).

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Hochuekkito Extract

補中益気湯エキス

Hochuekkito Extract contains not less than 16 mg and not more than 48 mg of hesperidin, not less than 0.3 mg and not more than 1.2 mg (for preparation prescribed 1 g of Bupleurum Root) or not less than 0.6 mg and not more than 2.4 mg (for preparation prescribed 2 g of Bupleurum Root) of saikosaponin b₂, and not less than 12 mg and not more than 36 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆; 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 0.5 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 3 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 2 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Cimicifuga Rhizome, or with 4 g of Ginseng, 4 g of Atractylodes Rhizome, 4 g of Astragalus Root, 3 g of Japanese Angelica Root, 2 g of Citrus Unshiu Peel, 2 g of Jujube, 1 g of Bupleurum Root, 1.5 g of Glycyrrhiza, 0.5 g of Processed Ginger and 0.5 g of Cimicifuga Rhizome.

Description Hochuekkito Extract occurs as a light brown to brown powder. It has a slight odor, and a sweet and bitter taste.

Identification (1) Ginseng—To 2.0 g of Hochuekkito Ex-

tract add 30 mL of water, shake, then add 50 mL of 1-butanol, and shake. Take the 1-butanol layer, evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rb₁ Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution.

(2) *Atractylodes rhizome* (for preparation prescribed *Atractylodes Rhizome*)—To 3.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the diethyl ether layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and 10 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the red spot from the standard solution.

(3) *Atractylodes lancea rhizome* (for preparation prescribed *Atractylodes Lancea Rhizome*)—To 2.0 g of Hochuekkito Extract add 10 mL of water, shake, then add 25 mL of hexane, shake, and take the hexane layer. To the hexane layer add anhydrous sodium sulfate to dry, filter, evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot appears around *R_f* 0.4, which shows a greenish brown color after spraying 4-dimethylaminobenzaldehyde TS for spraying, heating at 105°C for 5 minutes and allowing to cool.

(4) *Astragalus root*—To 3.0 g of Hochuekkito Extract add 40 mL of a solution of potassium hydroxide in methanol (1 in 50), shake for 15 minutes, centrifuge, and evaporate the supernatant liquid under reduced pressure. Add 30 mL of water to the residue, then add 20 mL of diethyl ether, shake, and take the water layer. To the water layer add 20 mL of 1-butanol, shake, and take the 1-butanol layer. To the 1-butanol layer add 20 mL of water, shake, take the 1-butanol layer, evaporate the layer under reduced pressure, add 1 mL of methanol to the residue, and use this solution as the sam-

ple solution. Separately, dissolve 1 mg of astragaloside IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of octadecylsilylated silica gel for thin-layer chromatography. Develop the plate with a mixture of methanol, water, 1-butanol and acetic acid (100) (60:30:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the red-brown spot from the standard solution.

(5) *Japanese angelica root*—To 3.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the diethyl ether under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*Z*)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-white fluorescent spot from the standard solution.

(6) *Citrus unshiu peel*—To 2.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of hesperidin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L of the sample solution and 20 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone, water and acetic acid (100) (10:6:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and expose to ammonia vapor: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue spot from the standard solution.

(7) *Bupleurum root*—To 2.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of 1-butanol, shake, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and 2 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the

spot among the several spots from the sample solution has the same color tone and *R_f* value with the red spot from the standard solution.

(8) Glycyrrhiza—To 2.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution.

(9) Ginger (for preparation prescribed Ginger)—To 3.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of diethyl ether, shake, and take the diethyl ether layer. Evaporate the diethyl ether under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution.

(10) Processed ginger (for preparation prescribed Processed Ginger)—Put 10 g of Hochuekkito Extract in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect an apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 60 μ L of the sample solution and 10 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of cyclohexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution.

(11) Cimicifuga rhizome—To 2.0 g of Hochuekkito Extract add 30 mL of water, shake, then add 50 mL of 1-butanol, and take the 1-butanol layer. Evaporate the layer

under reduced pressure, add 3 mL of methanol to the residue, and use this solution as the sample solution. Use 3-(3-hydroxy-4-methoxyphenyl)-2-(*E*)-propenic acid-(*E*)-ferulic acid TS for thin-layer chromatography as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution and 2 μ L of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, acetone and water (20:12:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow fluorescent spot from the standard solution.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Hochuekkito Extract as directed in (4) in Extracts, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Hochuekkito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 11.5% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 9.0%.

Assay (1) Hesperidin—Weigh accurately about 0.1 g of Hochuekkito Extract, add exactly 50 mL of diluted tetrahydrofuran (1 in 4), shake for 30 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 10 mg of hesperidin for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, add diluted tetrahydrofuran (1 in 4) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of peoniflorin.

$$\text{Amount (mg) of hesperidin} = W_S \times (A_T/A_S) \times (1/20)$$

W_S: Amount (mg) of hesperidin for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 285 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (82:18:1)

Flow rate: 1.0 mL/min. (the retention time of hesperidin is about 15 minutes.)

System suitability—

System performance: Dissolve 1 mg each of hesperidin for component determination and naringin for thin-layer chromatography in diluted methanol (1 in 2) to make 100 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, naringin and hesperidin are

eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of hespiridin is not more than 1.5%.

(2) Saikosaponin b_2 —Weigh accurately about 0.5 g of Hochuekkito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b_2 for component determination, previously dried in a desiccator (silica gel) for not less than 24 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of saikosaponin b_2 .

Amount (mg) of saikosaponin $b_2 = W_S \times (A_T/A_S) \times (1/20)$

W_S : Amount (mg) of saikosaponin b_2 for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3).

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b_2 is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Hochuekkito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
 $= W_S \times (A_T/A_S) \times (1/2)$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Stan-

dard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Honey

Mel

ハチミツ

Honey is the saccharine substances obtained from the honeycomb of *Apis mellifera* Linné or *Apis indica* Radoszkowski (*Apidae*).

Description Honey is a light yellow to light yellow-brown, syrupy liquid. Usually it is transparent, but often opaque with separated crystals.

It has a characteristic odor and a sweet taste.

Specific gravity <2.56> Mix 50.0 g of Honey with 100 mL of water: the specific gravity of the solution is not less than d_{20}^{20} : 1.111.

Purity (1) Acidity—Mix 10 g of Honey with 50 mL of water, and titrate <2.50> with 1 mol/L potassium hydroxide VS (indicator: 2 drops of phenolphthalein TS): not more than 0.5 mL is required.

(2) Sulfate—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 drops of barium chloride TS: the solution does not show any change immediately.

(3) Ammonia-coloring substances—Mix 1.0 g of Honey with 2.0 mL of water, and filter. To the filtrate add 2 mL of ammonia TS: the solution does not show any change immediately.

(4) Resorcinol-coloring substances—Mix well 5 g of Honey with 15 mL of diethyl ether, filter, and evaporate the diethyl ether solution at ordinary temperature. To the residue add 1 to 2 drops of resorcinol TS: a yellow-red color may develop in the solution of resorcinol and in the residue, and a red to red-purple color which does not persist more than 1

hour.

(5) Starch or dextrin—(i) Shake 7.5 g of Honey with 15 mL of water, warm the mixture on a water bath, and add 0.5 mL of tannic acid TS. After cooling, filter, and to 1.0 mL of the filtrate add 1.0 mL of ethanol (99.5) containing 2 drops of hydrochloric acid: no turbidity is produced.

(ii) To 2.0 g of Honey add 10 mL of water, warm in a water bath, mix, and allow to cool. Shake 1.0 mL of the mixture with 1 drop of iodine TS: no blue, green or red-brown color develops.

(6) Foreign matter—Mix 1.0 g of Honey with 2.0 mL of water, centrifuge the mixture, and examine the precipitate microscopically <5.01>: no foreign substance except pollen grains is observable.

Total ash <5.01> Not more than 0.4%.

Containers and storage Containers—Tight containers.

Houttuynia Herb

Houttuyniae Herba

ジュウヤク

Houttuynia Herb is the terrestrial part of *Houttuynia cordata* Thunberg (*Saururaceae*), collected during the flowering season.

Description Stem with alternate leaves and spikes; stem light brown, with longitudinal furrows and protruded nodes; when soaked in water and smoothed out, leaves wide ovate and cordate, 3–8 cm in length, 3–6 cm in width; light green-brown; margin entire, apex acuminate; petiole long, and membranous stipule at the base; spike, 1–3 cm in length, with numerous light yellow-brown achlamydeous florets, and the base enclosed by 4 long ovate, light yellow to light yellow-brown involucre.

Odor, slight; tasteless.

Identification Boil 2 g of pulverized Houttuynia Herb with 20 mL of ethyl acetate under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the filtrate to dryness, add 10 mL of water to the residue, warm the mixture on a water bath for 2 minutes, and, after cooling, filter. Shake well the filtrate with 20 mL of ethyl acetate in a separator, take 15 mL of ethyl acetate solution, and evaporate the solution on a water bath to dryness. Dissolve the residue in 5 mL of methanol, add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Purity Foreign matter <5.01>—The amount of the rhizome, roots and other foreign matter contained in Houttuynia Herb is not more than 2.0%.

Total ash <5.01> Not more than 14.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 10.0%.

Immature Orange

Aurantii Fructus Immaturus

キジツ

Immature Orange is the immature fruit or the fruit cut crosswise of *Citrus aurantium* Linné var. *daidai* Makino, *Citrus aurantium* Linné or *Citrus natsudaidai* Hayata (*Rutaceae*).

Description Nearly spherical fruit, 1–2 cm in diameter, or semispherical, 1.5–4.5 cm in diameter; external surface, deep green-brown to brown, and without luster, with numerous small dents associated with oil sacs; the outer portion of transverse section exhibits pericarp and mesocarp about 0.4 cm in thickness, yellow-brown in color in the region contacting epidermis, and light grayish brown color in the other parts; the central portion is radially divided into 8 to 16 small loculi; each loculus is brown and indented, often containing immature seeds.

Odor, characteristic; taste, bitter.

Identification To 0.5 g of pulverized Immature Orange add 10 mL of methanol, boil gently for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium ribbon and 1 mL of hydrochloric acid, and allow to stand: a red-purple color develops.

Total ash <5.01> Not more than 7.0%.

Imperata Rhizome

Imperatae Rhizoma

ポウコン

Imperata Rhizome is the rhizome of *Imperata cylindrica* Beauvois (*Gramineae*), from which rootlets and scale leaves have been removed.

Description Long and thin cylindrical rhizome, 0.3–0.5 cm in diameter; sometimes branched; externally yellowish white, with slight longitudinal wrinkles, and with nodes at 2–3-cm intervals; difficult to break; fractured surface fibrous. Cross section irregularly round; thickness of cortex is slightly smaller than the diameter of the stele; pith often forms a hollow. Under a magnifying glass, a transverse section reveals cortex, yellowish white, and with scattered brown spots; stele, yellow-brown in color.

Odorless, and tasteless at first, but later slightly sweet.

Identification To 1 g of pulverized Imperata Rhizome add 20 mL of hexane, allow the mixture to stand for 30 minutes with occasional shaking, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 5 mL of acetic anhydride, place 0.5 mL of this solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to blue-purple color.

Purity (1) Rootlet and scaly leaf—The amount of the

rootlets and scaly leaves contained in Imperata Rhizome is not more than 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than rootlets and scaly leaves contained in Imperata Rhizome is not more than 1.0%.

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Ipecac

Ipecacuanhae Radix

トコン

Ipecac is the root and rhizome of *Cephaelis ipecacuanha* (Broterol) A. Richard or *Cephaelis acuminata* Karsten (*Rubiaceae*).

It contains not less than 2.0% of the total alkaloids (emetine and cephaeline), calculated on the basis of dried material.

Description Slender, curved, cylindrical root, 3 – 15 cm in length, 0.3 – 0.9 cm in diameter; mostly twisted, and sometimes branched; outer surface gray, dark grayish brown, reddish brown in color and irregularly annulated; when root fractured, cortex easily separable from the xylem; the cortex on the fractured surface is grayish brown, and the xylem is light brown in color: thickness of cortex up to about two-thirds of radius in thickened portion. Scales in rhizome opposite.

Odor, slight; powder irritates the mucous membrane of the nose; taste, slightly bitter and unpleasant.

Under a microscope <5.01>, the transverse section of Ipecac reveals a cork layer, consisting of brown thin-walled cork cells; in the cortex, sclerenchyma cells are absent; in the xylem, vessels and tracheids arranged alternately; parenchyma cells filled with starch grains and sometimes with raphides of calcium oxalate.

Identification To 0.5 g of pulverized Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small piece of chlorinated lime: circumference of it turns red.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Component determination Weigh accurately about 0.5 g of pulverized Ipecac, in a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for component determination, previously dried in a desiccator (reduced below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L

hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μ L of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC} , of emetine and cephaeline in the sample solution, and the peak area, A_{SE} , of emetine in the standard solution.

$$\begin{aligned} & \text{Amount (mg) of total alkaloids} \\ & \text{(emetine and cephaeline)} \\ & = W_s \times \{A_{TE} + (A_{TC} \times 0.971)\} / A_{SE} \times 0.868 \end{aligned}$$

W_s : Amount (mg) of emetine hydrochloride for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength : 283 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.

Selection of column: Dissolve 1 mg each of emetine hydrochloride for component determination and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μ L of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

System repeatability: Repeat the test 6 times with the standard solution under the above operating conditions: the relative standard deviation of the peak area of emetine is not more than 1.5%.

Powdered Ipecac

Ipecacuanhae Radix Pulverata

トコン末

Powdered Ipecac is the powder of Ipecac or its powder diluted with Potato Starch.

It contains not less than 2.0% and not more than 2.6% of the total alkaloids (emetine and cephaeline).

Description Powdered Ipecac occurs as a light grayish yellow to light brown powder. It has a slight odor, which is irritating to the nasal mucosa, and has a somewhat bitter and unpleasant taste.

Under a microscope <5.01>, Powdered Ipecac reveals starch grains and needle crystals of calcium oxalate; fragments of parenchyma cells containing starch grains or the needle crystals; substitute fibers, thin-walled cork tissue; vessels and tracheids with simple or bordered pits; a few wood fibers and wood parenchyma. Starch grains inherent in Ipecac, mainly 2 – 8-compound grains, rarely simple grains 4

– 22 μm in diameter; and needle crystals of calcium oxalate 25 – 60 μm in length.

Identification To 0.5 g of Powdered Ipecac add 2.5 mL of hydrochloric acid, allow to stand for 1 hour with occasional shaking, and filter. Collect the filtrate into an evaporating dish, and add a small piece of chlorinated lime: circumference of it turns red.

Purity Foreign matter—Under a microscope <5.01>, groups of stone cells and thick-walled fibers are not observed.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Component determination Weigh accurately about 0.5 g of Powdered Ipecac, transfer into a glass-stoppered centrifuge tube, add 30 mL of 0.01 mol/L hydrochloric acid TS, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure twice with the residue using 30-mL portions of 0.01 mol/L hydrochloric acid TS. Combine all the extracts, add 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of emetine hydrochloride for component determination, previously dried in a desiccator (reduced below 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Pipet 10 μL of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC} , of emetine and cephaeline in the sample solution, and the peak area, A_{SE} , of emetine in the standard solution.

$$\begin{aligned} & \text{Amount (mg) of total alkaloids} \\ & \text{(emetine and cephaeline)} \\ & = W_S \times \{ [A_{\text{TE}} + (A_{\text{TC}} \times 0.971)] / A_{\text{SE}} \} \times 0.868 \end{aligned}$$

W_S : Amount (mg) of emetine hydrochloride for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength : 283 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.

Selection of column: Dissolve 1 mg each of emetine hydrochloride for component determination and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μL of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

System repeatability: Repeat the test 6 times with the stan-

dard solution under the above operating conditions: the relative standard deviation of the peak area of emetine is not more than 1.5%.

Ipecac Syrup

Syrupus Ipecacuanha

トコンシロップ

Ipecac Syrup is a syrup containing not less than 0.12 g and not more than 0.15 g of the total alkaloids (emetine and cephaeline) per 100 mL.

Method of preparation Take coarse powder of Ipecac, prepare the fluidextract as directed under Fluidextracts using a mixture of Ethanol and Purified Water (3:1), and evaporate the mixture under reduced pressure or add a suitable amount of Ethanol or Purified Water if necessary to get a solution containing 1.7 to 2.1 g of the total alkaloids (emetine and cephaeline) per 100 mL. To 70 mL of this solution add 100 mL of Glycerin and Simple Syrup to make 1000 mL, as directed under Syrups.

Description Ipecac Syrup is a yellow-brown, viscous liquid. It has a sweet taste and a bitter aftertaste.

Identification Take 2 mL of Ipecac Syrup into an evaporating dish, mix with 1 mL of hydrochloric acid, and add small pieces of chlorinated lime: circumference of it turns orange.

Purity Ethanol—Take exactly 5 mL of Ipecac Syrup, add 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the sample solution. Separately, pipet 5 mL of ethanol (99.5), and add water to make exactly 100 mL. To exactly 5 mL of this solution add exactly 5 mL of the internal standard solution and water to make 50 mL, and use this solution as the standard solution. Perform the test with 2 μL each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions, and determine the rate of peak height of ethanol to that of the internal standard, Q_T and Q_S : Q_T is not larger than Q_S .

Internal standard solution—A solution of acetonitrile (1 in 20).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass-column about 3 mm in inside diameter and about 1.5 m in length, packed with ethylvinylbenzene-divinylbenzene porous co-polymer for gas chromatography (150 to 180 μm in particle diameter).

Column temperature: A constant temperature of between 105°C and 115°C.

Carrier gas: Nitrogen

Flow rate: Adjust the flow rate so that the retention time of ethanol is 5 to 10 minutes.

Selection of column: Proceed with 2 μL of the standard solution under the above operating conditions. Use a column giving elution of ethanol and the internal standard in this order, and clearly separating each peak.

Component determination Take exactly 5 mL of Ipecac Syrup, add 0.01 mol/L hydrochloric acid TS to make exactly 50 mL, and use the solution as the sample solution. Separate-

ly, weigh accurately about 10 mg of emetine hydrochloride for component determination, previously dried in a desiccator (reduced pressure under 0.67 kPa, phosphorus (V) oxide, 50°C) for 5 hours, dissolve in 0.01 mol/L hydrochloric acid TS to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TC} , of emetine and cephaeline in the sample solution, and the peak area, A_{SE} , of emetine in the standard solution.

$$\text{Amount (mg) of total alkaloids (emetine and cephaeline)} \\ = W_S \times \{ [A_{TE} + (A_{TC} \times 0.971)] / A_{SE} \} \times (1/2) \times 0.868$$

W_S : Amount (mg) of emetine hydrochloride for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 283 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 10 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.0 g of sodium 1-heptane sulfonate in 500 mL of water, adjust the pH to 4.0 with acetic acid (100), and add 500 mL of methanol.

Flow rate: Adjust the flow rate so that the retention time of emetine is about 14 minutes.

Selection of column: Dissolve 1 mg each of emetine hydrochloride for component determination and cephaeline hydrobromide in 10 mL of 0.01 mol/L hydrochloric acid TS. Perform the test with 10 μ L of this solution under the above operating conditions. Use a column giving elution of cephaeline and emetine in this order, and clearly separating each peak.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of emetine is not more than 1.5%.

Microbial limit <4.05> Proceed with Ipecac Syrup: the total viable aerobic microbial count is not more than 1000 per mL, and the total count of fungi and yeast is not more than 100 per mL. *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* should not be observed.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Japanese Angelica Root

Angelicae Radix

トウキ

Japanese Angelica Root is the root of *Angelica acutiloba* Kitagawa or *Angelica acutiloba* Kitagawa var. *sugiyamae* Hikino (*Umbelliferae*), usually after being passed through hot water.

Description Thick and short main root, with numerous

branched roots, nearly fusiform; 10 – 25 cm in length; externally dark brown to red-brown, with longitudinal wrinkles and horizontal protrusions composed of numerous scars of fine rootlets; fractured surface is dark brown to yellow-brown in color, and smooth; and with a little remains of leaf sheath at the crown.

Odor, characteristic; taste, slightly sweet, followed by slight pungency.

Under a microscope <5.01>, a transverse section reveals 4 to 10 layers of cork, with several layers of collenchyma inside of the layer; the cortex exhibits many oil canals surrounded by secretory cells and often large hollows appear; boundary of phloem and xylem is distinct; in the xylem, numerous vessels radiate alternately with medullary rays; vessels in the outer part of the xylem are singly or in several groups, and disposed rather densely in a cuneiform pattern, but vessels in the region of the center are scattered very sparsely; starch grains are simple grains, not more than 20 μ m in diameter, and rarely 2- to 5-compound grains, up to 25 μ m in diameter; starch grains often gelatinized.

Purity (1) Leaf sheath—The amount of leaf sheath contained in Japanese Angelica Root does not exceed 3.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than leaf sheath contained in Japanese Angelica Root does not exceed 1.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Powdered Japanese Angelica Root

Angelicae Radix Pulverata

トウキ末

Powdered Japanese Angelica Root is the powder of Japanese Angelica Root.

Description Powdered Japanese Angelica Root occurs as a light grayish brown powder. It has a characteristic odor and a slight, sweet taste with a slightly pungent aftertaste.

Under a microscope <5.01>, Powdered Japanese Angelica Root reveals starch grains or masses of gelatinized starch, and fragments of parenchyma containing them; fragments of light yellow-brown cork tissue; fragments of rather thick-walled collenchyma and phloem tissue; fragments of resin duct surrounded by secretory cells; fragments, 20 – 60 μ m in diameter, of scalariform and reticulate vessels with simple perforation; starch grains composed of simple grains not more than 20 μ m in diameter, and rarely 2- to 3-compound grains.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of

Powdered Japanese Angelica Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Japanese Angelica Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Japanese Angelica Root does not show remarkably lignified sclerenchymatous cells.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Japanese Gentian

Gentianae Scabrae Radix

リュウタン

Japanese Gentian is the root and rhizome of *Gentiana scabra* Bunge, *Gentiana manshurica* Kitagawa or *Gentiana triflora* Pallas (*Gentianaceae*).

Description Irregular, cylindrical, short rhizome with numerous, slender roots around, and externally yellow-brown to grayish yellow-brown. The root is 10 to 15 cm in length, about 0.3 cm in diameter, and has longitudinal, coarse wrinkles on the outer surface; flexible; fractured surface, smooth and yellow-brown in color. The rhizome is about 2 cm in length, about 0.7 cm in diameter, and has buds or short remains of stems at the top.

Odor, slight; taste, extremely bitter and lasting.

Under a microscope <5.01>, a transverse section of the young root reveals epidermis, exodermis and a few layers of primary cortex; usually, the outermost layer is endodermis consisting of characteristic cells divided into a few daughter cells, often with collenchyma of 1 to 2 layers contacting the inner side; secondary cortex having rents here and there, and irregularly scattered sieve tubes; vessels arranged rather radially in xylem, sieve tubes existing in xylem; the rhizome has a large pith, rarely with sieve tubes; parenchyma cells contain needle, plate or sand crystals of calcium oxalate and oil drops; starch grains usually absent.

Identification To 0.5 g of Powdered Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and

a dark purple spot from the standard solution show the same color tone and the same Rf value.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Powdered Japanese Gentian

Gentianae Scabrae Radix Pulverata

リュウタン末

Powdered Japanese Gentian is the powder of Japanese Gentian.

Description Powdered Japanese Gentian occurs as a grayish yellow-brown powder. It has a slight odor and a lasting, extremely bitter taste.

Under a microscope <5.01>, Powdered Japanese Gentian reveals fragments of parenchyma cells containing oil droplets and fine crystals, fragments of endodermis and exodermis divided into daughter cells with suberized membrane, and fragments of vessels. Vessels mainly consist of reticulate vessels and scalariform vessels, 20 – 30 μ m in diameter.

Identification To 0.5 g of Powdered Japanese Gentian add 10 mL of methanol, shake for 20 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of gentiopicroside for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one spot among the spots from the sample solution and a dark purple spot from the standard solution show the same color tone and the same Rf value.

Purity Foreign matter—Under a microscope <5.01>, Powdered Japanese Gentian usually reveals no stone cells and fibers. No starch grains; if any, very few.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Japanese Valerian

Valerianae Radix

カノコソウ

Japanese Valerian is the root and rhizome of *Valeriana fauriei* Briquet (*Valerianaceae*).

Description Obovoid, short rhizome with numerous, fine and long roots; externally dark brown to grayish brown. The root, 10 – 15 cm in length, 0.1 – 0.3 cm in diameter; externally, with fine longitudinal wrinkles; brittle. The rhizome, 1 – 2 cm in length, 1 – 2 cm in diameter, with buds and remains of

stem at the crown; hard in texture and difficult to break; flank of rhizome sometimes accompanied with stolons having thick and short or thin, long and extremely small, scaly leaves. Under a magnifying glass, the transverse section reveals a thick, light grayish brown cortical layer, and a grayish brown stele.

Odor, strong and characteristic; taste, slightly bitter.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 5.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Japanese Valerian provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.3 mL.

Containers and storage Containers—Tight containers.

Powdered Japanese Valerian

Valerianae Radix Pulverata

カノコソウ末

Powdered Japanese Valerian is the powder of Japanese Valerian.

Description Powdered Japanese Valerian occurs as a dark grayish brown powder. It is somewhat moist to the touch. It has a strong, characteristic odor and a slightly bitter taste.

Under a microscope <5.01>, Powdered Japanese Valerian reveals starch grains and fragments of parenchyma cells containing them; fragments of pitted vessels, reticulate vessels, ring vessels, and spiral vessels; fragments of exodermis containing oil droplets and composed of cells suberized and divided into daughter cells; fragments of yellow stone cells from the rhizome and the stolon; and very rarely, some fragments of epidermis and phloem fibers. Starch grains, simple grains 10–20 μm in diameter and 2- to 4-compound grains; oil droplets stained red with sudan III TS.

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 5.0%.

Essential oil content <5.01> Perform the test with 50.0 g of Powdered Japanese Valerian provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Containers and storage Containers—Tight containers.

Jujube

Zizyphi Fructus

タイソウ

Jujube is the fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*).

Description Ellipsoidal or broad ovoid fruit, 2–3 cm in length, 1–2 cm in diameter; externally reddish brown with coarse wrinkles, or dark grayish red with fine wrinkles, and both lustrous; both ends slightly dented, with a scar of style

on one end and a scar of peduncle on the other; epicarp thin and leather; mesocarp thick, dark grayish brown in color, spongy, soft and adhesive; endocarp extremely hard, fusiform, and divided into two loculi; seeds flat and ovoid.

Odor, slight and characteristic; taste, sweet.

Purity (1) Rancidity—Jujube has no unpleasant, rancid odor and taste.

(2) Total BHC's and total DOT's <5.01> Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 3.0%.

Jujube Seed

Zizyphi Semen

サンソウニン

Jujube Seed is the seed of *Zizyphus jujuba* Miller var. *spinosa* (Bunge) Hu ex H. F. Chou (*Rhamnaceae*).

Description Jujube Seed is a compressed ovate to orbicular, lenticular seed, 5 to 9 mm in length, 4 to 6 mm in width, 2 to 3 mm in thickness, externally brown to dark red-brown, glossy; hilum at one end, charaza at the other end; seed coat slightly flexible, covering, milky white endosperm and light yellow embryo. 100 seeds weighing 3.0 to 4.5 g.

Odor, slightly oily; taste, mild and slightly oily.

Under a microscope <5.01>, transverse section reveals seed coat composed of an upper epidermis, parenchyma and lower epidermis; upper epidermal cells sclerified and elongated in radial direction; lower epidermis covered with cuticle; endosperm composed of parenchyma, containing aggregated crystals of calcium oxalate, aleurone grains and starch grains; cotyledons composed of parenchyma that contains aleurone grains, starch grains and oil drops.

Identification To 2 g of pulverized Jujube Seed add 10 mL of methanol, and heat under a reflux condenser for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of acetone, ethyl acetate, water and acetic acid (100) (10:10:3:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a purple spot appears at around *R_f* 0.3, which shows a yellow-green to grayish green color after spraying 1-naphthol-sulfuric acid TS on the plate and heating at 105°C for 5 minutes.

Purity Foreign matter <5.01>—Jujube Seed contains not more than 1.0% of the endocarp and other foreign matters.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 9.0%.

Kakkonto Extract

葛根湯エキス

Kakkonto Extract contains not less than 9 mg and not more than 27 mg (for preparation prescribed 3 g of Ephedra Herb) or not less than 12 mg and not more than 36 mg (for preparation prescribed 4 g of Ephedra Herb) of total alkaloids [ephedrine ($C_{10}H_{15}NO$: 165.23) and pseudoephedrine ($C_{10}H_{15}NO$: 165.23)], not less than 14 mg and not less than 42 mg (for preparation prescribed 2 g of Peony Root) or not less than 21 mg and not more than 63 mg (for preparation prescribed 3 g of Peony Root) of peoniflorin ($C_{23}H_{28}O_{11}$: 480.46), and not less than 19 mg and not more than 57 mg of glycyrrhizic acid ($C_{42}H_{62}O_{16}$: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 8 g of Pueraria Root, 4 g of Ephedra Herb, 4 g of Jujube, 3 g of Cinnamon Bark, 3 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 4 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 3 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 1 g of Ginger, or with 4 g of Pueraria Root, 3 g of Ephedra Herb, 3 g of Jujube, 2 g of Cinnamon Bark, 2 g of Peony Root, 2 g of Glycyrrhiza and 2 g of Ginger.

Description Kakkonto Extract occurs as a light brown to brown powder. It has a characteristic odor, and a sweet first, then hot, and slightly bitter taste.

Identification (1) Pueraria root—To 1.0 g of Kakkonto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Puerarin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-white fluorescent spot from the standard solution.

(2) Ephedra herb—To 1.0 g of Kakkonto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of ephedrine hydrochloride in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly ninhydrin TS on the plate, and heat at 105°C for 5 minutes: one

of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the red-purple spot from the standard solution.

(3) Cinnamon bark—Put 10 g of Kakkonto Extract in a 300-mL hard-glass flask, add 100 mL of water and 1 mL of silicone resin, connect the apparatus for essential oil determination, and heat to boil under a reflux condenser. The graduated tube of the apparatus is to be previously filled with water to the standard line, and 2 mL of hexane is added to the graduated tube. After heating under reflux for about 1 hour, separate the hexane layer, and use this as the sample solution. Separately, dissolve 1 mg of cinnamaldehyde for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution and 2 μ L the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 2,4-dinitrophenylhydrazine TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-orange spot from the standard solution.

(4) Peony Root—To 1.0 g of Kakkonto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Peoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution.

(5) Glycyrrhiza—To 1.0 g of Kakkonto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution.

(6) Ginger—To 1.0 g of Kakkonto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, shake, centrifuge, and take the diethyl ether layer. Evaporate the diethyl ether under reduced pressure, dissolve the residue in 2 mL of diethyl ether, and use the solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solu-

tions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Kakkonto Extract as directed in (4) in Extracts under the General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Kakkonto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%.

Assay (1) Total alkaloids (ephedrine and pseudoephedrine)—Weigh accurately about 0.5 g of Kakkonto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of ephedrine hydrochloride for assay, previously dried at 105°C for 3 hours, and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{TE} and A_{TP} , of ephedrine and pseudoephedrine with the sample solution, and the peak area, A_{S} , of ephedrine with the standard solution.

Amount (mg) of total alkaloids [ephedrine ($\text{C}_{10}\text{H}_{15}\text{NO}$) and pseudoephedrine ($\text{C}_{10}\text{H}_{15}\text{NO}$)]
 $= W_{\text{S}} \times \{(A_{\text{TE}} + A_{\text{TP}})/A_{\text{S}}\} \times 0.819 \times (1/10)$

W_{S} : Amount (mg) of ephedrine hydrochloride for assay

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of a solution of sodium lauryl sulfate (1 in 130), acetonitrile and phosphoric acid (650:350:1).

Flow rate: 1.0 mL/min. (the retention time of ephedrine is about 27 minutes.)

System suitability—

System performance: Dissolve 1 mg each of ephedrine hydrochloride for assay and pseudoephedrine hydrochloride in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above oper-

ating conditions, pseudoephedrine and ephedrine are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ephedrine is not more than 1.5%.

(2) Peoniflorin—Weigh accurately about 0.5 g of Kakkonto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, and filter. Pipet 5 mL of the filtrate, flow through in a column packed with 2 g of polyamide for column chromatography, elute with water to make exactly 20 mL of eluate, and use this as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately determine the water) and dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 5 mL of this solution, add diluted methanol (1 in 2) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{T} and A_{S} , of peoniflorin.

$$\begin{aligned} &\text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ &= W_{\text{S}} \times (A_{\text{T}}/A_{\text{S}}) \times (1/2) \end{aligned}$$

W_{S} : Amount (mg) of Peoniflorin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1)

Flow rate: 1.0 mL/min. (the retention time of peoniflorin is about 9 minutes.)

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and peoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Kakkonto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{T} and A_{S} , of glycyrrhizic

acid.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Kamishoyosan Extract

加味逍遙散エキス

Kamishoyosan Extract contains not less than 28 mg and not more than 84 mg of peoniflorin (C₂₃H₂₈O₁₁: 480.46), not less than 25 mg and not more than 75 mg of geniposide, and not less than 12 mg and not more than 36 mg (for preparation prescribed 1.5 g of Glycyrrhiza) or not less than 16 mg and not more than 48 mg (for preparation prescribed 2 g of Glycyrrhiza) of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 2 g of Glycyrrhiza, 1 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyrrhiza, 1 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome, 3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyrrhiza, 1.5 g of Ginger and 1 g of Mentha Herb, or with 3 g of Japanese Angelica Root, 3 g of Peony Root, 3 g of Atractylodes Rhizome,

3 g of Poria Sclerotium, 3 g of Bupleurum Root, 2 g of Moutan Bark, 2 g of Gardenia Fruit, 1.5 g of Glycyrrhiza, 0.5 g of Ginger and 1 g of Mentha Herb.

Description Kamishoyosan Extract occurs as a yellow-brown to brown powder. It has slightly a characteristic odor, and a sweet, slightly hot, then bitter taste.

Identification (1) Japanese angelica root—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of (Z)-ligustilide for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution.

(2) Peony root—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of albiflorin in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the orange fluorescent spot from the standard solution.

(3) Atractylodes rhizome (for preparation prescribed Atractylodes Rhizome)—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1-naphthol-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the red spot from the standard solution.

(4) Atractylodes lancea rhizome (for preparation prescribed Atractylodes Lancea Rhizome)—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the

residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around R_f 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool.

(5) Bupleurum root—To 2.0 g of Kamishoyosan Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b_2 for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the red spot from the standard solution.

(6) Moutan bark—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 15 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 1 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of peonol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and diethyl ether (5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the orange spot from the standard solution.

(7) Gardenia fruit—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of geniposide for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and ammonia solution (28) (6:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the purple spot from the standard solution.

(8) Glycyrrhiza—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution.

Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution.

(9) Ginger—To 2.0 g of Kamishoyosan Extract add 10 mL of water, shake, then add 5 mL of diethyl ether, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the blue-green spot from the standard solution.

(10) Mentha herb—To 2.0 g of Kamishoyosan Extract add 10 mL of diluted phosphoric acid (1 in 30), shake, then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, shake 0.2 g of pulverized Mentha Herb with 10 mL of diluted phosphoric acid (1 in 30), then add 15 mL of ethyl acetate, shake, centrifuge, and use the supernatant liquid as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (10:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the red-purple spot (around R_f 0.4) from the standard solution.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Kamishoyosan Extract as directed in (4) in Extracts, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Kamishoyosan Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 9.0% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 10.0%.

Assay (1) Peoniflorin—Weigh accurately about 0.5 g of Kamishoyosan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Peoniflorin Reference Standard (separately

determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of peoniflorin.

$$\begin{aligned} & \text{Amount (mg) of peoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Peoniflorin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1)

Flow rate: 1.0 mL/min. (the retention time of peoniflorin is about 9 minutes.)

System suitability—

System performance: Dissolve 1 mg each of Peoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, albiflorin and peoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of peoniflorin is not more than 1.5%.

(2) Geniposide—Weigh accurately about 0.5 g of Kamishoyosan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of geniposide for component determination, previously dried in a desiccator (in vacuum, phosphorous (V) oxide) for 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of geniposide.

$$\text{Amount (mg) of geniposide} = W_S \times (A_T/A_S) \times (1/2)$$

W_S : Amount (mg) of geniposide for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 240 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (900:100:1).

Flow rate: 1.0 mL/min. (the retention time of geniposide is about 10 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of geniposide are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of geniposide is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Kamishoyosan Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid.

$$\begin{aligned} & \text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5%, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Lindera Root

Linderae Radix

ウヤク

Lindera Root is the root of *Lindera strychnifolia* Fernandez-Villar (*Lauraceae*).

Description Fusiform or rosary-like root, 10–15 cm in length, 10–25 mm in diameter; externally yellowish brown to brown, with a few scars of rootlets; a transverse section reveals cortex brown, xylem light yellowish brown, concentric circles and radially arranged lines brown; dense and hard in texture.

Odor, camphor-like; taste, bitter.

Under a microscope <5.01>, a transverse section of the root with periderm reveals a cork layer several cells thick, partially consisting of cork stone cells; cortex parenchyma sometimes contains oil cells and fibers; in xylem, vessels-xylem fibers and rays are arranged alternately; parenchymatous cells of cortex and xylem contain sandy and columnar crystals of calcium oxalate, simple starch grains 1–15 μm in diameter, and 2- to 4- compound starch grains.

Identification To 3 g of pulverized Lindera Root add 40 mL of hexane, and warm under a reflux condenser on a water bath for 30 minutes. After cooling, filter, to the residue add 10 mL of ammonia TS and 30 mL of a mixture of ethyl acetate and diethyl ether (1:1), shake vigorously for 20 minutes, and centrifuge. Separate the supernatant liquid, add 10 g of anhydrous sodium sulfate, shake, and filter. Evaporate the filtrate, dissolve the residue with 0.5 mL of ethanol (99.5), and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, methanol and ammonia water (28) (10:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-brown spot appears at around Rf 0.4.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 2.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 6.0%.

Lithospermum Root

Lithospermi Radix

シコン

Lithospermum Root is the root of *Lithospermum erythrorhizon* Siebold et Zuccarini (*Boraginaceae*).

Description Rather slender conical root, often branched, 6–10 cm in length, 0.5–1.5 cm in diameter; externally dark purple, coarse in texture, thin and easily peeled; mostly with

twisted and deep longitudinal furrows, which sometimes reach to xylem; sometimes remains of stem at the crown; easily broken; fractured surface granular and with many clefts. Under a magnifying glass, a transverse section reveals a dark purple color at the outer portion of cortex, and light brown inner portion making irregular wave; xylem yellowish in color; the center of the crown is often cracked, and the surrounding part red-purple.

Odor, slight; taste, slightly sweet.

Identification (1) Heat 0.5 g of pulverized Lithospermum Root in a test tube: red vapor evolves, which condenses on the wall of the upper part of the tube into red-brown oil drops.

(2) Shake 0.5 g of pieces or powder of Lithospermum Root with 1 mL of ethanol (95), and to the red solution thereby obtained add 1 drop of sodium hydroxide TS: the red color changes to blue-purple. To this solution add 1 to 2 drops of dilute hydrochloric acid: the color turns red again.

(3) To 0.5 g of pulverized Lithospermum Root add 5 mL of ethanol (95), shake for 30 minutes, filter, and evaporate the filtrate at a temperature not higher than 40°C under reduced pressure. Add 1 mL of ethanol (95) to the residue, and use this solution as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and ethanol (95) (3:1) to a distance of about 10 cm, and air-dry the plate: a red-purple spot appears around the Rf 0.75.

Total ash <5.01> Not more than 11.0%.

Acid-insoluble ash <5.01> Not more than 3.5%.

Longgu

Fossilia Ossis Mastodi

リュウコツ

Longgu is the ossified bone of large mammal, and is mainly composed of calcium carbonate.

Description Irregular masses or fragments, occasionally cylindrical masses; externally light grayish white, sometimes with grayish black or yellow-brown spots here and there; the outer part consists of a layer 2–10 mm in thickness, and is minute in texture, surrounding the light brown, porous portion; heavy and hard, but somewhat fragile in texture; when crushed, it changes into pieces and powder.

Odorless, tasteless, and strongly adhesive to the tongue on licking.

Identification (1) Dissolve 0.5 g of pulverized Longgu in 10 mL of dilute hydrochloric acid: it evolves a gas, and forms a slightly brownish and turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The turbid solution, obtained in (1), has a characteristic odor. Filter this solution, and neutralize with ammonia TS: the solution responds to the Qualitative test <1.09> for calcium salt.

(3) Dissolve 0.1 g of pulverized Longgu in 5 mL of nitric

acid by warming, and add hexaammonium heptamolybdate TS: a yellow precipitate is produced.

Purity (1) Heavy metals <1.07>—To 2.0 g of pulverized Longgu add 5 mL of water, shake to mix, add carefully 6 mL of hydrochloric acid, and evaporate on a water bath to dryness. Dissolve the residue in 50 mL of water, and filter. To 25 mL of the filtrate add 2 mL of dilute acetic acid, 1 drop of ammonia TS and water to make 50 mL. Perform the test using this solution as the test solution. Prepare the control solution as follows: Evaporate 3 mL of hydrochloric acid on a water bath to dryness, add 2 mL of dilute acetic acid and 2.0 mL of Standard Lead Solution, and add water to make 50 mL (not more than 20 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.20 g of pulverized Longgu according to Method 2, and perform the test (not more than 10 ppm).

Lonicera Leaf and Stem

Lonicerae Folium Cum Caulis

ニンドウ

Lonicera Leaf and Stem is the leaves and stems of *Lonicera japonica* Thunberg (*Caprifoliaceae*).

Description Leaves and opposite leaves on short stem; leaf, ovate and entire, with short petiole, 3–7 cm in length, 1–3 cm in width; upper surface greenish brown, lower surface light grayish green; under a magnifying glass, both surfaces pubescent. Stem, 1–4 mm in diameter; externally grayish yellow-brown to purplish brown, a transverse section of stem, round and hollow.

Almost odorless; taste, slightly astringent, followed by a bitter bitterness.

Under a microscope <5.01>, a transverse section of leaf reveals the outermost layer of upper and lower surfaces to be composed of a single-layered epidermis, uni-cellular nonglandular hairs and multi-cellular glandular hairs on epidermis; in midvein, several-layered collenchyma present beneath the epidermis and vascular bundles in the center; in mesophyll, palisade layer adjacent to upper epidermis, spongy tissue adjacent to lower epidermis; glandular hairs contain brown secretion, parenchymatous cells contain aggregate crystals of calcium oxalate, and occasionally starch grains.

Identification To 1 g of pulverized Lonicera Leaf and Stem add 5 mL of methanol, shake for 5 minutes, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of chlorogenic acid for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of loganin for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions (1) and (2) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (6:1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the

several spots from the sample solution has the same color tone and *R_f* value with the blue-white fluorescent spot from the standard solution (1). Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the red-purple spot from the standard solution (2).

Purity Stem—Lonicera Leaf and Stem does not contain the stems larger than 5 mm in diameter.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 9.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 12.0%.

Loquat Leaf

Eriobotryae Folium

ビワヨウ

Loquat Leaf is the leaf of *Eriobotrya japonica* Lindley (*Rosaceae*).

Description Loquat Leaf is an oblong to wide lanceolate leaf, 12 to 30 cm in length, 4 to 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with short petiole; occasionally being cut into strips 5 to 10 mm in shorter diameter and several cm in longer diameter; upper surface green to green-brown in color, lower surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the lower surface of the leaf.

Odor, slight; practically tasteless.

Under a microscope <5.01>, a transverse section of Loquat Leaf reveals thick cuticle on both surfaces; palisade tissue, mostly 4 to 5 layers with several large cells without chloroplast; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fiber attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll; woolly hair, unicellular and curved, about 25 μ m in thickness, and up to 1.5 mm in length.

Identification To 0.3 g of pulverized Loquat Leaf add 10 mL of methanol, warm on a water bath for 5 minutes with occasional shaking, cool, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of water and acetonitrile (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: a red-purple principal spot appears at around *R_f* 0.5.

Purity Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 10.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 16.0%.

Lycium Bark

Lycii Cortex

ジコッピ

Lycium Bark is the root bark of *Lycium chinense* Miller or *Lycium barbarum* Linné (*Solanaceae*).

Description Tubular to semitubular bark, 1–6 mm in thickness; externally light brown to light yellowish brown, periderm peeled easily as scale; internally grayish brown, longitudinally striate; brittle in texture; fractured surface, grayish white, not fibrous.

Odor, weak and characteristic; taste, slightly sweet at first.

Under a microscope <5.01>, a transverse section reveals periderm composed of a cork layer of several layers of thin walled cork cells; in cortex parenchymatous cells containing sandy crystals of calcium oxalate sparsely distributed, occasionally a few fibers observed; parenchymatous cells contain starch grains, 1–10 μm in diameter; stone cells very rare.

Identification To 1.0 g of pulverized Lycium Bark add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water, pyridine and acetic acid (100) (3:1:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, heat at 105°C for 3 minutes, then spray evenly sodium nitrite TS: a dark brown principal spot appears at around Rf 0.5.

Loss on drying <5.01> Not more than 11.5% (6 hours).

Total ash <5.01> Not more than 20.0%.

Acid-insoluble ash <5.01> Not more than 3.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 10.0%.

Lycium Fruit

Lycii Fructus

クコシ

Lycium Fruit is the fruit of *Lycium chinense* Miller or *Lycium barbarum* Linne (*Solanaceae*).

Description Fusiform fruit with acute apex, 6–20 mm in length, 3–8 mm in diameter, pericarp red to dark red, externally roughly wrinkled; under a magnifying glass, a transverse section of fruit reveals two locules containing numerous seeds; seed light brown to light yellowish brown, about 2 mm in a diameter, compressed reniform.

Odor, characteristic; taste, sweet, later slightly bitter.

Identification To 1.0 g of powdered Lycium Fruit add 5 mL of ethyl acetate, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography. <2.03> Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of hexane and ethyl acetate (10:1) to a distance of about 10 cm, and air-dry the plate: a yellow principal spot appears at around Rf 0.6.

Purity Foreign matter <5.01>—It contains not more than 2.0% of foreign matter such as peduncle or others.

Total ash <5.01> Not more than 8.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 35.0%.

Magnolia Bark

コウボク

Magnolia Bark is the bark of the trunk of *Magnolia obovata* Thunberg, *Magnolia officinalis* Rehder et Wilson, *Magnolia officinalis* Rehder et Wilson var. *biloba* Rehder et Wilson (*Magnoliaceae*).

It contains not less than 0.8% of magnolol.

Description Plate-like or semi-tubular bark, 2–7 mm in thickness; externally grayish white to grayish brown, and rough, sometimes cork layer removed, and externally red-brown; internally light brown to dark purplish brown; cut surface extremely fibrous, and light red-brown to purplish brown.

Odor, slight; taste, bitter.

Under a microscope <5.01>, a transverse section reveals a thick cork layer or several thin cork layers, and internally adjoining the circular tissue of stone cells of approximately equal diameter; primary cortex thin; fiber groups scattered in the pericycle; phloem fibers lined stepwise between medullary rays in the secondary cortex, and then these tissues show a latticework; oil cells scattered in the primary and secondary cortex, but sometimes observed in the narrow medullary rays.

Identification To 1.0 g of pulverized Magnolia Bark add 10 mL of methanol, stir for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate, spray evenly the plate with Dragendorff's TS: a yellow spot appears at the Rf value of around 0.3.

Total ash <5.01> Not more than 6.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 11.0%.

Component determination Weigh accurately about 0.5 g of

pulverized Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for component determination in a desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of magnolol in each solution.

$$\begin{aligned} & \text{Amount (mg) of magnolol} \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of magnolol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

Selection of column: Dissolve 1 mg each of magnolol for component determination and honokiol in 10 mL of diluted methanol (7 in 10). Proceed with 10 μ L of this solution under the above operating conditions. Use a column giving elution of honokiol and magnolol in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of magnolol is not more than 1.5%.

Powdered Magnolia Bark

Magnoliae Cortex Pulveratus

コウボク末

Powdered Magnolia Bark is the powder of Magnolia Bark.

It contains not less than 0.8% of magnolol.

Description Powdered Magnolia Bark occurs as a yellow-brown powder, and has a slight odor and a bitter taste.

Under a microscope <5.01>, Powdered Magnolia Bark reveals starch grains and parenchyma cells containing them; stone cells of various sizes or its groups; fibers 12 to 25 μ m in diameter; yellowish red-brown cork tissue; oil cells containing a yellow-brown to red-brown substance. Simple starch grains about 10 μ m in diameter and 2- to 4-compound starch

grains.

Identification To 1.0 g of Powdered Magnolia Bark add 10 mL of methanol, stir for 10 minutes, centrifuge, and perform the test with the supernatant liquid as the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate, and spray evenly with Dragendorff's TS on the plate: a yellow spot appears at the R_f value of around 0.3.

Total ash <5.01> Not more than 6.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 11.0%.

Component determination Weigh accurately about 0.5 g of Powdered Magnolia Bark, add 40 mL of diluted methanol (7 in 10), heat under a reflux condenser on a water bath for 20 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of diluted methanol (7 in 10). Combine the whole filtrates, add diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the sample solution. Separately, dry magnolol for component determination in a desiccator (silica gel) for 1 hour or more. Weigh accurately about 10 mg of it, dissolve in diluted methanol (7 in 10) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of magnolol in each solution.

$$\begin{aligned} & \text{Amount (mg) of magnolol} \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of magnolol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 289 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and acetic acid (100) (50:50:1).

Flow rate: Adjust the flow rate so that the retention time of magnolol is about 14 minutes.

Selection of column: Dissolve 1 mg each of magnolol for component determination and honokiol in 10 mL of diluted methanol (7 in 10). Proceed with 10 μ L of this solution under the above operating conditions. Use a column giving elution of honokiol and magnolol in this order with the resolution between these peaks being not less than 5.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of magnolol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Magnolia Flower

Magnoliae Flos

シンイ

Magnolia Flower is the flower bud of *Magnolia salicifolia* Maximowicz, *Magnolia kobus* De Candolle, *Magnolia biondii* Pampanini, *Magnolia sprengeri* Pampanini or *Magnolia denudata* Desrousseaux (*Magnoliaceae*).

Description Magnolia Flower is a fusiform flower bud, 15 to 45 mm in length, 6 to 20 mm in diameter of central part; often having ligneous peduncles on base; usually 3 bracts, externally with sparse hairs; brown to dark brown; or with dense hairs, grayish white to light yellow-brown, and the inner surface smooth and dark brown in color; interior perianth of 9 pieces or 12 pieces, same size or exterior three pieces are smaller; 50 to 100 stamens and numerous pistils. Brittle in texture.

Odor, characteristic; taste, acrid and slightly bitter.

Identification To 1 g of pulverized Magnolia Flower add 10 mL of methanol, shake for 15 minutes, filter, and use the filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, acetone, water and formic acid (5:3:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate: a yellow-red spot appears at around *Rf* 0.3.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 5.5%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-extract: not less than 13.0%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Magnolia Flower: the volume of essential oil is not less than 0.5 mL.

Mallotus Bark

Malloti Cortex

アカメガシワ

Mallotus Bark is the bark of *Mallotus japonica* Mueller Arg. (*Euphorbiaceae*).

Description Mallotus Bark is flat or semitubular pieces of bark, 1 to 3 mm in thickness; externally green-gray to brown-gray brown in color, with a vertically striped shape gathering numerous lenticels; internal surface light yellowish brown to grayish brown in color, and smooth with numerous fine striped lines; easy to break; slightly fibrous at fracture surface.

Mallotus Bark has a slight odor, a bitter taste and slightly astringent.

Identification To 0.5 g pulverized Mallotus Bark add 10 mL of methanol, warm on a water bath for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of bergenin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (100:17:13) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a principal spot with a dark blue color which appears at *Rf* about 0.5 from the sample solution is the same as the spot from the standard solution in color and the *Rf*.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 12.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 11.0%.

Mentha Herb

Menthae Herba

ハッカ

Mentha Herb is the terrestrial part of *Mentha arvensis* Linné var. *piperascens* Malinvaud (*Labiatae*).

Description Stem with opposite leaves; stem, square, light brown to red-purple in color, and with fine hairs; when smoothed by immersing in water, leaf, ovate to oblong, with acute apex and base, 2 – 8 cm in length, 1 – 2.5 cm in width, margin irregularly serrated; the upper surface, light brown-yellow to light green-yellow, and the lower surface, light green to light green-yellow in color; petiole 0.3 – 1 cm in length. Under a magnifying glass, leaf reveals hairs, glandular hairs and scales.

It has a characteristic aroma and gives a cool feeling on keeping in the mouth.

Identification To 1 mL of the mixture of essential oil and xylene, obtained in the Essential oil content, add carefully 2 mL of sulfuric acid to make two layers: a deep red to red-brown color develops at the zone of contact.

Purity Foreign matter <5.01>—The amount of roots and other foreign matter contained in Mentha Herb does not exceed 2.0%.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 11.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Mentha Herb after adding 1 mL of silicone resin to the sample in the flask: the volume of essential oil is not

less than 0.4 mL.

Mentha Oil

Oleum Menthae Japonicae

ハッカ油

Mentha Oil is the essential oil which is distilled with steam from the terrestrial parts of *Mentha arvensis* Linné var. *piperascens* Malinvaud (*Labiatae*), and from which solids are removed after cooling.

It contains not less than 30.0% of menthol ($C_{10}H_{20}O$: 156.27).

Description Mentha Oil is a colorless or pale yellow, clear liquid. It has a characteristic, pleasant aroma and has a pungent taste, followed by a cool aftertaste.

It is miscible with ethanol (95), with ethanol (99.5), with warm ethanol (95), and with diethyl ether.

It is practically insoluble in water.

Refractive index <2.45> n_D^{20} : 1.455 – 1.467

Optical rotation <2.49> α_D^{20} : –17.0 – –36.0° (100 mm).

Specific gravity <1.13> d_{25}^{25} : 0.885 – 0.910

Acid value <1.13> Not more than 1.0.

Purity (1) Clarity and color of solution—To 1.0 mL of Mentha Oil add 3.5 mL of diluted ethanol (7 in 10), and shake: Mentha Oil dissolves clearly. To the solution add 10 mL of ethanol (95): the solution is clear or has no more turbidity, if any, than the following control solution.

Control solution: To 0.70 mL of 0.01 mol/L hydrochloric acid VS add 6 mL of dilute nitric acid and water to make 50 mL, add 1 mL of silver nitrate TS, and allow to stand for 5 minutes.

(2) Heavy metals <1.07>—Proceed with 1.0 mL of Mentha Oil according to Method 2, and perform the test. Prepare the control solution with 4.0 mL of Standard Lead Solution (not more than 40 ppm).

Assay Weigh accurately about 5 g of Mentha Oil, and dissolve in ethanol (95) to make exactly 20 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the sample solution. Separately, weigh accurately about 10 g of *l*-menthol for assay, and dissolve in ethanol (95) to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed under Gas Chromatography <2.02> according to the following conditions. Calculate the ratios, Q_T and Q_S , of the peak area of menthol to that of the internal standard.

$$\begin{aligned} & \text{Amount (mg) of } C_{10}H_{20}O \\ & = W_S \times (Q_T / Q_S) \end{aligned}$$

W_S : Amount (mg) of *l*-menthol for assay

Internal standard solution—A solution of *n*-ethyl caprylate in ethanol (95) (1 in 25).

Operating conditions—

Detector: A hydrogen flame-ionization detector.

Column: A glass column about 3 mm in inside diameter and about 2 m in length, packed with 25% of polyethylene glycol 6000 for gas chromatography supported on acid-washed 180 – 250 μ m siliceous earth for gas chromatography.

Column temperature: A constant temperature of about 150°C.

Carrier gas: Nitrogen.

Flow rate: Adjust the flow rate so that the retention time of the internal standard is about 10 minutes.

Selection of column: Proceed with 1 μ L of the standard solution under the above operating conditions, and calculate the resolution. Use a column giving elution of the internal standard and *l*-menthol in this order with the resolution between these peaks being not less than 5.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Mentha Water

ハッカ水

Method of preparation

Mentha Oil	2 mL
Purified Water	a sufficient quantity
To make 1000 mL	

Prepare as directed under Aromatic Waters, with the above ingredients.

Description Mentha Water is a clear, colorless liquid, having the odor of mentha oil.

Containers and storage Containers—Tight containers.

Moutan Bark

Moutan Cortex

ポタンビ

Moutan Bark is the root bark of *Paeonia suffruticosa* Andrews (*Paeonia moutan* Sims) (*Paeoniaceae*).

It contains not less than 1.0% of paeonol.

Description Tubular to semi-tubular bark, about 0.5 cm in thickness, 5 – 8 cm in length, 0.8 – 1.5 cm in diameter; externally dark brown to purplish brown, with small and transversely elongated ellipsoidal scars of lateral roots, and with longitudinal wrinkles; internally, light grayish brown to purplish brown and smooth; fractured surface coarse; white crystals often attached on the internal and fractured surfaces.

Odor, characteristic; taste, slightly pungent and bitter.

Identification To 2.0 g of pulverized Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of paeonol for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution and standard solu-

tion on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among several spots from the sample solution is similar with the spot from the standard solution in color tone and *R_f* value.

Purity (1) Xylem—The amount of the xylem contained in Moutan Bark is not more than 5.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

(4) Foreign matter <5.01>—The amount of foreign matter other than xylem contained in Moutan Bark is not exceed 1.0%.

(5) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Component determination Weigh accurately about 0.3 g of pulverized Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, dry paeonol for component determination in a desiccator (calcium chloride for drying) for more than 1 hour. Weigh accurately about 10 mg of it, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of paeonol in each solution.

$$\begin{aligned} & \text{Amount (mg) of paeonol} \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S: Amount (mg) of paeonol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

Selection of column: Dissolve 1 mg of paeonol for component determination and 5 mg of butyl parahydroxybenzoate in 25 mL of methanol. Proceed with 10 μ L of this solution

under the above operating conditions, and calculate the resolution. Use a column giving elution of paeonol and butyl parahydroxybenzoate in this order, with the resolution between these peaks being not less than 2.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of paeonol is not more than 1.5%.

Powdered Moutan Bark

Moutan Cortex Pulveratus

ポタンピ末

Powdered Moutan Bark is the powder of Moutan Bark.

It contains not less than 0.7% of paeonol.

Description Powdered Moutan Bark occurs as a light grayish yellow-brown powder. It has a characteristic odor and a slight, pungent and bitter taste.

Under a microscope <5.01>, Powdered Moutan Bark reveals starch grains and fragments of parenchyma containing them; fragments of cork tissue containing tannin; fragments of somewhat thick-walled collenchyma, medullary rays, and phloem parenchyma; rosette aggregates of calcium oxalate and also fragments of parenchyma cells containing them. Starch grains are simple or 2- to 10-compound grains, 10 – 25 μ m in diameter; rosette aggregates are 20 – 30 μ m in diameter.

Identification (1) To 2.0 g of Powdered Moutan Bark add 10 mL of hexane, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1mg of paeonol for thin-layer chromatography in 10 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of hexane and ethyl acetate (1:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a spot among several spots from the sample solution is similar with the spot from the standard solution in color tone and *R_f* value.

(2) Evaporate to dryness 1 mL of the sample solution obtained in (1), dissolve the residue in ethanol (95) to make 50 mL, and determine the absorption spectrum of this solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits maxima at around 228 nm, 274 nm and 313 nm.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Moutan Bark according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Moutan Bark according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, usually vessels and other thick-walled cells are not observable.

(4) Total BHC's and total DDT's <5.01>—Not more than

0.2 ppm, respectively.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Component determination Weigh accurately about 0.5 g of Powdered Moutan Bark, add 40 mL of methanol, heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure with the residue, using 40 mL of methanol. Combine the whole filtrates, add methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, dry paeonol for component determination in a desiccator (calcium chloride for drying) for more than 1 hour. Weigh accurately about 10 mg of it, dissolve in methanol to make exactly 100 mL, then pipet 10 mL of this solution, add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of paeonol in each solution.

$$\begin{aligned} & \text{Amount (mg) of paeonol} \\ & = W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of paeonol for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 274 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecyl-silanized silica gel (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile, and acetic acid (100) (65:35:2).

Flow rate: Adjust the flow rate so that the retention time of paeonol is about 14 minutes.

Selection of column: Dissolve 1 mg of paeonol for component determination and 5 mg of butyl parahydroxybenzoate in 25 mL of methanol. Proceed with 10 μ L of this solution under the above operating conditions, and calculate the resolution. Use a column giving elution of paeonol and butyl parahydroxybenzoate in this order, with the resolution between these peaks being not less than 2.

System repeatability: When the test is repeated 5 times with the standard solution under the above operating conditions, the relative deviation of the peak area of paeonol is not more than 1.5%.

Containers and storage Containers—Tight containers.

Mulberry Bark

Mori Cortex

ソウハクヒ

Mulberry Bark is the root bark of *Morus alba* Linné (*Moraceae*).

Description Tubular, semi-tubular or cord-like bark, 1 – 6

mm thick, often in fine lateral cuttings; externally, white to yellow-brown; in the case of the bark with periderm, its periderm is yellow-brown in color, easy to peel, with numerous longitudinal, fine wrinkles and numerous red-purple lenticels laterally elongated; inner surface, dark yellow-brown in color and flat; cross section, white to light brown in color, and fibrous.

Odor, slight; taste, slight.

Under a microscope <5.01>, a transverse section of bark with periderm reveals 5 to 12 layers of cork cells in the outer portion; phloem fibers or their bundles scattered in the cortex, arranged alternately and stepwise with phloem parenchyma; lactiferous tubes; solitary crystals of calcium oxalate; starch grains as spheroidal or ellipsoidal, simple or compound grains, simple grain 1 – 7 μ m in diameter.

Identification Heat 1 g of pulverized Mulberry Bark with 20 mL of hexane under a reflux condenser on a water bath for 15 minutes, and filter. Evaporate the hexane of the filtrate under reduced pressure, dissolve the residue in 10 mL of acetic anhydride, place 0.5 mL of the solution in a test tube, and add carefully 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

Purity Foreign matter <5.01>—The amount of the root xylem and other foreign matter contained in Mulberry Bark does not exceed 1.0%.

Total ash <5.01> Not more than 11.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Nelumbo Seed

Nelumbis Semen

レンニク

Nelumbo Seed is the seed of *Nelumbo nucifera* Gaertner (*Nymphaeaceae*), usually with the endocarp, sometime being removed the embryo.

Description Ovoid to ellipsoidal seed, at the base a papillate protuberance surrounded with shallow depression, 1.0 – 1.7 cm in length, 0.5 – 1.2 cm in width; externally light reddish brown to light yellowish brown; projection part dark reddish brown; endocarp not lustrous and hardly peeled off; endosperm yellowish white, a green embryo in the center.

Almost odorless; taste, slightly sweet and oily, embryo is extremely bitter.

Under a microscope <5.01>, a transverse section of the seed at central portion reveals endocarp composed of parenchyma or endocarp occasionally left out; seed coat composed of epidermis and parenchyma of compressed cells; vascular bundles scattered in parenchyma; endosperm composed of epidermis and parenchyma; aggregate crystals of calcium oxalate and tannin-like substances contained in endocarp remained; parenchymatous cells of seed coat contain tannin-like substances; parenchyma of endosperm contain starch grains.

Identification To 0.5 g of pulverized Nelumbo Seed add 5 mL of water, shake for 5 minutes, and centrifuge. To 0.5 mL of the supernatant liquid add 1 drop of a solution of 1-

naphthol in ethanol (99.5) (1 in 5), mix, then add gently 1 mL of sulfuric acid: the solution shows a purple color.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 14.5%.

Notopterygium Rhizome

Notopterygium Rhizoma

キヨウカツ

Notopterygium Rhizome is the rhizome and root of *Notopterygium incisum* Ting ex H. T. Chang or *Notopterygium forbesii* Boissieu (*Umbelliferae*).

Description Notopterygium Rhizome is slightly curved, cylindrical to conical, 3 to 10 cm in length, 5 to 20 mm in diameter; rhizome occasionally branched; externally yellow-brown to dark brown. The rhizome with nearly orbicular, hollowed stem scars at the apex, sometimes having short residue of stem; externally node rising, internode short; root scars in warty processes on the node; externally root has coarse longitudinal wrinkles and lateral root scars in warty processes; light and slightly brittle in texture, easy to break. The transverse section of the rhizome reveals numerous radial cracks; cortex yellow-brown to brown; xylem light yellow to light grayish yellow; pith grayish white to light brown. Under a magnifying glass, the rhizome reveals brown, fine points of resin canals in the cortex and pith.

Odor, characteristic; taste, slightly acid at first, followed by a slightly pungent and slightly numbing aftertaste.

Under a microscope <5.01>, transverse section shows the outermost layer to be composed of a cork layer several to a dozen or so cells thick; collenchyma just inside of the cork layer; oil canals scattered in cortex, large ones more than 300 μm in diameter; intercellular space occurring in radial direction in cortex; oil canals scattered in pith, large ones more than 500 μm in diameter; parenchymatous cells contain simple and 2- to 3-compound starch grains.

Identification To 0.3 g of pulverized Notopterygium Rhizome add 3 mL of hexane in a glass-stoppered centrifuge tube, shake for 10 minutes, centrifuge, and use the supernatant liquid as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of octadecylsilanized silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of methanol and water (9:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a bluish white fluorescent spot appears at around R_f 0.5, which shows a dark purple color under ultraviolet light (main wavelength: 254 nm).

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 20.0%.

Nuphar Rhizome

Nupharis Rhizoma

センコツ

Nuphar Rhizome is the longitudinally split rhizome of *Nuphar japonicum* De Candolle.

Description Usually, longitudinally split irregular column, twisted, bent or somewhat pressed, 20 – 30 cm in length, about 2 cm in width; the outer surface, dark brown, and the cross section, white to grayish white in color; one side shows nearly round to blunt triangular scars of petiole about 1 cm in diameter, and the other side numerous scars of roots less than 0.3 cm in diameter; light, spongy in texture, and easily broken; fractured surface flat and powdery. Under a magnifying glass, a transverse section reveals a black outer portion, and porous tissue with scattered vascular bundles in the inner portion.

Odor, slight; taste, slightly bitter and unpleasant.

Identification Boil 1 g of pulverized Nuphar Rhizome with 20 mL of methanol under a reflux condenser on a water bath for 15 minutes, cool, and filter. Evaporate the filtrate to dryness, warm the residue with 5 mL of dilute acetic acid on a water bath for 1 minute, cool, and filter. Spot 1 drop of the filtrate on a piece of filter paper, air-dry the paper, spray Dragendorff's TS for spraying on it, and allow it to stand: a yellow-red color appears.

Purity (1) Petiole—The amount of its petioles contained in Nuphar Rhizome does not exceed 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than petiole contained in Nuphar Rhizome does not exceed 1.0%.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 10.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Nux Vomica

Strychni Semen

ホミカ

Nux Vomica is the seed of *Strychnos nux-vomica* Linné (*Loganiaceae*).

When dried, it contains not less than 1.07% of strychnine ($\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_2$: 334.41).

Description Disk, often slightly bent, 1 – 3 cm in diameter, 0.3 – 0.5 cm in thickness; externally light grayish yellow-green to light grayish brown, covered densely with lustrous appressed hairs radiating from the center to the circumference; on both sides, the margin and the central part bulged a little; the dot-like micropyle situated at one point on the margin, and from which usually a raised line runs to the center on one side; extremely hard in texture; when cracked upon soak-

ing in water, the seed coat thin, the interior consisting of two horny, light grayish yellow endosperms, and leaving a central narrow cavity at the center; a white embryo, about 0.7 cm in length, situated at one end between the inner surfaces of the endosperms.

Odorless; taste, very bitter and persisting.

Identification (1) To 3 g of pulverized Nux Vomica add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

Total ash <5.01> Not more than 3.0%.

Assay Weigh accurately about 1 g of pulverized Nux Vomica, previously dried at 60°C for 8 hours, place in a glass-stoppered centrifuge tube, and moisten with 1 mL of ammonia solution (28). To this solution add 20 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure three times with the residue using 20-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make exactly 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μm, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (previously determine the loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solution, add exactly 10 mL of the internal standard solution, then add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with 5 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratio, Q_T and Q_S , of the peak area of strychnine to that of the internal standard in each solution.

$$\begin{aligned} &\text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_2\text{O}_2\text{)} \\ &= W_S \times (Q_T/Q_S) \times (1/5) \times 0.8414 \end{aligned}$$

W_S : Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside di-

ameter and about 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: Room temperature.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in water to make 1000 mL, and mix with acetonitrile and triethylamine (45:5:1), and adjust the mixture with phosphoric acid to pH 3.0.

Flow rate: Adjust the flow rate so that the retention time of Strychnine is about 17 minutes.

Selection of column: Proceed with 5 μL of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly separating each peak.

Nux Vomica Extract

ホミカエキス

Nux Vomica Extract contains not less than 6.15% and not more than 6.81% of strychnine (C₂₁H₂₂N₂O₂: 334.41).

Method of preparation After defatting 1000 g of coarse powder of Nux Vomica with hexane, extract with the percolation method, using a mixture of 750 mL of Ethanol, 10 mL of Acetic Acid and 240 mL of Purified Water as the first solvent, and 70 vol% ethanol as the second solvent. Combine the extracts, and prepare the dry extract as directed under Extracts. Where, an appropriate quantity of Ethanol and Purified Water may be used instead of 70 vol% ethanol.

Description Nux Vomica Extract occurs as yellow-brown to brown powder. It has a slight characteristic odor, and an extremely bitter taste.

Identification Extract 0.5 g of Nux Vomica Extract with 0.5 mL of ammonia TS and 10 mL of chloroform with occasional shaking. Filter the chloroform extract, evaporate the filtrate on a water bath until most of the chloroform is removed, and proceed as directed in the Identification under Nux Vomica.

Assay Weigh accurately about 0.2 g of Nux Vomica Extract, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to disperse the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make exactly 100 mL. Proceed as directed in the Assay under Nux Vomica.

$$\begin{aligned} &\text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_2\text{O}_2\text{)} \\ &= W_S \times (Q_T/Q_S) \times (1/5) \times 0.8414 \end{aligned}$$

W_S : Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nux Vomica Extract Powder

ホミカエキス散

Nux Vomica Extract Powder contains not less than 0.61% and not more than 0.68% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica Extract	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
<hr/>	
To make	1000 g

To Nux Vomica Extract add 100 mL of Purified Water, then warm, and soften with stirring. Cool, add 800 g of Starch, Lactose Hydrate or their mixture little by little, and mix well. Dry, preferably at a low temperature, and dilute with a sufficient additional quantity of Starch, Lactose or their mixture to make 1000 g of the homogeneous powder.

Description Nux Vomica Extract Powder occurs as a yellow-brown to grayish brown powder. It has a slight, characteristic odor and a bitter taste.

Identification (1) To 3 g of Nux Vomica Extract Powder add 3 mL of ammonia TS and 20 mL of chloroform, macerate for 30 minutes with occasional shaking, and filter. Remove most of the chloroform from the filtrate by warming on a water bath, add 5 mL of diluted sulfuric acid (1 in 10), and warm on a water bath while shaking well until the odor of chloroform is no longer perceptible. After cooling, filter through a pledget of absorbent cotton, and add 2 mL of nitric acid to 1 mL of the filtrate: a red color develops.

(2) To the remaining filtrate obtained in (1) add 1 mL of potassium dichromate TS, and allow to stand for 1 hour: a yellow-red precipitate is produced. Collect the precipitate by filtration, and wash with 1 mL of water. Transfer a part of the precipitate to a small test tube, add 1 mL of water, dissolve by warming, cool, and add 5 drops of sulfuric acid dropwise carefully along the wall of the test tube: the layer of sulfuric acid shows a purple color which turns immediately red to red-brown.

Assay Weigh accurately about 2.0 g of Nux Vomica Extract Powder, place in a glass-stoppered centrifuge tube, add 15 mL of ammonia TS, and shake. Add 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure three times with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 10 mL of the mobile phase, add exactly 10 mL of the internal standard solution, and add the mobile phase to make exactly 100 mL. Filter this solution through a membrane filter with a porosity not more than 0.8 μ m, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (previously determine the loss on drying), and dissolve in the mobile phase to make exactly 50 mL. Pipet 10 mL of this solu-

tion, add exactly 10 mL of the internal standard solution, then add the mobile phase to make exactly 100 mL, and use this solution as the standard solution. Perform the test with the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratio, Q_T and Q_S , of the peak area of strychnine to that of the internal standard in each solution.

$$\begin{aligned} &\text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_2\text{O}_2) \\ &= W_S \times (Q_T/Q_S) \times (1/5) \times 0.8414 \end{aligned}$$

W_S : Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: Room temperature.

Mobile phase: A mixture of a solution of potassium dihydrogenphosphate (6.8 in 1000), acetonitrile and triethylamine (45:5:1), adjusted the pH to 3.0 with phosphoric acid.

Flow rate: Adjust the flow rate so that the retention time of strychnine is about 17 minutes.

Selection of column: Proceed with 5 μ L of the standard solution under the above operating conditions. Use a column giving elution of the internal standard and strychnine in this order, and clearly dividing each peak.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Nux Vomica Tincture

ホミカチンキ

Nux Vomica Tincture contains not less than 0.097 w/v% and not more than 0.116 w/v% of strychnine ($C_{21}H_{22}N_2O_2$: 334.41).

Method of preparation

Nux Vomica, in coarse powder	100 g
70 vol% Ethanol	a sufficient quantity
<hr/>	
To make	1000 mL

Prepare as directed under Tinctures, with the above ingredients. May be prepared with an appropriate quantity of Ethanol and Purified Water.

Description Nux Vomica Tincture is a yellow-brown liquid. It has an extremely bitter taste.

Specific gravity d_{20}^{20} : about 0.90

Identification Heat 20 mL of Nux Vomica Tincture on a water bath to remove ethanol, cool, transfer to a separator, add 2 mL of ammonia TS and 20 mL of chloroform, and shake well for 2 to 3 minutes. Filter the chloroform layer through a pledget of absorbent cotton, warm the filtrate on a water bath to remove most of chloroform, and proceed as

directed in the Identification under Nux Vomica.

Alcohol number <1.01> Not less than 6.7 (Method 2).

Assay Pipet 3 mL of Nux Vomica Tincture into a glass-stoppered centrifuge tube, add 10 mL of ammonia TS and 20 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 20-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue with 10 mL of the mobile phase, add exactly 5 mL of the internal standard solution, and add the mobile phase to make exactly 50 mL. Filter the solution through a membrane filter with a pore size not exceeding 0.8- μ m, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 75 mg of strychnine nitrate for assay (previously determine the loss on drying), and dissolve in the mobile phase to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 5 mL of the internal standard solution, add the mobile phase to make exactly 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Nux Vomica.

$$\begin{aligned} & \text{Amount (mg) of strychnine (C}_{21}\text{H}_{22}\text{N}_2\text{O}_2) \\ & = W_S \times (Q_T/Q_S) \times (1/20) \times 0.8414 \end{aligned}$$

W_S : Amount (mg) of strychnine nitrate for assay, calculated on the dried basis

Internal standard solution—A solution of barbital sodium in the mobile phase (1 in 500).

Containers and storage Containers—Tight containers.
Storage—Light-resistant.

Ophiopogon Tuber

Ophiopogonis Tuber

バクモンドウ

Ophiopogon Tuber is the enlarged part of the root of *Ophiopogon japonicus* Ker-Gawler (*Liliaceae*).

Description Fusiform root, 1 – 2.5 cm in length, 0.3 – 0.5 cm in diameter, somewhat sharp at one end, and somewhat rounded at the other; externally light yellow to light yellow-brown, with longitudinal wrinkles of various sizes; when fractured, cortex flexible and friable, stele strong; fractured surface of cortex light yellow-brown in color, slightly translucent and viscous.

Odor, slight; taste, slightly sweet and mucous.

Under a microscope <5.01>, a transverse section reveals brown, 4- to 5-layer velamen internally adjoining the epidermis; a single-layer exodermis inside the velamen, and cortex of parenchyma cells inside the exodermis; endodermis is distinct; about 20 protoxylems in actionstele; cortex parenchyma contains columnar crystals and needle raphides of calcium oxalate; oil drops in the exodermis.

Purity (1) Rootlets—The amount of the rootlets contained in Ophiopogon Tuber is not exceed 1.0%.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulver-

ized Ophiopogon Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Ophiopogon Tuber according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 3.0%.

Opium Ipecac Powder

Dover's Powder

アヘン・トコ散

Opium Ipecac Powder contains not less than 0.90% and not more than 1.10% of morphine ($\text{C}_{17}\text{H}_{19}\text{NO}_3$: 285.34).

Method of preparation

Powdered Opium	100 g
Powdered Ipecac	100 g
Starch or a suitable ingredient	a sufficient quantity
To make	
	1000 g

Prepare as directed under Powders, with the above ingredients. Lactose Hydrate should not be used.

Description Opium Ipecac Powder occurs as a light brown powder.

Identification (1) Proceed with 1 g of Opium Ipecac Powder as directed in the Identification (1) under Powdered Opium.

(2) Proceed with 1 g of Opium Ipecac Powder as directed in the Identification (2) under Powdered Opium.

(3) Shake frequently a mixture of 3 g of Opium Ipecac Powder and 5 mL of hydrochloric acid, and allow to stand for 1 hour. Filter the solution into an evaporating dish. Add 5 mg of chlorinated lime to the filtrate: an orange color is produced at the circumference of the chlorinated lime (emetine).

Assay Weigh accurately about 50 g of Opium Ipecac Powder in a glass stoppered flask, add 250 mL of dilute ethanol, warm in a water bath at 40°C for 1 hour with stirring, and filter through a glass filter (G3). Transfer the residue on the filter to the first glass-stoppered flask, add 50 mL of dilute ethanol, warm in a water bath at 40°C for 10 minutes with stirring, and filter through the glass filter. Repeat the extraction with three 50-mL portions of dilute ethanol. Combine all the filtrates in a mortar, evaporate on a water bath to dryness, add 10 mL of ethanol (99.5) to the residue, and evaporate again. After cooling, triturate the residue with an exactly measured 10 mL of water, add 2 g of calcium hydroxide and an exactly measured 40 mL of water, stir the mixture for 20 minutes, and filter. To 30 mL of the filtrate add 0.1 g of magnesium sulfate heptahydrate, shake for 1 minute, then add 0.3 g of calcium hydroxide, shake for 1 minute, allow to stand for 1 hour, and filter. To an exactly measured 20 mL of the filtrate add 5 mL of sodium hydroxide TS, and adjust the pH to between 9.0 and 9.2 with ammonium chloride. Extract the solution successively with 60 mL, 40 mL and 30 mL of a mixture of chloroform and

ethanol (95) (3:1). Combine all the extracts, distil, then evaporate off the solvent on a water bath. Dissolve the residue in 20 mL of dilute sodium hydroxide TS and 10 mL of diethyl ether with shaking, add 0.5 g of ammonium chloride, shake vigorously with caution, and proceed as directed in the Assay under Powdered Opium.

Each mL of 0.05 mol/L sulfuric acid VS
= 28.53 mg of $C_{17}H_{19}NO_3$

Containers and storage Containers—Tight containers.

Orange Peel Syrup

トウヒシロップ

Method of preparation

Orange Peel Tincture	200 mL
Simple Syrup	a sufficient quantity
To make 1000 mL	

Prepare as directed under Syrups, with the above ingredients. An appropriate quantity of Sucrose and Purified Water may be used in place of Simple Syrup.

Description Orange Peel Syrup is a brownish yellow to reddish brown liquid. It has a characteristic odor, a sweet taste and a bitter aftertaste.

Specific gravity d_{20}^{20} : about 1.25

Identification To 25 mL of Orange Peel Syrup add 50 mL of ethyl acetate, shake for 5 minutes, allow to stand until clear ethyl acetate layer separate, and take the ethyl acetate layer, and evaporate on a water bath to dryness. Dissolve the residue in 10 mL of ethanol (95), filter if necessary, and use this solution as the sample solution. Separately, dissolve 10 mg of naringin for thin-layer chromatography in 10 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute 2,6-dibromo-*N*-chloro-1,4-benzoquinone monoimine TS on the plate, and allow to stand in ammonia gas: a spot from the sample solution and a grayish green spot from the standard solution show the same color tone and the same *R_f* value.

Containers and storage Containers—Tight containers.

Orange Peel Tincture

トウヒチンキ

Method of preparation

Bitter Orange Peel, in coarse powder	200 g
70 vol% Ethanol	a sufficient quantity
To make 1000 mL	

Prepare as directed under Tinctures, with the above in-

redients. An appropriate quantity of Ethanol and Purified Water may be used in place of 70 vol% Ethanol.

Description Orange Peel Tincture is a yellowish brown liquid. It has a characteristic odor, and a bitter taste.

Specific gravity d_{20}^{20} : about 0.90

Identification To 5.0 mL of Orange Peel Tincture add 5 mL of ethanol (95), filter if necessary, and use the filtrate as the sample solution. Proceed as directed in the Identification under Bitter Orange Peel.

Alcohol number <1.01> Not less than 6.6 (Method 2).

Containers and storage Containers—Tight containers.

Oriental Bezoar

Bezoar Bovis

ゴオウ

Oriental Bezoar is a stone formed in the gall sac of *Bos taurus* Linné var. *domesticus* Gmelin (*Bovidae*).

Description Spherical or massive stone, 1–4 cm in diameter; externally yellow-brown to red-brown; light, fragile and easily broken. Fractured surface shows yellow-brown to red-brown annular rings, often containing white granular substances or thin layers in these annular rings.

Odor, slight; taste, slightly bitter, followed by slight sweetness.

Identification (1) Shake 0.1 g of pulverized Oriental Bezoar with 10 mL of petroleum ether for 30 minutes, filter, and wash the residue with 10 mL of petroleum ether. Shake 0.01 g of the residue with 3 mL of acetic anhydride for 1 to 2 minutes, add a mixture of 0.5 mL of acetic anhydride and 2 drops of sulfuric acid, and shake: a yellow-red to deep red color develops, and changes through dark red-purple to dark red-brown.

(2) Shake well 0.01 g of Oriental Bezoar with 1 mL of hydrochloric acid and 10 mL of chloroform, separate the chloroform layer when it acquires a yellow-brown color, and shake with 5 mL of barium hydroxide TS: a yellow-brown precipitate is produced.

Purity (1) Synthetic dye—To 2 mg of pulverized Oriental Bezoar add 1 mL dilute hydrochloric acid: no violet color develops.

(2) Starch—To 5 mg of pulverized Oriental Bezoar add 2 mL of water, and heat on a water bath for 5 minutes. Cool and add 2 to 3 drops of iodine TS: no blue-purple color develops.

(3) Sucrose—To 0.02 g of pulverized Oriental Bezoar add 10 mL of water, shake for 15 minutes, and filter. To 1 mL of the filtrate add 2 mL of anthrone TS, and shake: no deep blue-green to dark green color develops.

Total ash <5.01> Not more than 10.0%.

Content of the active principle Weigh accurately about 0.5 g of pulverized Oriental Bezoar in a flask, add 50 mL of petroleum ether, warm under a reflux condenser on a water bath for 2 hours, and filter. Place the residue along with the filter paper in the flask, add 2 mL of hydrochloric acid and 40

mL of chloroform, warm under a reflux condenser on a water bath for 1 hour, and filter into a tared flask. Wash the filter paper with a small quantity of chloroform, combine the washings with the filtrate, and distil off the chloroform. Dry the residue in a desiccator (silica gel) for 24 hours, and weigh: the mass of the residue is not less than 12.0%.

Oyster Shell

Ostrea Testa

ボレイ

Oyster Shell is the shell of *Ostrea gigas* Thunberg (*Ostreidae*).

Description Irregularly curved, foliaceous or lamellated broken pieces. The unbroken oyster shell forms a bivalve 6 – 10 cm in length and 2 – 5 cm in width. The upper valve is flat and the lower one is somewhat hollow. Both the upper and lower edges of the valve are irregularly curved and bite with each other. The surface of the valve is externally light greenish gray-brown and internally milky in color.

Odorless and tasteless.

Identification (1) Dissolve 1 g of sample pieces of Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution in which a transparent, thin suspended matter remains. Pass the evolved gas through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

(3) Ignite 1 g of pulverized Oyster Shell: it turns blackish brown in color at first, and evolves a characteristic odor. Ignite it further: it becomes almost white.

Purity Barium—Dissolve 1 g of pulverized Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not respond to the Qualitative Tests (1) <1.09> for barium salt.

Powdered Oyster Shell

Ostrea Testa Pulverata

ボレイ末

Powdered Oyster Shell is the powder of Oyster Shell.

Description Powdered Oyster Shell occurs as a grayish white powder. It is odorless and tasteless.

Identification (1) Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid by heating: it evolves a gas, and forms a very slightly red, turbid solution. Pass the gas evolved through calcium hydroxide TS: a white precipitate is produced.

(2) The solution obtained in (1) has a slight, characteristic odor. Filter this solution, and neutralize with ammonia TS: the solution responds to the Qualitative Tests <1.09> for calcium salt.

(3) Ignite 1 g of Powdered Oyster Shell: it turns blackish brown in color at first evolving a characteristic odor, and becomes almost white by further ignition.

Purity (1) Water-soluble substances—Shake 3.0 g of Powdered Oyster Shell with 50 mL of freshly boiled and cooled water for 5 minutes, filter, and evaporate 25 mL of the filtrate to dryness. Dry the residue at 105°C for 1 hour, cool, and weigh: the mass of the residue does not exceed 15 mg.

(2) Acid-insoluble substances—To 5.0 g of Powdered Oyster Shell add 100 mL of water, and add hydrochloric acid in small portions with stirring until the solution becomes acid. Boil the acidic mixture with additional 1 mL of hydrochloric acid. After cooling, collect the insoluble substance by filtration, and wash it with hot water until the last washing no longer gives any reaction in Qualitative Tests <1.09> (2) for chloride. Ignite the residue and weigh: the mass of the residue does not exceed 25 mg.

(3) Barium—Dissolve 1 g of Powdered Oyster Shell in 10 mL of dilute hydrochloric acid: the solution does not respond to the Qualitative Tests <1.09> (1) for barium salt.

Loss on drying <2.41> Not more than 4.0% (1 g, 180°C, 4 hours).

Containers and storage Containers—Tight containers.

Panax Japonicus Rhizome

Panacis Japonici Rhizoma

チクセツニンジン

Panax Japonicus Rhizome is the rhizome of *Panax japonicus* C. A. Meyer (*Araliaceae*), usually after being treated with hot water.

Description Irregularly cylindrical rhizome with distinct nodes, 3 – 20 cm in length, 1 – 1.5 cm in diameter, internode 1 – 2 cm; externally light yellow-brown, with fine longitudinal wrinkles; stem scars, hollowed at the center, protruding on the upper surface, and root scars protruding as knobs on internodes; easily broken; fractured surface approximately flat, and light yellow-brown in color; horny in texture.

Odor, slight; taste, slightly bitter.

Identification Shake 0.5 g of pulverized Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 µL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of several spots obtained from the sample solution shows the same color and the same R_f value with the purple-red spot from the standard solution.

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 30.0%.

Powdered Panax Japonicus Rhizome

Panacis Japonici Rhizoma Pulveratum

チクセツニンジン末

Powdered Panax Japonicus Rhizome is the powder of Panax Rhizome.

Description Powdered Panax Japonicus Rhizome occurs as a light grayish yellow-brown powder, and has a slight odor and a slightly bitter taste.

Under a microscope <5.01>, Powdered Panax Japonicus Rhizome reveals mainly starch grains or gelatinized starch masses, and fragments of parenchyma cells containing them; also fragments of cork tissue, somewhat thick-walled collenchyma, phloem tissue, and reticulate vessels; rarely fragments of scalariform vessels with a simple perforation, fibers and fiber bundles, rosette aggregates of calcium oxalate, and parenchyma cells containing them; yellow to orange-yellow resin; starch grains consisting of simple grains or 2- to 4-compound grains, simple grains, 3 – 18 μm in diameter; rosette aggregates of calcium oxalate are 20 – 60 μm in diameter.

Identification Shake 0.5 g of Powdered Panax Japonicus Rhizome with 10 mL of methanol for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of chikusetsusaponin IV for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, water and formic acid (5:1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat the plate at 110°C for 5 minutes: one of several spots obtained from the sample solution shows the same color tone and the same *R_f* value with the purple-red spot from the standard solution.

Total ash <5.01> Not more than 5.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Peach Kernel

Persicae Semen

トウニン

Peach Kernel is the seed of *Prunus persica* Batsch or *Prunus persica* Batsch var. *davidiana* Maximowicz (*Rosaceae*).

Description Flattened, asymmetric ovoid seed, 1.2 – 2.0 cm in length, 0.6 – 1.2 cm in width, and 0.3 – 0.7 cm in thickness; somewhat sharp at one end, and round at the other end with chalaza; seed coat red-brown to light brown; externally,

its surface being powdery by easily detachable stone cells of epidermis; numerous vascular bundles running and rarely branching from chalaza through the seed coat, and, appearing as dented longitudinal wrinkles; when soaked in boiling water and softened, the seed coat and thin, translucent, white albumen easily separated from the cotyledone; cotyledone white in color.

Almost odorless; taste, slightly bitter and oily.

Under a microscope <5.01>, the outer surface of seed coat reveals polygonal, long polygonal, or obtuse triangular stone cells on the protrusion from vascular bundles, shape of which considerably different according to the position, and their membranes almost equally thickened; in lateral view, appearing as a square, rectangle or obtuse triangle.

Identification To 1.0 g of ground Peach Kernel add 10 mL of methanol, immediately heat under a reflux condenser on a water bath for 10 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid upon the plate, and heat at 105°C for 10 minutes: one spot among the spots from the sample solution and a brown to dark green spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Rancidity—Grind Peach Kernel with boiling water: no odor of rancid oil is perceptible.

(2) Foreign matter <5.01>—Peach Kernel does not contain broken pieces of endocarp or other foreign matter.

Powdered Peach Kernel

Persicae Semen Pulveratum

トウニン末

Powdered Peach Kernel is the powder of the Peach Kernel.

Description Powdered Peach Kernel occurs as a reddish-light brown to light brown powder. It is almost odorless and is oily and has slightly a bitter taste.

Under a microscope <5.01>, Powdered Peach Kernel fragments of outer seed coat epidermis; elliptical to ovoid, containing yellowish brown compound 50 to 80 μm in diameter and stone cell; cap-like shape to ovoid, yellowish brown in color. The stone cell is element of epidermis, 50 to 80 μm in diameter and 70 to 80 μm in height, cell wall of the top, 12 to 25 μm thickness, the base 4 μm in thickness, with obvious and numerous pits. Inner seed coat, yellowish brown, irregular and somewhat long polygon, 15 to 30 μm in diameter; and fragments of cotyledon and albumen containing aleurone grains and fatted oil, Aleurone grains are almost spherical grains, 5 to 10 μm in diameter.

Identification (1) Grind Powdered Peach Kernel with

water: the odor of benzaldehyde is perceptible.

(2) To 1.0 g of Powdered Peach Kernel add 10 mL of methanol, and immediately heat under a reflux condenser on a water bath for 10 minutes. After cooling, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of amygdalin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (7:3:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 10 minutes: one spot among the spots shows the same in color tone and *R_f* value with the brown to dark green spot obtained with the standard solution.

Loss on drying <5.01> Not more than 8.5% (6 hours).

Total ash <5.01> Not more than 3.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Containers and storage Containers—Tight containers.

Peony Root

Paeoniae Radix

シャクヤク

Peony Root is the root of *Paeonia lactiflora* Pallas (*Paeoniaceae*).

It contains not less than 2.0% of peoniflorin, ($C_{23}H_{28}O_{11}$: 480.46) calculated on the dried basis.

Description Cylindrical root, 7–20 cm in length, 1–2.5 cm in diameter; externally brown to light grayish brown, with distinct longitudinal wrinkles, with warty scars of lateral roots, and with laterally elongated lenticels; fractured surface dense in texture, light grayish brown, and with light brown radial lines in xylem.

Odor, characteristic; taste, slightly sweet at first, followed by an astringency and a slight bitterness.

Identification (1) Shake 0.5 g of pulverized Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. Shake 3 mL of the filtrate with 1 drop of iron (III) chloride TS: a blue-purple to blue-green color is produced, and it changes to dark blue-purple to dark green.

(2) To 2 g of pulverized Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS upon the plate, and heat at 105°C for 5 minutes: one spot among the spots from

the sample solution and the purple-red spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Peony Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of pulverized Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin Reference Standard (previously determine the water) dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of paeoniflorin in each solution.

Amount (mg) of paeoniflorin ($C_{23}H_{28}O_{11}$) = $W_S \times (A_T/A_S)$

W_S : Amount (mg) of Paeoniflorin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust the flow rate so that the retention time of paeoniflorin is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Powdered Peony Root

Paeoniae Radix Pulverata

シャクヤク末

Powdered Peony Root is the powder of Peony Root.

It contains not less than 2.0% of paeoniflorin ($C_{23}H_{28}O_{11}$: 480.46), calculated on the dried basis.

Description Powdered Peony Root occurs as a light grayish brown powder, and has a characteristic odor and a slightly sweet taste at first, followed by an astringency and a slight bitterness.

Under a microscope <5.01>, Powdered Peony Root reveals starch grains and fragments of parenchyma cells containing them; fragments of cork cells, vessels, tracheids and xylem fibers; rosette aggregates of calcium oxalate, and fragments of rows of crystal cells containing them. Starch grains consist of simple grains, 5–25 μm in diameter, occasionally 2- to 3-compound grains.

Identification (1) Shake 0.5 g of Powdered Peony Root with 30 mL of ethanol (95) for 15 minutes, and filter. To 3 mL of the filtrate add 1 drop of iron (III) chloride TS, and mix: a blue-purple to blue-green color is produced, and thereafter it changes to dark blue-purple to dark green.

(2) To 2 g of Powdered Peony Root add 10 mL of methanol, warm on a water bath for 5 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Paeoniflorin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, ethyl acetate and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-methoxybenzaldehyde-sulfuric acid TS on the plate, and heat at 105°C for 5 minutes: one spot among the spots from the sample solution and the purple spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Peony Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Peony Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Peony Root does not show groups of light yellow stone cells and fibers.

Loss on drying <5.01> Not less than 14.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of Powdered Peony Root, add 50 mL of diluted methanol (1 in 2), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2),

and proceed in the same manner. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Paeoniflorin Reference Standard (previously determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_T and A_S , of paeoniflorin in each solution.

$$\begin{aligned} &\text{Amount (mg) of paeoniflorin (C}_{23}\text{H}_{28}\text{O}_{11}) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Paeoniflorin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 232 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (850:150:1).

Flow rate: Adjust the flow rate so that the retention time of paeoniflorin is about 10 minutes.

System suitability—

System performance: Dissolve 1 mg each of Paeoniflorin Reference Standard and albiflorin in diluted methanol (1 in 2) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, albiflorin and paeoniflorin are eluted in this order with the resolution between these peaks being not less than 2.5.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of paeoniflorin is not more than 1.5%.

Perilla Herb

Perillae Herba

ソヨウ

Perilla Herb is the leaf and twig of *Perilla frutescens* Britton var. *acuta* Kudo or *Perilla frutescens* Britton var. *crispa* Decaisne (*Labiatae*).

Description Usually, contracted and wrinkled leaves, often with thin stems. Both surfaces of the leaf are brownish purple, or the upper surface is grayish green to brownish green, and the lower surface is brownish purple in color. When smoothed by immersion in water, the lamina is ovate to obcordate, 5–12 cm in length, 5–8 cm in width; the apex, acuminate; the margin, serrate; the base, broadly cuneate; petiole, 3–5 cm in length; cross sections of stem and petiole, square. Under a magnifying glass, hairs are observed on both surfaces of the leaf, but abundantly on the vein and sparsely

on other parts; small glandular hairs are observed on the lower surface.

Odor, characteristic; taste slightly bitter.

Identification To 0.3 mL of the mixture of essential oil and xylene, obtained in Essential oil content, add 1 mL of acetic anhydride, shake, and add 1 drop of sulfuric acid: a red-purple to dark red-purple color develops.

Purity (1) Stem—The amount of its stems, which are not less than 3 mm in diameter, contained in Perilla Herb does not exceed 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the stems contained in Perilla Herb does not exceed 1.0%.

(3) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 16.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Essential oil content <5.01> Perform the test with 50.0 g of pulverized Perilla Herb after adding 1 mL of silicone resin to the sample in the flask: the volume of essential oil is not less than 0.2 mL.

Pharbitis Seed

Pharbitidis Semen

ケンゴン

Pharbitis Seed is the seed of *Pharbitis nil* Choisy (*Convolvulaceae*).

Description Longitudinally quartered or separtite globe, 6–8 mm in length, 3–5 mm in width; externally black to grayish red-brown or grayish white, smooth, but slightly shrunken and coarsely wrinkled. The transverse section almost fan-shaped, light yellow-brown to light grayish brown, and dense in texture. Under a magnifying glass, the surface of the seed coat reveals dense, short hairs; dented hilum at the bottom of the ridge. Seed coat thin, the outer layer dark gray, and the inner layer light gray; two irregularly folded cotyledons in the transverse section at one end; two thin membranes from the center of the dorsal side to the ridge separating cotyledons but unrecognizable in the transverse section of the other end having hilum; dark gray secretory pits in the section of the cotyledon. 100 seeds weighing about 4.5 g.

When cracked, odor, slight; taste, oily and slightly pungent.

Total ash <5.01> Not more than 6.0%.

Phellodendron Bark

Phellodendri Cortex

オウバク

Phellodendron Bark is the bark of *Phellodendron*

amurense Ruprecht or *Phellodendron chinense* Schneider (*Rutaceae*), from which the periderm has been removed.

It contains not less than 1.2% of berberine [as berberine chloride ($C_{20}H_{18}ClNO_4$; 371.81)], calculated on the basis of dried material.

Description Flat or rolled semi-tubular pieces of bark, 2–4 mm in thickness; externally grayish yellow-brown to grayish brown, with numerous traces of lenticel; the internal surface yellow to dark yellow-brown in color, with fine vertical lines, and smooth; fractured surface fibrous and bright yellow. Under a magnifying glass, the transverse section of Phellodendron Bark reveals a thin and yellow outer cortex, scattered with stone cells appearing as yellow-brown dots; inner cortex thick; primary medullary rays expanding its width towards the outer end, the phloem appearing as a nearly triangular part between these medullary rays in secondary cortex, and many secondary medullary rays radiating and gathering to the tip of the triangle; brown phloem fiber bundles lined in tangential direction, crossed over the secondary medullary rays, and then these tissues show a latticework.

Odor, slight; taste, extremely bitter; mucilaginous; it colors the saliva yellow on chewing.

Identification (1) To 1 g of pulverized Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R_f* value.

(3) Stir up pulverized Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

Loss on drying <5.01> Not more than 11.0% (105°C, 6 hours).

Total ash <5.01> Not more than 7.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of pulverized Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To the last residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution

as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (previously determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of berberine in each solution.

$$\begin{aligned} &\text{Amount (mg) of berberine [as berberine chloride} \\ &(\text{C}_{20}\text{H}_{18}\text{ClNO}_4)] \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Berberine Chloride Reference Standards, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel (5 to 10 mm in particle diameter).

Column temperature: A constant temperature of about 40°C

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride Reference Standard and palmatine chloride in 10 mL of methanol. Perform the test with 20 mL of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly separating each peak.

System repeatability: Repeat the test 5 times with the standard solution under the above operating conditions the relative deviation of the peak area of berberine is not more than 1.5%.

Powdered Phellodendron Bark

Phellodendri Cortex Pulveratus

オウバク末

Powdered Phellodendron Bark is the powder of Phellodendron Bark.

It contains not less than 1.2% of berberine chloride [as berberine chloride ($\text{C}_{20}\text{H}_{18}\text{ClNO}_4$: 371.81)], calculated on the basis of dried material.

Description Powdered Phellodendron Bark occurs as a bright yellow to yellow powder. It has a slight odor and an extremely bitter taste, is mucilaginous, and colors the saliva yellow on chewing.

Under a microscope <5.01>, Powdered Phellodendron Bark reveals fragments of yellow, thick-walled fiber bundles or fibers, and fibers often accompanied by crystal cell rows; fewer groups of stone cells together with idioblasts; fragments of parenchyma cells containing starch grains and oil droplets; fragments of medullary ray and phloem; mucilage cells and

mucilage masses. Numerous solitary crystals of calcium oxalate, 7 – 20 μ m in diameter; starch grains, simple grains and 2- to 4-compound grains, simple grain, 2 – 6 μ m in diameter; oil droplets, stained red with sudan III TS.

Identification (1) To 1 g of Powdered Phellodendron Bark add 10 mL of diethyl ether, allow to stand for 10 minutes with occasional shaking, and filter to remove the diethyl ether. Collect the powder on the filter paper, add 10 mL of ethanol (95), allow to stand for 10 minutes with occasional shaking, and filter. To 2 to 3 drops of the filtrate add 1 mL of hydrochloric acid, add 1 to 2 drops of hydrogen peroxide TS, and shake: a red-purple color develops.

(2) Use the filtrate obtained in (1) as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same R_f value.

(3) Stir up Powdered Phellodendron Bark with water: the solution becomes gelatinous owing to mucilage.

Purity Curcuma—Place Powdered Phellodendron Bark on filter paper, drop diethyl ether on it, and allow to stand. Take the powder off the filter paper, and drip 1 drop of potassium hydroxide TS: no red-purple color develops. Under a microscope <5.01>, Powdered Phellodendron Bark does not contain gelatinized starch or secretory cells containing yellow-red resin.

Loss on drying <5.01> Not more than 11.0% (105°C 6 hours).

Total ash <5.01> Not more than 7.5%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Assay Weigh accurately about 0.5 g of Powdered Phellodendron Bark, add 30 mL of a mixture of methanol and dilute hydrochloric acid (100:1), heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. Repeat the above procedure twice with the residue, using 30-mL and 20-mL portions of a mixture of methanol and dilute hydrochloric acid (100:1). To obtained residue add 10 mL of methanol, shake well, and filter. Combine the whole filtrates, add methanol to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Berberine Chloride Reference Standard (separately determine the water <2.48> in the same manner as Berberine Chloride Hydrate), dissolve in methanol to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 20 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of berberine in each solution.

$$\begin{aligned} &\text{Amount (mg) of berberine ([as berberine chloride} \\ &(\text{C}_{20}\text{H}_{18}\text{ClNO}_4)] \end{aligned}$$

$$= W_s \times (A_T/A_S)$$

W_s : Amount (mg) of berberine chloride for component determination Berberine Chloride Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 345 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.4 g of potassium dihydrogenphosphate and 1.7 g of sodium lauryl sulfate in 1000 mL of a mixture of water and acetonitrile (1:1).

Flow rate: Adjust the flow rate so that the retention time of berberine is about 10 minutes.

Selection of column: Dissolve 1 mg each of Berberine Chloride Reference Standard and palmatine chloride in 10 mL of methanol. Proceed with 20 μ L of this solution under the above operating conditions. Use a column giving elution of palmatine and berberine in this order, and clearly dividing each peak.

System repeatability: When repeat the test 5 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of berberine is not more than 1.5%.

Compound Phellodendron Powder for Cataplasm

パップ用複方オウバク散

Method of preparation

Powdered Phellodendron Bark	660 g
Powdered Gardenia Fruit	325 g
<i>d</i> - or <i>dl</i> -Camphor	10 g
<i>dl</i> - or <i>l</i> -Menthol	5 g
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Phellodendron Powder for Cataplasm occurs as a yellow-brown powder, having a characteristic odor.

Identification Shake thoroughly 0.2 g of Compound Phellodendron Powder for Cataplasm with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use the solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot

among the several spots from the sample solution has the same color tone and *R_f* value with the yellow to yellow-green fluorescent spot from the standard solution (phellodendron bark).

Containers and storage Containers—Tight containers.

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder

オウバク・タンナルビン・ビスマス散

Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder contains not less than 12.9% and not more than 16.3% of bismuth (Bi: 208.98).

Method of preparation

Powdered Phellodendron Bark	300 g
Albumin Tannate	300 g
Bismuth Subnitrate	200 g
Scopolia Extract	10 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make	1000 g

Prepare as directed under Powders, with the above ingredients. Scopolia Extract Powder may be used in place of Scopolia Extract.

Description Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder is brownish yellow in color, and has a bitter taste.

Identification (1) Shake thoroughly 0.1 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder with 5 mL of methanol, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Berberine Chloride Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a spot with yellow to yellow-green fluorescence from the standard solution show the same color tone and the same *R_f* value (phellodendron bark).

(2) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 20 mL of ethanol (95), heat in a water bath for 3 minutes with shaking, cool, and filter. To 10 mL of the filtrate add 1 drop of iron (III) chloride TS: a blue-green color is produced. Allow to stand: a bluish black precipitate is produced (albumin tannate).

(3) To 0.3 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder add 10 mL of diluted pyridine (1 in 5), warm in a water bath for 3 minutes with shaking, cool, and filter. Add 1 mL of ninhydrin-ascorbic acid TS to the filtrate, and heat in a water bath: a blue color is produced (albumin tannate).

(4) To 0.5 g of Phellodendron, Albumin Tannate and

Bismuth Subnitrate Powder add 5 mL of dilute hydrochloric acid and 10 mL of water, warm, shake thoroughly, and filter. The filtrate responds to the Qualitative Tests <1.09> for bismuth salt.

Assay Weigh accurately about 0.7 g of Phellodendron, Albumin Tannate and Bismuth Subnitrate Powder, shake well with 10 mL of water and 20 mL of diluted nitric acid (1 in 3), add water to make exactly 100 mL, and filter. Discard the first 20 mL of the filtrate, pipet the subsequent 10 mL of the filtrate, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 0.23 g of bismuth nitrate pentahydrate, add 20 mL of diluted nitric acid (1 in 3) and water to make exactly 100 mL. Pipet 10 mL of this solution, and add water to make exactly 100 mL. Pipet 25 mL of this solution, add diluted nitric acid (1 in 100) to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances, A_T and A_S , of the sample solution and standard solution according to Atomic Absorption Spectrophotometry <2.23> under the following conditions. On the other hand, determine the absorbance A_0 of the solution prepared in the same manner using 20 mL of diluted nitric acid (1 in 3) instead of the standard solution.

Gas: Combustible gas—Acetylene

Supporting gas—Air

Lamp: A bismuth hollow-cathode lamp

Wavelength: 223.1 nm

$$\begin{aligned} & \text{Amount (mg) of bismuth (Bi)} \\ & = W \times \{(A_T - A_0)/(A_S - A_0)\} \times 0.4308 \end{aligned}$$

W : Amount (mg) of bismuth nitrate pentahydrate

Containers and storage Containers—Well-closed containers.

Picrasma Wood

Picrasmae Lignum

ニガキ

Picrasma Wood is the wood of *Picrasma quassioides* Bennet (*Simaroubaceae*).

Description Light yellow chips, slices or short pieces of wood; a transverse section reveals distinct annual rings and thin medullary rays; tissue dense in texture.

Odorless; taste, extremely bitter and lasting.

Under a microscope <5.01>, it reveals medullary rays consisting of 1–5 cells wide for transverse section, and 5–50 cells high for longitudinal section; vessels of spring wood up to about 150 μm in diameter, but those of autumn wood only one-fifth as wide; vessels, single or in groups, scattered in the xylem parenchyma; membrane of wood fibers extremely thickened; medullary rays and xylem parenchyma cells contain rosette aggregates of calcium oxalate and starch grains. Vivid yellow or red-brown, resinous substance often present in the vessels.

Purity Foreign matter <5.01>—The amount of foreign matter contained in Picrasma Wood does not exceed 1.0%.

Total ash <5.01> Not more than 4.0%.

Powdered Picrasma Wood

Picrasmae Lignum Pulveratum

ニガキ末

Powdered Picrasma Wood is the powder of Picrasma Wood.

Description Powdered Picrasma occurs as a grayish white to light yellow powder. It is odorless, and has an extremely bitter and lasting taste.

Under a microscope <5.01>, Powdered Picrasma Wood reveals fragments of vessels of various sizes, xylem fibers and xylem parenchyma cells; fragments of medullary rays containing starch grains; all tissues lignified; a few crystals of calcium oxalate observed. Starch grains are 5 to 15 μm in diameter.

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Pinellia Tuber

Pinelliae Tuber

ハンゲ

Pinellia Tuber is the tuber of *Pinellia ternata* Breitenbach (*Araceae*), from which the cork layer has been removed.

Description Slightly flattened spherical to irregular-shaped tuber; 0.7–2.5 cm in diameter and 0.7–1.5 cm in height; externally white to grayish white-yellow; the upper end dented, where the stem has been removed, with root scars dented as numerous small spots on the circumference; dense in texture; cross section white and powdery.

Almost odorless; tasteless at first, slightly mucous, but leaving a strong acrid taste.

Under a microscope <5.01>, a transverse section reveals mainly tissue of parenchyma filled with starch grains, and scattered with a few mucilage cells containing raphides of calcium oxalate. Starch grains mostly 2- to 3-compound grains, usually 10–15 μm in diameter, and simple grains, usually 3–7 μm in diameter; raphides of calcium oxalate 25–150 μm in length.

Purity (1) Rhizome of *Arisaema* species and others—Under a microscope <5.01>, no mucilage canal is revealed on the outer layer of cortex.

(2) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Pinellia Tuber according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Pinellia Tuber according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 3.5%.

Plantago Herb

Plantaginis Herba

シャゼンソウ

Plantago Herb is the entire plant of *Plantago asiatica* Linné (*Plantaginaceae*), collected during the flowering season.

Description Usually wrinkled and contracted leaf and spike, grayish green to dark yellow-green in color; when soaked in water and smoothed out, the lamina is ovate to orbicular-ovate, 4 – 15 cm in length, 3 – 8 cm in width; apex acute, and base sharply narrowed; margin slightly wavy, with distinct parallel veins; glabrous or nearly glabrous; petiole is rather longer than the lamina, and its base is slightly expanded with thin-walled leaf-sheath; scape is 10 – 50 cm in length, one-third to one-half of the upper part forming the spike, with dense florets; the lower part of inflorescence often shows pyxidial; roots usually removed, but, if any, fine roots are closely packed.

Odor, slight; tasteless.

Identification To 2.0 g of pulverized Plantago Herb add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (7:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride TS on the plate: a dark blue spot appears at the R_f value about 0.5.

Total ash <5.01> Not more than 15.0%.

Acid-insoluble ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 14.0%.

Plantago Seed

Plantaginis Semen

シャゼンシ

Plantago Seed is the seed of *Plantago asiatica* Linné (*Plantaginaceae*).

Description Flattened ellipsoidal seed, 2 – 2.5 mm in length, 0.7 – 1 mm in width, 0.3 – 0.5 mm in thickness; externally brown to yellow-brown and lustrous. Under a magnifying glass, the surface of the seed is practically smooth, with the dorsal side protruding like a bow, and with the ventral side somewhat dented; micropyle and raphe not observable. 100 seeds weigh about 0.05 g.

Odorless; taste, slightly bitter and mucous.

Under a microscope <5.01>, a transverse section reveals a seed coat consisting of three layers of epidermis composed of

cells containing mucilage, a vegetative layer, and a pigment layer of approximately equidiameter cells; in the interior, endosperm thicker than seed coat, enclosing two cotyledons.

Identification (1) To 1 g of Plantago Seed add 2 mL of warm water, and allow the mixture to stand for 10 minutes: the seed coat swells to discharge mucilage.

(2) Boil gently 1 g of Plantago Seed with 10 mL of dilute hydrochloric acid for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS, to 3 mL of this solution add 1 mL of Fehling's TS, and warm the mixture: a red precipitate is produced.

Purity Foreign matter <5.01>—The amount of foreign matter contained in Plantago Seed does not exceed 2.0%.

Total ash <5.01> Not more than 5.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Platycodon Fluidextract

キキヨウ流エキス

Method of preparation Take coarse powder of platycodon, and prepare the fluidextract as directed under Fluidextracts using 25 vol% ethanol. An appropriate quantity of Ethanol and Purified Water may be used in place of 25 vol% ethanol.

Description Platycodon Fluidextract is a red-brown liquid. It is miscible with water, producing slight turbidity. It has a mild taste at first, followed by an acrid and bitter taste.

Identification (1) Shake vigorously 0.5 mL of Platycodon Fluidextract with 10 mL of water: a lasting fine foam is produced.

(2) Dissolve 1 drop of Platycodon Fluidextract in 2 mL of acetic anhydride, and add gently 0.5 mL of sulfuric acid: a red to red-brown color develops at the zone of contact.

Purity Starch—Mix 1 mL of Platycodon Fluidextract with 4 mL of water, and add 1 drop of dilute iodine TS: no purple or blue color develops.

Content of the active principle Transfer exactly 5 mL of Platycodon Fluidextract to a tared beaker, evaporate to dryness on a water bath, and dry at 105°C for 5 hours: the mass of the residue is not less than 0.50 g.

Containers and storage Containers—Tight containers.

Storage—Light-resistant.

Platycodon Root

Platycodi Radix

キキヨウ

Platycodon Root is the root of *Platycodon grandiflorum* A. De Candolle (*Campanulaceae*).

Description Irregular, somewhat thin and long fusiform to conical root, often branched; externally grayish brown, light brown or white; main root 10 – 15 cm in length, 1 – 3 cm in diameter; the upper end, with dented scars of removed stems;

the neighborhood, with fine lateral wrinkles and longitudinal furrows and also slightly constricted; the greater part of the root, except the crown, covered with coarse longitudinal wrinkles, lateral furrows and lenticel-like lateral lines; hard in texture, but brittle; fractured surface not fibrous, often with cracks. Under a magnifying glass, a transverse section reveals cambium and its neighborhood often brown in color; cortex slightly thinner than xylem, almost white and with scattered cracks; xylem white to light brown in color, and the tissue slightly denser than cortex.

Odor, slight; tasteless at first, later acrid and bitter.

Identification (1) Boil 0.5 g of pulverized Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of pulverized Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red to red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to green color.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 25.0%.

Powdered Platycodon Root

Platycodi Radix Pulverata

キキヨウ末

Powdered Platycodon Root is the powder of Platycodon Root.

Description Powdered Platycodon Root occurs as a light grayish yellow to light grayish brown powder. It has a slight odor, and is tasteless at first, later acrid and bitter.

Under a microscope <5.01>, Powdered Platycodon Root reveals numerous fragments of colorless parenchyma cells; fragments of reticulate vessels and scalariform vessels; fragments of sieve tubes and lactiferous tubes; fragments of cork layer are sometimes observed. Usually, starch grains are not observed, but very rarely simple grain.

Identification (1) Boil 0.5 g of Powdered Platycodon Root with 10 mL of water for a while, allow to cool, and shake the mixture vigorously: a lasting fine foam is produced.

(2) Warm 0.2 g of Powdered Platycodon Root with 2 mL of acetic anhydride on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add carefully 0.5 mL of sulfuric acid to make two layers: a red to red-brown color develops at the zone of contact, and the upper layer acquires a blue-green to green color.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of

Powdered Platycodon Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Platycodon Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Platycodon Root does not show fibers, stone cells or other foreign matter.

Total ash <5.01> Not more than 4.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 25.0%.

Polygala Root

Polygalae Radix

オンジ

Polygala Root is the root of *Polygala tenuifolia* Willdenow (*Polygalaceae*).

Description Thin, long and bent, cylindrical or tubular root; main root, 10 – 20 cm in length, 0.2 – 1 cm in diameter, sometimes with one to several lateral roots; externally light grayish brown, with coarse longitudinal wrinkles, and with deep lateral furrows cracked to some degree here and there; brittle, and fractured surface not fibrous; margin of the transverse section irregularly undulate; cortex, comparatively thick, with large cracks here and there; xylem usually round to elliptical, light brown in color, and often tears in a wedge-like shape.

Odor, slight; taste, slightly acrid.

Identification (1) Shake vigorously 0.5 g of pulverized Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of pulverized Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and changes to dark green.

Purity (1) Stem—The amount of the stems contained in Polygala Root does not exceed 10.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the stems contained in Polygala Root is not more than 1.0%.

(3) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 6.0%.

Powdered Polygala Root

Polygalae Radix Pulverata

オンジ末

Powdered Polygala Root is the powder of Polygala

Root.

Description Powdered Polygala Root occurs as a light grayish yellow-brown powder. It has a slight odor and a slightly acid taste.

Under a microscope <5.01>, Powdered Polygala Root reveals fragments of cork layers, pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers and xylem parenchyma cells with a small number of simple pits; fragments of parenchyma cells containing substances such as oil droplets, rosette aggregates and solitary crystals of calcium oxalate. Oil drop-like contents stained red with sudan III TS.

Identification (1) Shake vigorously 0.5 g of Powdered Polygala Root with 10 mL of water: a lasting fine foam is produced.

(2) To 0.5 g of Powdered Polygala Root add 2 mL of acetic anhydride. After shaking well, allow to stand for 2 minutes, and filter. To the filtrate add carefully 1 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact, and changes to dark green later.

Purity (1) Foreign matter <5.01>—Under a microscope, Powdered Polygala Root does not show stone cells or starch grains.

(2) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Total ash <5.01> Not more than 6.0%.

Polygonatum Rhizome

Polygonati Rhizoma

オウセイ

Polygonatum Rhizome is the rhizome of *Polygonatum falcatum* A. Gray, *Polygonatum sibiricum* Redoute, *Polygonatum kingianum* Collett et Hemsley or *Polygonatum cyrtoneura* Hua (*Liliaceae*), usually after being steamed.

Description Irregularly cylindrical rhizome, 3–10 cm in length, 0.5–3 cm in diameter; or irregular massive rhizome, 5–10 cm in length, 2–6 cm in diameter, occasionally branched; both rhizomes with many cyclic nodes and longitudinally striate; externally yellowish brown to dark brown; stem scars, round, concave at their center, and protuberant on the upper surface; root scars on the lower surface; cut surface flat and horny.

Odor, slight; taste, slightly sweet.

Under a microscope <5.01>, a transverse section of the rhizome reveals epidermis coated with cuticle; inside of epidermis parenchyma lie; numerous vascular bundles and mucilage cells scattered in parenchyma; vascular bundles collateral or amphivasal concentric; mucilage cells contain raphides of calcium oxalate.

Identification (1) To 0.5 g of fine cutted Polygonatum Rhizome add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid: a red-brown color appears at the zone of contact.

(2) To 1.0 g of fine cutted Polygonatum Rhizome add 10

mL of dilute hydrochloric acid, boil gently for 2 minutes, and filter. Neutralize the filtrate with sodium hydroxide TS. To 3 mL of this solution add 1 mL of Fehling's TS, and warm: red precipitates appear.

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Polygonum Root

Polygoni Multiflori Radix

カシュウ

Polygonum Root is the root of *Polygonum multiflorum* Thunberg (*Polygonaceae*), often being cut into round slices.

Description Polygonum Root is nearly fusiform, 10 to 15 cm in length, 2 to 5 cm in diameter; externally red-brown to dark brown; roughly wrinkled; a cross section light red-brown or light grayish brown, with numerous abnormal vascular bundles scattering irregularly around the large vascular bundles near center; heavy and hard in texture.

Odor, slight and characteristic; taste, astringent and slightly bitter.

Under a microscope <5.01>, transverse section reveals the outermost layer to be several cells thick and composed of cork; cork cells contain brown substances; cortex composed of parenchyma; abnormal vascular bundles, exhibiting a ring of cambium; xylem lies inside of the cambium, and phloem outside; fibers lie outside the phloem; central portion of root lignified; parenchymatous cells contain aggregated crystals of calcium oxalate, and both simple and 2- to 8-compound starch grains; navel of starch grain obvious.

Identification To 1 g of pulverized Polygonum Root add 10 mL of methanol, shake for 15 minutes, and filter. Evaporate the filtrate to dryness, dissolve the residue in 2 mL of methanol, and use this as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, water, methanol and acetic acid (100) (200:10:10:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): a fluorescent bluish white spot appears at around *R_f* 0.3.

Loss on drying <5.01> Not more than 14.0% (6 hours).

Total ash <5.01> Not more than 5.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 17.0%.

Polyporus Sclerotium

Polyporus

チヨレイ

Polyporus Sclerotium is the sclerotium of *Polyporus*

umbellatus Fries (*Polyporaceae*).

Description Irregularly shaped mass, usually 5 – 15 cm in length; externally blackish brown to grayish brown, with numerous dents and coarse wrinkles; breakable; fractured surface rather soft and cork-like, and almost white to light brown in color, and a white speckled pattern on the inner region; light in texture.

Odorless and tasteless.

Identification Warm, while shaking, 0.5 g of pulverized *Polyporus Sclerotium* with 5 mL of acetone on a water bath for 2 minutes, filter, and evaporate the filtrate to dryness. Dissolve the residue in 5 drops of acetic anhydride, and add 1 drop of sulfuric acid: a red-purple color develops, and immediately changes to dark green.

Total ash <5.01> Not more than 16.0%.

Acid-insoluble ash <5.01> Not more than 4.0%.

Powdered Polyporus Sclerotium

Polyporus Pulveratus

チヨレイ末

Powdered *Polyporus Sclerotium* is the powder of the *Polyporus Sclerotium*.

Description Powdered *Polyporus Sclerotium* occurs as a light grayish brown to light brown powder. It has a slight odor, and a slightly bitter and acid taste, is gritty between the teeth on chewing.

Under a microscope <5.01>, Powdered *Polyporus Sclerotium* reveals hypha, 1 to 2 μm , rarely up to 13 μm in diameter, and colorless transparent; granule strongly refracting light; and a few mucilage plates; sometimes fragments of false tissue consisting of them; somewhat brown false tissues; and solitary crystal. Solitary crystal is 10 to 40 μm in diameter, sometimes 100 μm in diameter.

Identification Warm, while shaking, 0.5 g of Powdered *Polyporus Sclerotium* with 5 mL of acetone on a water bath for 2 minutes, filter and evaporate the filtrate to dryness. Dissolve the residue in 5 drops of acetic anhydride, and add 1 drop of sulfuric acid: a red-purple color develops, and immediately changes to dark green.

Total ash <5.01> Not more than 16.0%.

Acid-insoluble ash <5.01> Not more than 4.0%.

Containers and storage Containers—Tight containers.

Poria Sclerotium

Poria

ブクリヨウ

Poria Sclerotium is the sclerotium of *Poria cocos* Wolf (*Polyporaceae*), from which usually the external layer has been mostly removed.

Description Mass, about 10 – 30 cm in diameter, up to 0.1 –

2 kg in mass; usually it appears as broken or chipped pieces; white or slightly reddish white; sclerotium with remaining outer layer is dark brown to dark red-brown in color, coarse, which fissures; hard in texture, but brittle.

Almost odorless, tasteless, and slightly mucous.

Identification (1) Warm 1 g of pulverized *Poria Sclerotium* with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To a section or powder of *Poria Sclerotium* add 1 drop of iodine TS: a deep red-brown color is produced.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of *Poria Sclerotium* according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of *Poria Sclerotium* according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 1.0%.

Powdered Poria Sclerotium

Poria Pulveratum

ブクリヨウ末

Powdered *Poria Sclerotium* is the powder of *Poria Sclerotium*.

Description Powdered *Poria Sclerotium* occurs as a white to grayish white powder. It is almost odorless and tasteless, but is slightly mucous.

Under a microscope <5.01>, Powdered *Poria Sclerotium* reveals colorless and transparent hyphae strongly refracting light, and fragments of false tissue consisting of granules and mucilage plates. Thin hyphae, 2 – 4 μm in diameter; thick ones, usually 10 – 20 μm , up to 30 μm .

Identification (1) Warm 1 g of Powdered *Poria Sclerotium* with 5 mL of acetone on a water bath for 2 minutes with shaking, and filter. Evaporate the filtrate to dryness, dissolve the residue in 0.5 mL of acetic anhydride, and add 1 drop of sulfuric acid: a light red color develops, which changes immediately to dark green.

(2) To Powdered *Poria Sclerotium* add 1 drop of iodine TS: a deep red-brown color is produced.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered *Poria Sclerotium* according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered *Poria Sclerotium* according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered *Poria Sclerotium* does not show starch grains.

Total ash <5.01> Not more than 1.0%.

Processed Aconite Root

Processi Aconiti Radix

フシ

Processed Aconite Root is the tuberous root of *Aconitum carmichaeli* Debeaux or *Aconitum japonicum* Thunberg (*Ranunculaceae*) prepared by the following processes.

Process 1: Autoclaving. [Processed Aconite Root 1]

Process 2: Heating or autoclaving after rinsing in salt or rock salt solution. [Processed Aconite Root 2]

Process 3: Treating with lime after rinsing in salt solution. [Processed Aconite Root 3]

Processed Aconite Root 1, Processed Aconite Root 2 and Processed Aconite Root 3 contain the total alkaloid [as benzoyl aconin ($C_{32}H_{45}NO_{10}$: 603.70)] of not less than 0.7% and not more than 1.5%, not less than 0.1% and not more than 0.6%, and not less than 0.5% and not more than 0.9%, calculated on the dried bases, respectively.

The label indicates the treating process.

Description Processed Aconite Root 1: Cut pieces irregularly polygonal, less than 10 mm in diameter; externally dark grayish brown to blackish brown; hard in texture; cut surface flat, light brown to dark brown, usually horny and lustrous.

Odor, weak and characteristic.

Under a microscope <5.0I>, transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains in parenchymatous cells usually gelatinized but sometimes not gelatinized; starch grains, simple, spherical or ellipsoid, 2 – 25 μm in diameter, or 2- to a dozen or so-compound, hilum of starch grain distinct.

Processed Aconite Root 2: Nearly obconical root, 15 – 30 mm in length, 12 – 16 mm in diameter, slices cut longitudinally or transversely, 20 – 60 mm in length, 15 – 40 mm in width, and 200 – 700 μm in thickness, or cut pieces irregularly polygonal, less than 12 mm in diameter; externally light brown to dark brown or yellowish brown; hard in texture, usually without wrinkles; cut surface flat, light brown to dark brown or yellowish white to light yellowish brown, usually horny, semi-transparent and lustrous.

Odor, weak and characteristic.

Under a microscope <5.0I>, transverse and longitudinal sections reveal metaderm, primary cortex, endodermis, secondary cortex, cambium, and xylem; primary cortex contains oblong to oblong-square sclerenchymatous cells, 30 – 75 μm in short axis, 60 – 150 μm in long axis; endodermis single layered, endodermal cells elongated in tangential direction; cambium, star shaped or irregular polygons to orbicular; a group of vessel in xylem v-shaped; sometimes isolated ring of cambium appears in secondary cortex or in pith; vessels, pitted, scaraliform, reticulate and spiral; starch grains in parenchymatous cells gelatinized.

Processed Aconite Root 3: Cut pieces irregularly polygonal, less than 5 mm in diameter; externally grayish brown; hard in texture; cut surface flat, light grayish brown to grayish white,

not lustrous.

Odor, weak and characteristic.

Under a microscope <5.0I>, transverse and longitudinal sections reveal pitted, scaraliform, reticulate and spiral vessels; starch grains, simple, spherical or ellipsoid, 2 – 25 μm in diameter, or 2- to a dozen or so-compound, hilum of starch grain distinct.

Identification To 3 g of pulverized Processed Aconite Root add 20 mL of diethyl ether and 2 mL of ammonia TS, shake for 10 minutes, centrifuge, and take the ether layer. Evaporate the ether layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution.

Purity Aconitum diester alkaloids (aconitine, jesaconitine, hypaconitine and mesaconitine)—Weigh accurately about 0.5 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process twice more. Combine all extracts, evaporate to dryness under reduced pressure below 40°C, and dissolve the residue with exactly 10 mL of a mixture of the phosphate buffer solution for aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μL each of the sample solution and aconitum diester alkaloids standard solution as directed under Liquid Chromatography <2.0I> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA} , jesaconitine, H_{TJ} and H_{SJ} , hypaconitine, H_{TH} and H_{SH} , and mesaconitine, H_{TM} and H_{SM} , respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hypaconitine and mesaconitine per g calculated on the dried basis are not more than 60 μg , 60 μg , 280 μg and 140 μg , respectively, and the total amount of them is not more than 450 μg .

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of aconitine } (C_{34}H_{47}NO_{11}) \\ = (C_{SA}/W) \times (H_{TA}/H_{SA}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of jesaconitine } (C_{35}H_{49}NO_{12}) \\ = (C_{SJ}/W) \times (H_{TJ}/H_{SJ}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of hypaconitine } (C_{33}H_{45}NO_{10}) \\ = (C_{SH}/W) \times (H_{TH}/H_{SH}) \times 10 \end{aligned}$$

$$\text{Amount } (\mu\text{g}) \text{ of mesaconitine } (C_{33}H_{45}NO_{11})$$

$$= (C_{SM}/W) \times (H_{TM}/H_{SM}) \times 10$$

C_{SA} : Concentration ($\mu\text{g}/\text{mL}$) of aconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SJ} : Concentration ($\mu\text{g}/\text{mL}$) of jesaconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SH} : Concentration ($\mu\text{g}/\text{mL}$) of hypaconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SM} : Concentration ($\mu\text{g}/\text{mL}$) of mesaconitine for purity in the aconitum diester alkaloids standard solution for purity

W : Amount (g) of the sample, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hypaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography ($5\ \mu\text{m}$ in particle diameter).

Column temperature: A constant temperature of about 40°C .

Mobile phase: A mixture of the phosphate buffer solution for aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 31 minutes.

System suitability—

System performance: When the procedure is run with $20\ \mu\text{L}$ of the aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hypaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of the phosphate buffer solution for aconite root and acetonitrile (1:1) to make 10 mL. When the test is repeated 6 times with $20\ \mu\text{L}$ of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01>

Processed Aconite Root 1: Not more than 4.0%.

Processed Aconite Root 2: Not more than 12.0%.

Processed Aconite Root 3: Not more than 19.0%.

Acid-insoluble ash <5.01> Not more than 0.9%.

Assay Weigh accurately about 2 g of pulverized Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate <2.50> with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3

drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

Each mL of 0.01 mol/L hydrochloric acid VS
= 6.037 mg of total alkaloid [as benzoyleaconine
($\text{C}_{32}\text{H}_{45}\text{NO}_{10}$)]

Powdered Processed Aconite Root

Processi Aconiti Radix Pulverata

フシ末

Powdered Processed Aconite Root is the powder of Processed Aconite Root prepared by the process 1 or process 2, the powder of Processed Aconite Root prepared by process 1, or the powder of Processed Aconite Root prepared by the process 1 to which Corn Starch or Lactose Hydrate is added.

Process 1: Autoclaving. [Powdered Processed Aconite Root 1]

Process 2: Heating or autoclaving after rinsing in salt or rock salt solution. [Powdered Processed Aconite Root 2]

Powdered Processed Aconite Root 1 and Powdered Processed Aconite Root 2 contain the total alkaloid [as benzoyl aconin ($\text{C}_{32}\text{H}_{45}\text{NO}_{10}$: 603.70)] of not less than 0.4% and not more than 1.2%, and not less than 0.1% and not more than 0.3%, calculated on the dried bases, respectively.

The label indicates the treating process.

Description Powdered Processed Aconite Root 1: Powdered Processed Aconite Root 1 occurs as a light grayish brown powder. It has a characteristic odor.

Under a microscope <5.01>, Powdered Processed Aconite Root 1 reveals gelatinized starch masses or starch grains and parenchymatous cells containing them, fragments of reddish brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, $30 - 150\ \mu\text{m}$ in diameter, $100 - 250\ \mu\text{m}$ in length, cell wall of sclerenchymatous cells, $6 - 12\ \mu\text{m}$ in thickness; starch grains of *Aconitum carmichaeli* Debeaux or *Aconitum japonicum* Thunberg (*Ranunculaceae*) origin, simple, spherical or ellipsoid, $2 - 25\ \mu\text{m}$ in diameter, or 2- to a dozen or so- compound, hilum of starch grain distinct.

Powdered Processed Aconite Root 2: Powdered Processed Aconite Root 2 occurs as a light yellowish white powder. It has a characteristic odor.

Under a microscope <5.01>, Powdered Processed Aconite Root 2 reveals gelatinized starch masses and parenchymatous cells containing them, fragments of reddish brown metaderm, fragments of pitted, scaraliform, reticulate and spiral vessels; also square to oblong-square sclerenchymatous cells, $30 - 150\ \mu\text{m}$ in diameter, $100 - 250\ \mu\text{m}$ in length, cell wall of sclerenchymatous cells, $6 - 12\ \mu\text{m}$ in thickness.

Identification To 3 g of Powdered Processed Aconite Root add 2 mL of ammonia TS and 20 mL of diethyl ether, shake for 10 minutes, and centrifuge. Evaporate the ether layer to dryness under reduced pressure, dissolve the residue in 1 mL of diethyl ether, and use this solution as the sample solution. Separately, dissolve 1 mg of benzoylmesaconine hydrochloride

ride for thin-layer chromatography in 10 mL of ethanol (99.5), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate, ethanol (99.5) and ammonia water (28) (40:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly Dragendorff's TS for spraying on the plate, air-dry the plate, and spray evenly sodium nitrite TS: one of the spot among the several spots from the sample solution has the same color tone and Rf value with the yellow-brown spot from the standard solution.

Purity Aconitum diester alkaloids (aconitine, jesaconitine, hyaconitine and mesaconitine)—Weigh accurately about 0.5 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, suspend in 3.0 mL of water by shaking, and add 1.0 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 1.0 mL of ammonia TS and 20 mL of diethyl ether, and repeat the above process two times. Combine all extracts, evaporate to dryness under reduced pressure at not more than 40°C, and dissolve the residue with exactly 10 mL of a mixture of phosphate buffer solution for aconite root and acetonitrile (1:1). Centrifuge this solution, and use the supernatant liquid as the sample solution. Perform the test with exactly 20 μ L each of the sample solution and aconitum diester alkaloids standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the heights of the peaks corresponding to aconitine, H_{TA} and H_{SA} , jesaconitine, H_{TJ} and H_{SJ} , hyaconitine, H_{TH} and H_{SH} , and mesaconitine, H_{TM} and H_{SM} , respectively, and calculate the amounts of them by the following formulae: the amounts of aconitine, jesaconitine, hyaconitine and mesaconitine per g calculated on the dried basis are not more than 55 μ g, 40 μ g, 55 μ g and 120 μ g, respectively, and the total amount of them is not more than 230 μ g.

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of aconitine } (\text{C}_{34}\text{H}_{47}\text{NO}_{11}) \\ = (C_{SA}/W) \times (H_{TA}/H_{SA}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of jesaconitine } (\text{C}_{35}\text{H}_{49}\text{NO}_{12}) \\ = (C_{SJ}/W) \times (H_{TJ}/H_{SJ}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of hyaconitine } (\text{C}_{33}\text{H}_{45}\text{NO}_{10}) \\ = (C_{SH}/W) \times (H_{TH}/H_{SH}) \times 10 \end{aligned}$$

$$\begin{aligned} \text{Amount } (\mu\text{g}) \text{ of mesaconitine } (\text{C}_{33}\text{H}_{45}\text{NO}_{11}) \\ = (C_{SM}/W) \times (H_{TM}/H_{SM}) \times 10 \end{aligned}$$

C_{SA} : Concentration (μ g/mL) of aconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SJ} : Concentration (μ g/mL) of jesaconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SH} : Concentration (μ g/mL) of hyaconitine for purity in the aconitum diester alkaloids standard solution for purity

C_{SM} : Concentration (μ g/mL) of mesaconitine for purity in the aconitum diester alkaloids standard solution for purity

W : Amount (g) of the sample, calculated on the dried basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 231 nm for aconitine, hyaconitine and mesaconitine; 254 nm for jesaconitine).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of the phosphate buffer solution for aconite root and tetrahydrofuran (183:17).

Flow rate: Adjust the flow rate so that the retention time of mesaconitine is about 31 minutes.

System suitability—

System performance: When the procedure is run with 20 μ L of the aconitum diester alkaloids standard solution for purity under the above operating conditions, using 254 nm, mesaconitine, hyaconitine, aconitine and jesaconitine are eluted in this order, and each resolution between their peaks is not less than 1.5, respectively.

System repeatability: To 1 mL of aconitum diester alkaloids standard solution for purity add a mixture of phosphate buffer solution for aconite root and acetonitrile (1:1) to make 10 mL. When the test is repeated 6 times with 20 μ L of this solution under the above operating conditions, using 231 nm, the relative standard deviation of the peak height of mesaconitine is not more than 1.5%.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01>

Powdered Processed Aconite Root 1: Not more than 4.0%.

Powdered Processed Aconite Root 2: Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.7%.

Assay Weigh accurately about 2 g of Powdered Processed Aconite Root, put in a glass-stoppered centrifuge tube, and add 1.6 mL of ammonia TS and 20 mL of diethyl ether. Stopper tightly the tube, shake for 30 minutes, centrifuge, and separate the ether layer. To the residue add 0.8 mL of ammonia TS and 20 mL of diethyl ether, and proceed as above. Repeat this process more three times. Combine all extracts, evaporate to dryness under reduced pressure, dissolve the residue in 5 mL of ethanol (99.5), add 30 mL of freshly boiled and cooled water, and titrate <2.50> with 0.01 mol/L hydrochloric acid VS until the color of the solution changes from green to gray-blue through blue-green (indicator: 3 drops of methyl red-methylene blue TS). Perform a blank determination and make any necessary correction.

$$\begin{aligned} \text{Each mL of 0.01 mol/L hydrochloric acid VS} \\ = 6.037 \text{ mg of total alkaloid [as benzoylaconine} \\ (\text{C}_{32}\text{H}_{45}\text{NO}_{10})] \end{aligned}$$

Processed Ginger

Zingiber Processum Rhizoma

カンキョウ

Processed Ginger is the rhizome of *Zingiber officinale* Roscoe (*Zingiberaceae*), after being passed through hot water or being steamed.

Description Irregularly compressed and often branched massive rhizome; branched parts slightly curved ovoid or oblong-ovoid, 2–4 cm in length, and 1–2 cm in diameter; external surface grayish yellow to grayish yellow-brown, with wrinkles and ring node; fractured surface brown to dark brown, transparent and horny; under a magnifying glass, a transverse section reveals cortex and stele distinctly divided; vascular bundles scattered throughout the surface.

Odor, characteristic; taste, extremely pungent.

Under a microscope <5.01>, a transverse section reveals cork layer, cortex and stele in this order from the outside; cortex and stele, divided by a single-layered endodermis, composed of parenchyma, vascular bundles scattered and surrounded by fiber bundles; oil cells contain yellow oil-like substances, scattered in parenchyma; parenchymatous cells contain solitary crystals of calcium oxalate, and gelatinized starch.

Identification To 2 g of pulverized Processed Ginger add 5 mL of diethyl ether, shake for 10 minutes, filter, and use the filtrate as the sample solution (1). To the residue add 5 mL of methanol, proceed in the same manner as above, and use so obtained solution as the sample solution (2). Separately, dissolve 1 mg of [6]-shogaol for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution (1). Separately, dissolve 1 mg of Sucrose in 2 mL of methanol, and use this solution as the standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution (1) and standard solution (1) on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution (1) has the same color tone and *R_f* value with the green spot from the standard solution (1). Spot 10 μ L each of the sample solution (2) and standard solution (2) on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of 1-butanol, water and acetic acid (100) (8:5:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly 1,3-naphthalenediol TS on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution (2) has the same color tone and *R_f* value with the red-purple spot from the standard solution (2).

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not

less than 8.0%.

Prunella Spike

Prunellae Spica

カゴソウ

Prunella Spike is the spike of *Prunella vulgaris* Linné var. *lilacina* Nakai (*Labiatae*).

Description Spikes in nearly cylindrical and wheat ear-like shape, 3–6 cm in length, 1–1.5 cm in diameter, externally grayish brown; spikes composed of a floral axis having numerous bracts and calyxes; corollas often remaining on the upper part; a calyx usually enclosing four mericarps; bract, cordate to eccentric, and exhibiting white hairs on the vein, as on the calyx; light in texture.

Almost odorless and tasteless.

Purity (1) Stem—The amount of the stems contained in Prunella Spike does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than the stems contained in Prunella Spike does not exceed 1.0%.

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 5.0%.

Pueraria Root

Puerariae Radix

カッコン

Pueraria Root is the root of *Pueraria lobata* Ohwi (*Leguminosae*), from which periderm has been removed.

It contains not less than 2.0% of puerarin (C₂₁H₂₀O₉: 416.38), calculated on the basis of dried material.

Description Usually cut into small pieces of irregular hexagons of about 0.5 cm cube, or cut into longitudinally plate-like pieces 20–30 cm in length, 5–10 cm in width, and about 1 cm in thickness; externally light grayish yellow to grayish white; transverse section showing concentric annulate ring or part of it formed by abnormal growth of cambium. Under a magnifying glass, phloem light grayish yellow in color; in xylem, numerous vessels appearing as small dots; medullary rays slightly dented; vertical section showing longitudinal patterns formed alternately by fibrous xylem and parenchyma; easily breakable lengthwise, and its section extremely fibrous.

Odorless; taste, slightly sweet.

Under a microscope <5.01>, a transverse section reveals fiber bundles accompanied by crystal cells in phloem; distinct vessels and xylem fibers in xylem; starch grains numerous in parenchyma, mainly composed of polygonal simple grains, rarely 2- to 3-compound grains, 2–18 μ m, mostly 8–12 μ m, in size, with hilum or cleft in the center, and also with striae.

Identification To 2 g of pulverized Pueraria Root add 10

mL of methanol, shake for 3 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Puerarin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 2 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (12:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and Rf value with the blue-white fluorescent spot from the standard solution.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Pueraria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Pueraria Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not less than 13.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Assay Weigh accurately about 0.3 g of pulverized Pueraria Root, add 50 mL of diluted methanol (1 in 2), and heat under a reflux condenser on a water bath for 30 minutes, cool, and filter. To the residue add 50 mL of diluted methanol (1 in 2), and perform as the same as above. Combine the filtrates, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Puerarin Reference Standard (separately determine the water), add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and measure the peak areas of puerarin, A_T and A_S , of each solution.

$$\text{Amount (mg) of puerarin (C}_{21}\text{H}_{20}\text{O}_9) = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of Puerarin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 250 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of puerarin is about 15 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmet-

ry coefficient of the peak of puerarin are not less than 3000 and not more than 2.0, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of puerarin is not more than 1.5%.

Red Ginseng

Ginseng Radix Rubra

コウジン

Red Ginseng is the root of *Panax ginseng* C. A. Meyer (*Panax schinseng* Nees) (*Araliaceae*), after being steamed.

It contains not less than 0.10% of ginsenoside Rg₁ (C₄₂H₇₂O₁₄: 801.01) and not less than 0.20% of ginsenoside Rb₁ (C₅₄H₉₂O₂₃: 1109.29), calculated on the basis of dried material.

Description Thin and long cylindrical to fusiform root, often branching out into 2 to 5 lateral roots from the middle; 5 – 25 cm in length, main root 0.5 – 3 cm in diameter; externally light yellow-brown to red-brown, and translucent and with longitudinal wrinkles; crown somewhat constricted, and sometimes with short remains of stem; fractured surface flat; horny and hard in texture.

Odor, characteristic; taste, at first slightly sweet, followed by a slight bitterness.

Identification (1) To 0.2 g of pulverized Red Ginseng add 2 mL of acetic anhydride, warm on a water bath for 2 minutes, and filter. To 1 mL of the filtrate add gently 0.5 mL of sulfuric acid to make two layers: a red-brown color develops at the zone of contact.

(2) To 2.0 g of pulverized Red Ginseng add 20 mL of methanol, boil gently under a reflux condenser on a water bath for 15 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Ginsenoside Rg₁ Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with the lower layer of a mixture of chloroform, methanol and water (13:7:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 110°C for 5 minutes: one spot among the spots from the sample solution and a red-purple spot from the standard solution show the same color tone and the same Rf value.

Purity (1) Foreign matter <5.01>—The amount of stems and other foreign matter contained in Red Ginseng does not exceed 2.0%.

(2) Heavy metals <1.07>—Proceed with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test. Prepare the control solution with 1.5 mL of Standard Lead Solution (not more than 15 ppm).

(3) Arsenic <1.11>—Prepare the test solution with 1.0 g of pulverized Red Ginseng according to Method 4, and perform the test (not more than 2 ppm).

(4) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 15.5% (6 hours).

Total ash <5.01> Not more than 4.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 18.0%.

Assay (1) Ginsenoside Rg₁—Weigh accurately about 1 g of pulverized Red Ginseng, put in a glass-stoppered centrifuge tube, add 30 mL of diluted methanol (3 in 5), shake for 15 minutes, centrifuge, and separate the supernatant liquid. Repeat the procedure with the residue using 15 mL of diluted methanol (3 in 5), combine the supernatant liquids, and add diluted methanol (3 in 5) to make exactly 50 mL. Pipet 10 mL of this solution, add 3 mL of dilute sodium hydroxide TS, allow to stand for 30 minutes, add 3 mL of 0.1 mol/L hydrochloric acid TS and diluted methanol (3 in 5) to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rg₁ Reference Standard (separately determine the water) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rg₁.

Amount (mg) of ginsenoside Rg₁ (C₄₂H₇₂O₁₄) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of Ginsenoside Rg₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 30°C.

Mobile phase: A mixture of water and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rg₁ is about 25 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rg₁ Reference Standard and ginsenoside Re in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rg₁ and ginsenoside Re are eluted in this order with the resolution between these peaks being not less than 1.5.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rg₁ is not more than 1.5%.

(2) Ginsenoside Rb₁—Use the sample solution obtained in (1) as the sample solution. Separately, weigh accurately about 10 mg of Ginsenoside Rb₁ Reference Standard (separately determine the water) dissolve in diluted methanol (3 in 5) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the follow-

ing conditions, and determine the peak areas, A_T and A_S, of ginsenoside Rb₁.

Amount (mg) of ginsenoside Rb₁ (C₅₄H₉₂O₂₃) = $W_S \times (A_T/A_S)$

W_S: Amount (mg) of Ginsenoside Rb₁ Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 203 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water and acetonitrile (7:3).

Flow rate: Adjust the flow rate so that the retention time of ginsenoside Rb₁ is about 20 minutes.

System suitability—

System performance: Dissolve 1 mg each of Ginsenoside Rb₁ Reference Standard and ginsenoside Rc in diluted methanol (3 in 5) to make 10 mL. When the procedure is run with 10 μL of this solution under the above operating conditions, ginsenoside Rb₁ and ginsenoside Rc are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of ginsenoside Rb₁ is not more than 1.5%.

Rehmannia Root

Rehmanniae Radix

ジオウ

Rehmannia Root is the root of *Rehmannia glutinosa* Liboschitz var. *purpurea* Makino or *Rehmania glutinosa* Liboschitz (*Scrophulariaceae*), with or without the application of steaming.

Description Thin and long, usually, fusiform root, 5–10 cm in length, 0.5–1.5 cm in diameter, often broken or markedly deformed in shape; externally yellow-brown to blackish brown, with deep, longitudinal wrinkles and constrictions; soft in texture and mucous; cross section yellow-brown to blackish brown, and cortex darker than xylem in color; pith hardly observable.

Odor, characteristic; taste, slightly sweet at first, followed by a slight bitterness.

Under a microscope <5.01>, a transverse section reveals 7 to 15 layers of cork; cortex composed entirely of parenchyma cells; outer region of cortex with scattered cells containing brown secretes; xylem practically filled with parenchyma; vessels radially lined, mainly reticulate vessels.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rehmannia Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g

of pulverized *Rehmannia* Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Rhubarb

Rhei Rhizoma

ダイオウ

Rhubarb is usually the rhizome of *Rheum palmatum* Linné, *Rheum tanguticum* Maximowicz, *Rheum officinale* Baillon, *Rheum coreanum* Nakai or their interspecific hybrids (*Polygonaceae*).

It contains not less than 0.25% of sennosides A ($C_{42}H_{38}O_{20}$: 862.74), calculated on the basis of dried material.

Description Ovoid, oblong-ovoid or cylindrical rhizome, often cut crosswise or longitudinally, 4 – 10 cm in diameter, 5 – 15 cm in length. In the case of Rhubarb without most part of cortex, the outer surface is flat and smooth, yellow-brown to light brown in color, and sometimes exhibiting white, fine reticulations; thick and hard in texture. In the case of Rhubarb with cork layer, externally dark brown or reddish black, and with coarse wrinkles; rough and brittle in texture. The fractured surface of Rhubarb is not fibrous; transverse section grayish brown, light grayish brown or brown in color, having patterns of dark brown tissue complicated with white and light brown tissues; near the cambium, the patterns often radiate, and in pith, consist of whirls of tissues radiated from the center of a small brown circle 1 – 3 mm in diameter and arranged in a ring or scattered irregularly.

Odor, characteristic; taste, slightly astringent and bitter; when chewed, gritty between the teeth, and coloring the saliva yellow.

Under a microscope <5.01>, the transverse section reveals mostly parenchyma cells; small abnormal cambium-rings scattered here and there in the pith; the cambium-rings produce phloem inside and xylem outside, accompanied with 2 to 4 rows of medullary rays containing brown-colored substances, and the rays run radiately from the center of the ring towards the outside forming whirls of tissues; parenchyma cells contain starch grains, brown-colored substances or crystal druses of calcium oxalate.

Identification To 2 g of pulverized Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 μ L each of the

sample solution and standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

(3) Raponticin—To 0.5 g of pulverized Rhubarb add exactly 10 mL of ethanol (95), heat on a water bath with a reflux condenser for 10 minutes, and filter. Perform the test as directed under Thin-layer Chromatography <2.03>, using the filtrate as the sample solution. Spot 10 μ L of the sample solution on a plate of silica gel for thin-layer chromatography <2.03>. Develop the plate with a mixture of isopropyl ether, 1-butanol and methanol (26:7:7) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength 365 nm): no spot with blue-purple fluorescence is observed at an *R_f* value between 0.3 and 0.6, though a bluish white fluorescence may appear.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Assay Weigh accurately about 0.5 g of pulverized Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, (separately determine the water) dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL and use this solution as the standard solution. Perform the test with exactly 10 μ L of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of sennoside A in each solution.

$$\begin{aligned} &\text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) \\ &= W_S \times (A_T/A_S) \times (1/4) \end{aligned}$$

W_S : Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

Operating conditions –

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4 – 6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A Reference Standard and naringin for thin-layer chromatography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Powdered Rhubarb

Rhei Rhizoma Pulveratum

ダイオウ末

Powdered Rhubarb is the powder of Rhubarb.

It contains not less than 0.25% of sennoside A ($C_{42}H_{38}O_{20}$: 862.74), calculated on the basis of dried materials.

Description Powdered Rhubarb occurs as a brown powder. It has a characteristic odor and a slightly astringent and bitter taste; is gritty between the teeth and colors the saliva yellow on chewing.

Under a microscope <5.01>, Powdered Rhubarb reveals starch grains, dark brown substances or druses of calcium oxalate, fragments of parenchyma cells containing them, and reticulate vessels. The starch grains are spherical, simple, or 2- to 4-compound grains. Simple grain, 3–18 μ m in diameter, rarely 30 μ m; crystal druses of calcium oxalate, 30–60 μ m in diameter, sometimes exceeding 100 μ m.

Identification To 2 g of Powdered Rhubarb add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, add 30 mL of tetrahydrofuran, shake for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 4 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography at 10 mm along the initial line. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one of the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Rhubarb according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Rhubarb according to Method 4, and perform the test (not more than 5 ppm).

(3) Raponticin—To 0.5 g of Powdered Rhubarb add exactly 10 mL of ethanol (95), heat on a water bath under a reflux condenser for 10 minutes, and filter. Perform the test as directed under Thin-layer Chromatography <2.03>, using the filtrate as the sample solution. Spot 10 μ L of the sample solution on a plate of silica gel for thin-layer chromatography <2.03>. Develop the plate with a mixture of isopropyl ether, methanol and 1-butanol (26:7:7) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): no spot with blue-purple fluorescence is observed at the *R_f* value of between 0.3 and 0.6, though a bluish white fluorescence may appear.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Assay Weigh accurately about 0.5 g of Powdered Rhubarb, add exactly 50 mL of a solution of sodium hydrogen carbonate (1 in 1000), shake for 30 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard, (separately determine the water) dissolve in a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 50 mL. Pipet 5 mL of this solution, add a solution of sodium hydrogen carbonate (1 in 1000) to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, *A_T* and *A_S*, of sennoside A in each solution.

$$\begin{aligned} &\text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) \\ &= W_S \times (A_T/A_S) \times (1/4) \end{aligned}$$

W_S: Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column about 4–6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (100) (1 in 80) and acetonitrile (4:1).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 15 minutes.

System suitability—

System performance: Dissolve 1 mg each of Sennoside A Reference Standard and naringin for thin-layer chro-

matography in a solution of sodium hydrogen carbonate (1 in 1000) to make 10 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, sennoside A and naringin are eluted in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Compound Rhubarb and Senna Powder

複方ダイオウ・センナ散

Method of preparation

Powdered Senna Leaves	110 g
Powdered Rhubarb	110 g
Sulfur	555 g
Magnesium Oxide	225 g
To make	1000 g

Prepare as directed under Powders, with the above ingredients.

Description Compound Rhubarb and Senna Powder occurs as a yellow-brown powder, having a characteristic odor and a bitter taste.

Identification To 2 g of Compound Rhubarb and Senna Powder add 50 mL of water, warm on a water bath for 30 minutes, and filter. Add 2 drops of dilute hydrochloric acid to the filtrate, shake with two 20-mL portions of diethyl ether, and remove the diethyl ether layer. Add 5 mL of hydrochloric acid to the aqueous layer, and heat it on a water bath for 30 minutes. Cool, shake with 20 mL of diethyl ether, take the diethyl ether layer, add 10 mL of sodium hydrogen carbonate TS, and shake: the aqueous layer is red in color.

Containers and storage Containers—Well-closed containers.

Rice Starch

Amylum Oryzae

コメデンプン

Rice Starch consists of the starch granules obtained from the seeds of *Oryza sativa* Linné (*Gramineae*).

Description Rice Starch occurs as white masses or powder. It is odorless and tasteless.

Under a microscope <5.01>, Rice Starch appears as polyhedral, simple grains 3 – 10 μ m, mostly 4 – 6 μ m, in size. These simple grains often gather in ellipsoidal, compound grains 50 – 100 μ m in diameter. Hilum and striation are not observable.

It is practically insoluble in water and in ethanol (95).

Identification (1) To 1 g of Rice Starch add 50 mL of

water, boil, and allow to cool: a turbid, neutral and pasty liquid is formed.

(2) To a portion of Rice Starch add iodine TS: a dark blue-purple color is produced.

Purity Foreign matter—Under a microscope <5.01>, Rice Starch does not contain starch grains of any other origin. It may contain a minute quantity, if any, of fragments of the tissue of the original plant.

Loss on drying <5.01> Not more than 15.0% (6 hours).

Total ash <5.01> Not more than 1.0%.

Rose Fruit

Rosae Fructus

エイジツ

Rose Fruit is the pseudocarp of fruit of *Rosa multiflora* Thunberg (*Rosaceae*).

Description The pseudocarp, spherical, ellipsoidal or spheroidal, 5 – 9.5 mm in length, 3.5 – 8 mm in diameter; the external surface red to dark brown in color, smooth and lustrous; often with peduncle about 10 mm in length at one end, and with pentagonal remains of calyx without sepal at the other end; internal wall of receptacle covered densely with silvery hairs; the interior containing 5 – 10 mature nuts; the nut, irregularly angular ovoid, about 4 mm in length, about 2 mm in diameter; external surface, light yellow-brown; obtuse at one end, and slightly acute at the other.

Odor, slight; taste of receptacle, sweet and acid, and of nut, mucilaginous at first, later astringent, bitter and irritative.

Identification Boil gently 1 g of pulverized Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Purity Foreign matter <5.01>—The amount of the peduncle and other foreign matter contained in Rose Fruit is not more than 1.0%.

Total ash <5.01> Not more than 6.0%.

Powdered Rose Fruit

Rosae Fructus Pulveratus

エイジツ末

Powdered Rose Fruit is the powder of Rose Fruit.

Description Powdered Rose Fruit occurs as a grayish yellow-brown powder. It has a slight odor, and has a slightly mucilaginous, astringent, bitter, and slightly acid taste.

Under a microscope <5.01>, Powdered Rose Fruit reveals fragments of extremely thick-walled hairs 35 – 70 μ m in diameter, fragments of epidermis and hypodermis containing brown tannin masses, fragments of thin-walled fundamental

tissue containing grayish brown substances, fragments of fine vessels, and solitary or twin crystals or rosette aggregates of calcium oxalate (components of receptacle); fragments of sclerenchyma, fiber groups, fine vessels, and fragments of epidermis containing brown tannin and mucilage (components of pericarp); fragments of endosperm composed of polygonal cells containing aleuron grains and fatty oil, fragments of outer epidermis composed of polygonal cells containing tannin, and fragments of inner epidermis composed of elongated cells having wavy lateral walls (components of seed).

Identification Boil gently 1 g of Powdered Rose Fruit with 20 mL of methanol for 2 minutes, and filter. To 5 mL of the filtrate add 0.1 g of magnesium in ribbon form and 0.5 mL of hydrochloric acid, and allow the mixture to stand: a light red to red color develops.

Total ash <5.01> Not more than 6.0%.

Rosin

Colophonium

Resina Pini

ロジン

Rosin is the resin obtained from the exudation of plants of *Pinus* species (*Pinaceae*) from which essential oil has been removed.

Description Rosin occurs as a light yellow to light brown, glassily transparent, brittle mass, the surfaces of which are often covered with a yellow powder. The fractured surface is shell-like and lustrous.

It has a slight odor.

It melts easily, and burns with a yellow-brown flame.

It is freely soluble in ethanol (95), in acetic acid (100) and in diethyl ether.

A solution of Rosin in ethanol (95) is acidic.

Acid value <1.13> 150 – 177

Total ash <5.01> Not more than 0.1%.

Ryokeijutsukanto Extract

茶桂朮甘湯エキス

Ryokeijutsukanto Extract contains not less than 1 mg and not more than 4 mg of (*E*)-cinnamic acid, and not less than 21 mg and not more than 63 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 6 g of Poria Sclerotium, 4 g of Cinnamon Bark, 3 g of Atractylodes Rhizome or Atractylodes Lancea Rhizome and 2 g of Glycyrrhiza.

Description Ryokeijutsukanto Extract occurs as a brown powder. It has an odor, and a sweet first then bitter taste.

Identification (1) Cinnamon bark—To 1.0 g of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-purple spot from the standard solution.

(2) Atractylodes rhizome (for preparation prescribed Atractylodes Rhizome)—To 1.0 g of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-white fluorescent spot from the standard solution.

(3) Atractylodes lancea rhizome (for preparation prescribed Atractylodes Lancea Rhizome)—To 2.0 g of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around *R_f* 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool.

(4) Glycyrrhiza—To 1.0 g of Ryokeijutsukanto Extract add 10 mL of water, shake, then add 10 mL of 1-butanol, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>.

Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the yellow-brown spot from the standard solution.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Ryokeijutsukanto Extract as directed in (4) in Extracts under the General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Ryokeijutsukanto Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 8.5% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 8.0%.

Assay (1) (*E*)-Cinnamic acid—Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately about 0.5 g of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of (*E*)-cinnamic acid for component determination, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of (*E*)-cinnamic acid.

$$\begin{aligned} &\text{Amount (mg) of (E)-cinnamic acid} \\ &= W_S \times (A_T/A_S) \times (1/20) \end{aligned}$$

W_S : Amount (mg) of (*E*)-cinnamic acid for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 273 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of water, acetonitrile and phosphoric acid (750:250:1)

Flow rate: 1.0 mL/min. (the retention time of (*E*)-cinnamic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of (*E*)-cinnamic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with

10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of (*E*)-cinnamic acid is not more than 1.5%.

(2) Glycyrrhizic acid—Weigh accurately about 0.5 g of Ryokeijutsukanto Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid.

$$\begin{aligned} &\text{Amount (mg) of glycyrrhizic acid (C}_{42}\text{H}_{62}\text{O}_{16}) \\ &= W_S \times (A_T/A_S) \times (1/2) \end{aligned}$$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Safflower

Carthami Flos

コウカ

Safflower is the tubulous flower of *Carthamus tinctorius* Linné (*Compositae*) without any treatment or with most of the yellow pigment removed, and pressed into a flat slab.

Description Red to red-brown corolla, yellow style and stamens, rarely mixed with immature ovary; total length about 1 cm; corolla, tubular and with 5 lobes; 5 stamens surrounding long pistil; pollen grains yellow and approximately spherical, about 50 μm in diameter, with fine protrusions on the sur-

face. The pressed slab, about 0.5 cm in thickness, consists of a collection of numerous corollas.

Odor, characteristic; taste, slightly bitter.

Identification Boil 0.2 g of Safflower with 10 mL of dilute ethanol under a reflux condenser for 15 minutes, and after cooling, filter. Place 3 mL of the filtrate in a small glass vessel about 3 cm in both internal diameter and height, hang a piece of filter paper, 20 mm by 300 mm, so that one end of the filter paper reaches the bottom of the vessel, and allow the paper to soak up the liquid for 1 hour. Transfer and immediately hang the paper in another glass vessel of the same type, containing 3 mL of water, and allow the paper to soak up the water for 1 hour: most of the upper part of the paper is colored light yellow, and the lower portion, light red.

Purity Foreign matter <5.01>—The amount of ovaries, stems, leaves and other foreign matter contained in Safflower does not exceed 2.0%.

Total ash <5.01> Not more than 18.0%.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Saffron

Crocus

サフラン

Saffron is the stigma of *Crocus sativus* Linné (*Iridaceae*).

Description Thin cord-like stigma, externally dark yellow-red to red-brown, 1.5–3.5 cm in length, tripartite or separate; the end of partite part widened and the other end narrowed gradually.

Odor, strong and characteristic; taste, bitter; colors the saliva yellow on chewing.

Under a microscope <5.01>, when softened by immersion in water, the upper end has numerous tubular protrusions about 150 μm in length, with a small number of pollen grains.

Identification Add 1 drop of sulfuric acid to Saffron: the color changes to dark blue which gradually turns red-brown through purple.

Purity (1) Aniline dyes—Shake 0.05 g of Saffron with 10 mL of chloroform: the solution is colorless, or only slightly yellow.

(2) Glycerol, sugar or honey—Saffron has no sweet taste. Press it between two pieces of paper: no spot is left on the paper.

(3) Yellow style—The yellow style in Saffron does not exceed 10.0%.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 7.5%.

Content of the active principle Crocin—Dry Saffron in a desiccator (silica gel) for 24 hours, and powder. To exactly 0.100 g of the powder add 150 mL of warm water, warm the mixture between 60°C and 70°C for 30 minutes with frequent

shaking, cool, and filter. Pipet 1 mL of the filtrate, add water to make exactly 10 mL, and use this solution as the sample solution. Separately, dissolve exactly 98 mg of carbazochrome sodium sulfonate for content of the active principle in water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 100 mL, and use this solution as the standard solution. Determine the absorbances of the sample solution and standard solution at 438 nm as directed under Ultraviolet-visible Spectrophotometry <2.24>: the absorbance of the sample solution is larger than that of the standard solution.

Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Saireito Extract

柴苓湯エキス

Saireito Extract contains not less than 2 mg and not more than 8 mg of saikosaponin b₂, not less than 80 mg and not more than 240 mg of baicalin (C₂₁H₁₈O₁₁: 446.37), and not less than 17 mg and not more than 51 mg of glycyrrhizic acid (C₄₂H₆₂O₁₆: 822.93) per a dried extract prepared as directed in the Method of preparation.

Method of preparation Prepare a dried extract as directed under Extracts, with 7 g of Bupleurum Root, 5 g of Pinellia Tuber, 1 g of Ginger, 3 g of Scutellaria Root, 3 g of Jujube, 3 g of Ginseng, 2 g of Glycyrrhiza, 6 g of Alisma Rhizome, 4.5 g of Polyporus Sclerotium, 4.5 g of Poria Sclerotium, 4.5 g of Atractylodes Rhizome and 3 g of Cinnamon Bark, or with 7 g of Bupleurum Root, 5 g of Pinellia Tuber, 1 g of Ginger, 3 g of Scutellaria Root, 3 g of Jujube, 3 g of Ginseng, 2 g of Glycyrrhiza, 5 g of Alisma Rhizome, 3 g of Polyporus Sclerotium, 3 g of Poria Sclerotium, 3 g of Atractylodes Lancea Rhizome and 2 g of Cinnamon Bark.

Description Saireito Extract occurs as a light yellow-brown powder. It has slightly a characteristic odor, and a sweet, then bitter taste.

Identification (1) Bupleurum root—To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of saikosaponin b₂ for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (99.5) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and R_f value with the red spot from the standard solution.

(2) Ginger—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether layer

under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of [6]-gingerol for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 15 μL of the sample solution and 5 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly 4-dimethylaminobenzaldehyde TS for spraying on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-green spot from the standard solution.

(3) Scutellaria root—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the diethyl ether layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of wogonin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, air-dry the plate, and spray evenly iron (III) chloride-methanol TS on the plate: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solution.

(4) Ginseng—To 2.0 g of Saireito Extract add 10 mL of sodium hydroxide TS, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of Ginsenoside R_{b1} Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol, water and acetic acid (100) (7:5:4:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution.

(5) Glycyrrhiza—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 5 mL of 1-butanol, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of liquiritin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, methanol and water (20:3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the yellow-brown spot from the standard solu-

tion.

(6) Alisma rhizome—To 2.0 g of Saireito Extract add 10 mL of sodium carbonate TS, shake, then add 10 mL of diethyl ether, shake, centrifuge, and use the supernatant liquid as the sample solution. Separately, dissolve 1 mg of alisol A for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 μL of the sample solution and 2 μL of the standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:3) to a distance of about 10 cm, and air-dry the plate. Spray evenly vanillin-sulfuric acid TS on the plate, heat at 105°C for 5 minutes, and allow to cool: one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the purple spot from the standard solution.

(7) Atractylodes rhizome (for preparation prescribed Atractylodes Rhizome)—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of atractylenolide III for thin-layer chromatography in 2 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μL each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and hexane (1:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, examine under ultraviolet light (main wavelength: 365 nm): one of the spot among the several spots from the sample solution has the same color tone and *R_f* value with the blue-white fluorescent spot from the standard solution.

(8) Atractylodes lancea rhizome (for preparation prescribed Atractylodes Lancea Rhizome)—To 2.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of hexane, and shake. Take the hexane layer, add anhydrous sodium sulfate to dry, and filter. Evaporate the filtrate under reduced pressure, add 2 mL of hexane to the residue, and use this solution as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 20 μL of the sample solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane and acetone (7:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): a dark purple spot is observed at around *R_f* 0.4. The spot shows a greenish brown color after being sprayed 4-dimethylaminobenzaldehyde TS for spraying, heated at 105°C for 5 minutes, and allowed to cool.

(9) Cinnamon bark—To 1.0 g of Saireito Extract add 10 mL of water, shake, then add 25 mL of diethyl ether, and shake. Take the diethyl ether layer, evaporate the layer under reduced pressure, add 2 mL of diethyl ether to the residue, and use this solution as the sample solution. Separately, dissolve 1 mg of (*E*)-cinnamic acid for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 40 μL of the sample solution and 2 μL of the standard solution

on a plate of silica gel with fluorescent indicator for thin-layer chromatography, develop the plate with a mixture of hexane, ethyl acetate, formic acid and water (60:40:4:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spot among the several spots from the sample solution has the same color tone and R_f value with the dark purple spot from the standard solution.

Purity (1) Heavy metals <1.07>—Prepare the test solution with 1.0 g of Saireito Extract as directed in (4) in Extracts under the General Rules for Preparations, and perform the test (not more than 30 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.67 g of Saireito Extract according to Method 3, and perform the test (not more than 3 ppm).

Loss on drying <2.41> Not more than 10.0% (1 g, 105°C, 5 hours).

Total ash <5.01> Not more than 9.0%.

Assay (1) Saikosaponin b_2 —Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of saikosaponin b_2 for component determination, previously dried in a desiccator (silica gel) for more than 24 hours, dissolve in diluted methanol (1 in 2) to make exactly 100 mL. Pipet 10 mL of this solution, add diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of saikosaponin b_2 .

Amount (mg) of saikosaponin $b_2 = W_S \times (A_T/A_S) \times (1/20)$

W_S : Amount (mg) of saikosaponin b_2 for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 0.05 mol/L sodium dihydrogen phosphate TS and acetonitrile (5:3)

Flow rate: 1.0 mL/min. (the retention time of saikosaponin b_2 is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of saikosaponin b_2 are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of saikosaponin b_2 is not more than 1.5%.

(2) Baicalin—Weigh accurately about 0.1 g of Saireito

Extract, add exactly 50 mL of diluted methanol (7 in 10), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (separately determine the water), dissolve in diluted methanol (7 in 10) to make exactly 200 mL, and use this solution as the standard solution. Perform test with exactly 10 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of baicalin.

Amount (mg) of baicalin ($C_{21}H_{18}O_{11}$)
 $= W_S \times (A_T/A_S) \times (1/4)$

W_S : Amount (mg) of Baicalin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 200) and acetonitrile (19:6)

Flow rate: 1.0 mL/min. (the retention time of baicalin is about 10 minutes.)

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of baicalin are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

(3) Glycyrrhizic acid—Weigh accurately about 0.5 g of Saireito Extract, add exactly 50 mL of diluted methanol (1 in 2), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, weigh accurately about 10 mg of Glycyrrhizic Acid Reference Standard (separately determine the water), dissolve in diluted methanol (1 in 2) to make exactly 100 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and the standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of glycyrrhizic acid.

Amount (mg) of glycyrrhizic acid ($C_{42}H_{62}O_{16}$)
 $= W_S \times (A_T/A_S) \times (1/2)$

W_S : Amount (mg) of Glycyrrhizic Acid Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 254 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted acetic acid (31) (1 in 15) and acetonitrile (13:7).

Flow rate: 1.0 mL/min. (the retention time of glycyrrhizic acid is about 12 minutes.)

System suitability—

System performance: When the procedure is run with 10 µL of the standard solution under the above operating conditions, the number of theoretical plates and the symmetry factor of the peak of glycyrrhizic acid are not less than 5000 and not more than 1.5, respectively.

System repeatability: When the test is repeated 6 times with 10 µL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of glycyrrhizic acid is not more than 1.5%.

Containers and storage Containers—Tight containers.

Saposhnikovia Root

Saposhnikoviae Radix

ボウフウ

Saposhnikovia Root is the root and rhizome of *Saposhnikovia divaricata* Schischkin (*Umbelliferae*).

Description Long and narrow, conical rhizome and root, 15 – 20 cm in length, 0.7 – 1.5 cm in diameter; externally light brown; rhizome reveals dense crosswise wrinkles like ring nodes, and sometimes reveals brown and hair-like remains of leaf sheath; the root reveals many longitudinal wrinkles and scars of rootlets; in a cross section, cortex is grayish brown in color and reveals many lacunae, and xylem is yellow in color.

Odor, slight; taste, slightly sweet.

Purity Foreign matter <5.01>—The amount of stems and other foreign matter contained in Saposhnikovia Root is not more than 2.0%.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 20.0%.

Sappan Wood

Sappan Lignum

ソボク

Sappan Wood is the duramen of *Caesalpinia sappan* Linné (*Leguminosae*).

Description Chips, slices or short pieces of wood; yellowish red to grayish yellow-brown, sometimes with light brown to grayish white splint woods; hard in texture; a transverse section shows a pattern like annual ring.

Almost odorless; almost tasteless.

Under a microscope <5.01>, a transverse section reveals ray

composed of 1 – 2 rows of slender and long cells; the area between rays filled with fiber cells, and large and oblong vessels scattered there; solitary crystals of calcium oxalate in parenchymatous cells of the innermost of xylem.

Identification To 0.5 g of pulverized Sappan Wood add 10 mL of dilute ethanol, shake, and filter. To 5 mL of the filtrate add 2 to 3 drops of sodium hydroxide TS: a dark red color develops.

Purity Put a small piece of Sappan Wood in calcium hydroxide TS: no purple-blue color develops.

Loss on drying <5.01> Not more than 11.5% (6 hours).

Total ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 7.0%.

Saussurea Root

Saussureae Radix

モッコウ

Saussurea Root is the root of *Saussurea lappa* Clarke (*Compositae*).

Description Nearly cylindrical roots, 5 – 20 cm in length, 1 – 6 cm in diameter; some of them slightly bent, and sometimes longitudinally cut; scar of stem dented on the top of the root with crown; externally yellow-brown to grayish brown, with coarse longitudinal wrinkles and fine reticulate furrows, and also with remains of lateral roots; sometimes root from which periderm has been removed; hard and dense in texture, and difficult to break. A cross section is yellow-brown to dark brown, and cambium part has a dark color. Under a magnifying glass, medullary rays distinct, here and there, large clefts, and brown oil sacs scattered; in old root, pith existing in the center, and often forming a hollow.

Odor, characteristic; taste, bitter.

Identification Warm 0.5 g of pulverized Saussurea Root with 10 mL of ethanol (95) for 1 minute, cool, and filter. Shake 1 mL of the filtrate with 0.5 mL of hydrochloric acid: a purple color is produced.

Purity Foreign matter—Add iodine TS dropwise to a transverse section: no blue-purple color develops.

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 17.0%.

Schisandra Fruit

Schisandrae Fructus

ゴミシ

Schisandra Fruit is the fruit of *Schisandra chinensis* Baillon (*Schisandraceae*).

Description Sap fruit of irregular sphere or spheroid, about

6 mm in diameter; externally dark red to blackish brown in color, with wrinkles, and occasionally with white powder; seeds, kidney-shaped, externally yellow-brown to dark red-brown, lustrous, with distinct raphe on the dorsal side; external seed coat easily peeled but internal seed coat adhering closely to the albumen.

Odor, slight; taste, acid, later astringent and bitter.

Identification To 1.0 g of pulverized Schisandra Fruit add 10 mL of methanol, warm on a water bath for 3 minutes with shaking, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of schisandrin for thin-layer chromatography in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (10:10:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 254 nm): one of the spots from the sample solution and a blue-violet spot from the standard solution show the same color tone and *R_f* value.

Purity Foreign matter <5.01>—The amount of receptacle, peduncle and other foreign matter contained in Schisandra Fruit is not more than 1.0%.

Total ash <5.01> Not more than 5.0%.

Schizonepeta Spike

Schizonepetae Spica

ケイガイ

Schizonepeta Spike is the spike of *Schizonepeta tenuifolia* Briquet (*Labiatae*).

Description Oblong spike, 5 – 10 cm in length, 0.5 – 0.8 cm in diameter, purplish green-brown to green-brown in color. Spike, 5 – 10 cm in length, with calyx-tubes containing small labiate flower or often fruits; sometimes leaves under spike; leaf, linear or small lanceolate; stem, prismatic, purple-brown in color. Under a magnifying glass, it reveals short hairs.

It has a characteristic aroma and slightly cool feeling on keeping in the mouth.

Identification To 2 g of pulverized Schizonepeta Spike add 20 mL of water, shake well, and distill. To 3 mL of the distillate add 2 or 3 drops of 2,4-dinitrophenylhydrazine-ethanol TS: an orange-red precipitate is formed.

Total ash <5.05> Not more than 11.0%.

Acid-insoluble ash <5.05> Not more than 3.0%.

Extract content <5.05> Dilute ethanol-soluble extract: not less than 8.0%.

Scopolia Extract

ロートエキス

Scopolia Extract contains not less than 0.90% and not more than 1.09% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Method of preparation Extract the coarse powder of Scopolia Rhizome with 35 vol% ethanol, Water, or Purified Water, and prepare the viscous extract as directed under Extracts.

Description Scopolia Extract is brown to dark brown in color. It has a characteristic odor, and a bitter taste.

It dissolves in water with a slight turbidity.

Identification (1) Dissolve 4 g of Scopolia Extract in 10 mL of water, add 8 mL of ammonia TS and 80 mL of diethyl ether, stopper tightly, shake for 1 hour, add 2.5 g of powdered tragacanth, shake vigorously, allow to stand for 5 minutes, and separate the diethyl ether layer into a porcelain dish. Evaporate the diethyl ether on a water bath, add 5 drops of fuming nitric acid, and evaporate on a water bath to dryness. After cooling, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a red-purple to purple color develops.

(2) Mix 0.5 g of Scopolia Extract with 30 mL of ammonia TS in a flask, and transfer the mixture to a separator. Add 40 mL of ethyl acetate to the separator, and shake the mixture. After drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in Identification (2) under Scopolia Rhizome.

Assay Weigh accurately about 0.4 g of Scopolia Extract, place in a glass-stoppered, centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the water layer, using 25 mL each of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Proceed as directed under Scopolia Rhizome.

$$\begin{aligned} \text{Amount (mg) of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_3) \\ = W_{SA} \times (Q_{TA}/Q_{SA}) \times (1/5) \times 0.8551 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of scopolamine (C}_{17}\text{H}_{21}\text{NO}_4) \\ = W_{SS} \times (Q_{TS}/Q_{SS}) \times (1/25) \times 0.7894 \end{aligned}$$

W_{SA} : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

W_{SS} : amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate

in the mobile phase (1 in 2500).

Containers and storage Containers—Tight containers.

Storage—Light-resistant, and in a cold place.

Scopolia Extract Powder

ロートエキス散

Scopolia Extract Powder contains not less than 0.085% and not more than 0.110% of total alkaloids [hyoscyamine ($C_{17}H_{23}NO_3$: 289.37) and scopolamine ($C_{17}H_{21}NO_4$: 303.35)].

Method of preparation

Scopolia Extract	100 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
<hr/>	
	To make 1000 g

To Scopolia Extract add 100 mL of Purified Water, then warm and soften the mixture with stirring. Cool, add 800 g of starch, Lactose Hydrate or their mixture little by little, and mix well. Dry preferably at a low temperature, and dilute with a sufficient additional quantity of starch, Lactose Hydrate or their mixture to make 1000 g of homogeneous powder.

Description Scopolia Extract Powder is a brownish yellow to grayish yellow-brown powder. It has a faint, characteristic odor and a slightly bitter taste.

Identification (1) To 20 g of Scopolia Extract Powder add 15 mL of water and 8 mL of ammonia TS, mix homogeneously, add 100 mL of diethyl ether and 7 g of sodium chloride, stopper tightly, shake for 1 hour, add 5 g of Powdered Tragacanth, and shake vigorously. Allow to stand for 5 minutes, take the clearly separated diethyl ether layer, and filter. Proceed with the filtrate as directed in the Identification (1) under Scopolia Extract.

(2) Place 5.0 g of Scopolia Extract Powder in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Proceed as directed in the Identification (2) under Scopolia Rhizome.

Assay Weigh accurately about 4 g of Scopolia Extract Powder, place in a glass-stoppered, centrifuge tube, add 15 mL of ammonia TS, and shake. Add 25 mL of diethyl ether, stopper tightly, shake for 15 minutes, centrifuge to take the diethyl ether layer. Repeat this procedure three times with the water layer, using 25-mL portions of diethyl ether. Combine the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make exactly 25 mL. Filter this solution through a membrane filter with a pore size not exceeding $0.8 \mu\text{m}$, dis-

card the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate Reference Standard (separately determine the loss on drying <2.41> in the same manner as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 25 mg of Scopolamine Hydrobromide Reference Standard (separately determine the loss on drying <2.41> in the same manner as Scopolamine Hydrobromide Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of the standard stock solution A and 1 mL of the standard stock solution B, add exactly 3 mL of the internal standard solution, then add the mobile phase to make exactly 25 mL, and use this solution as the standard solution. Perform the test with 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratios, Q_{TA} and Q_{SA} , of the peak area of hyoscyamine (atropine), and ratios, Q_{TS} and Q_{SS} , of the peak area of scopolamine to that of the internal standard in each solution, calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

$$\begin{aligned} \text{Amount (mg) of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_3) \\ = W_{SA} \times (Q_{TA}/Q_{SA}) \times (1/5) \times 0.8551 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of scopolamine (C}_{17}\text{H}_{21}\text{NO}_4) \\ = W_{SS} \times (Q_{TS}/Q_{SS}) \times (1/25) \times 0.7894 \end{aligned}$$

W_{SA} : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

W_{SS} : Amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column about 4 mm in inside diameter and about 15 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: A mixture of a solution obtained by dissolving 6.8 g of potassium dihydrogenphosphate in 900 mL of water, adding 10 mL of triethylamine, adjusting the pH to 3.5 with phosphoric acid, and adding water to make 1000 mL, and acetonitrile (9:1).

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

Selection of column: Proceed with 10 μL of the standard solution under the above operating conditions, and determine the resolution. Use a column giving elution of scopolamine, atropine and the internal standard in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and the resolution between the peaks of atropine and the internal standard being not less than 4.

Containers and storage Containers—Tight containers.

Scopolia Extract and Carbon Powder

ロートエキス・カーボン散

Method of preparation

Scopolia Extract	5 g
Medicinal Carbon	550 g
Natural Aluminum Silicate	345 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
<hr/>	
To make	1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract and Carbon Powder is easily dustable and black in color. It is tasteless.

Containers and storage Containers—Well-closed containers.

Compound Scopolia Extract and Diastase Powder

複方ロートエキス・ジアスターゼ散

Method of preparation

Scopolia Extract	8 g
Diastase	200 g
Precipitate Calcium Carbonate	300 g
Sodium Bicarbonate	250 g
Magnesium Oxide	100 g
Powdered Gentian	50 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
<hr/>	
To make	1000 g

Prepare before use as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Compound Scopolia Extract and Diastase Powder is light yellow in color. It has a bitter taste.

Containers and storage Containers—Well-closed containers.

Scopolia Extract and Ethyl Aminobenzoate Powder

ロートエキス・アネスタミン散

Scopolia Extract and Ethyl Aminobenzoate Powder

contains not less than 22.5% and not more than 27.5% of ethyl aminobenzoate ($C_9H_{11}NO_2$; 165.19).

Method of preparation

Scopolia Extract	10 g
Ethyl Aminobenzoate	250 g
Magnesium Oxide	150 g
Sodium Bicarbonate	500 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
<hr/>	
To make	1000 g

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract and Ethyl Aminobenzoate Powder is slightly brownish white in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

Identification (1) To 2 g of Scopolia Extract and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate).

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 30 mL of water, shake gently, and filter: the filtrate responds to the Qualitative Tests <1.09> for sodium salt and for bicarbonate.

(3) To the water-insoluble residue obtained in (2) add 10 mL of dilute hydrochloric acid, shake, and filter: the filtrate responds to the Qualitative Tests <1.09> for magnesium salt.

(4) Place 30 g of Scopolia Extract and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 100 mL of water, shake for 30 minutes, and filter immediately by suction through a glass filter (G3). Transfer the residue in the flask to the same glass filter with the filtrate, and filter the residue by suction while pressing vigorously the residue on the same glass filter. Place 75 mL of the filtrate in a 300-mL beaker, and add cautiously 10 mL of diluted sulfuric acid (1 in 3). Add 0.2 mL of bromocresol green TS to this solution, and add dilute sulfuric acid dropwise while shaking thoroughly, until the color of the solution changes from green to yellow-green. After cooling, place this solution in a separator, wash with two 25-mL portions of a mixture of hexane and diethyl ether (1:1) by shaking well, and place the water layer in another separator. Make slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, separate the diethyl ether layer, add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol (95), and use this solution as the sample solution. Separately,

dissolve 2 mg of Atropine Sulfate Reference Standard and 1 mg of Scopolamine Hydrobromide Reference Standard in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia solution (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution show the same color tone and the same *R_f* value with each yellow-red spot from the standard solutions, respectively.

Assay Weigh accurately about 0.3 g of Scopolia Extract and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Weigh accurately about 75 mg of Ethyl Aminobenzoate Reference Standard, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, to each add 10 mL of 1 mol/L hydrochloric acid TS, then add 1 mL of a solution of sodium nitrite (1 in 200), prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of *N,N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, determine the absorbances, A_T and A_S , of these solutions at 550 nm, as directed under Ultraviolet-visible Spectrophotometry <2.24> using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

$$\begin{aligned} & \text{Amount (mg) of ethyl aminobenzoate (C}_9\text{H}_{11}\text{NO}_2) \\ & = W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ethyl Aminobenzoate Reference Standard

Containers and storage Containers—Well-closed containers.

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder

ロートエキス・パパベリン・アネスタミン散

Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder contains not less than 10.8% and not more than 13.2% of ethyl aminobenzoate (C₉H₁₁NO₂; 165.19).

Method of preparation

Scopolia Extract	15 g
Papaverine Hydrochloride	15 g
Ethyl Aminobenzoate	120 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity

To make 1000 g

Prepare as directed under Powders, with the above ingredients. May be prepared with an equivalent amount of Scopolia Extract Powder in place of Scopolia Extract.

Description Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder is brownish yellow to grayish yellow-brown in color. It has a slightly bitter taste, leaving a sensation of numbness on the tongue.

Identification (1) To 4 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder add 20 mL of diethyl ether, shake, and filter through a glass filter (G4). Wash the residue with three 10-mL portions of diethyl ether, combine the filtrate and the washings, evaporate to dryness, and perform the following test with the residue (ethyl aminobenzoate):

(i) Dissolve 0.01 g of the residue in 1 mL of dilute hydrochloric acid and 4 mL of water: the solution responds to the Qualitative Tests <1.09> for primary aromatic amines.

(ii) Dissolve 0.1 g of the residue in 5 mL of water with the aid of dilute hydrochloric acid added dropwise, and add iodine TS dropwise: a brown precipitate is produced.

(iii) Warm 0.05 g of the residue with 2 drops of acetic acid (31) and 5 drops of sulfuric acid: the odor of ethyl acetate is perceptible.

(2) To the diethyl ether-insoluble residue obtained in (1) add 20 mL of chloroform, shake well, filter, and further wash the residue with 10 mL of chloroform. Combine the filtrate and the washing, transfer this solution to a separator, and add 10 mL of 0.1 mol/L hydrochloric acid TS. After shaking, separate the chloroform layer, add 2 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dry the residue at 105°C for 3 hours, and perform the following tests (papaverine hydrochloride):

(i) To 1 mg of the residue add 1 drop of formaldehyde solution-sulfuric acid TS: a colorless or light yellow-green color, changing to red-purple, is produced.

(ii) Dissolve 1 mg of the residue in 3 mL of acetic anhydride and 5 drops of sulfuric acid, heat in a water bath for 1 minute, and view under ultraviolet light: the solution shows a yellow-green fluorescence.

(3) Place 20 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder in a glass-stoppered conical flask, add 80 mL of water, shake for 15 minutes, and filter by suction through a glass filter (G3). Transfer 60 mL of the filtrate to a separator, add 0.5 mL of 1 mol/L hydrochloric acid TS, and extract with three 20-mL portions of chloroform by shaking. Make the aqueous layer slightly alkaline with ammonia TS, add immediately 30 mL of diethyl ether, and shake well. Wash the diethyl ether layer with two 10-mL portions of a saturated solution of sodium chloride, and separate the diethyl ether layer. Add 3 g of anhydrous sodium sulfate, shake, and filter through a pledget of cotton. Evaporate the filtrate to dryness, dissolve the residue in 0.2 mL of ethanol

(95), and use the solution as the sample solution. Dissolve 20 mg of atropine sulfate for thin-layer chromatography, 10 mg of scopolamine hydrobromide and 20 mg of papaverine hydrochloride in 10 mL each of ethanol (95), and use these solutions as standard solutions (1), (2) and (3). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of chloroform, methanol, acetone and ammonia solution (28) (73:15:10:2) to a distance of about 10 cm, and dry the plate at 80°C for 20 minutes. After cooling, spray Dragendorff's TS for spraying upon the plate evenly: three yellow-red principal spots obtained from the sample solution and the corresponding spots from standard solutions (1), (2) and (3) show the same *R_f* values.

Assay Weigh accurately about 0.6 g of Scopolia Extract, Papaverine and Ethyl Aminobenzoate Powder, transfer to a Soxhlet extractor, and extract with 100 mL of diethyl ether for 1 hour, and evaporate the diethyl ether on a water bath. Dissolve the residue in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the sample solution. Separately, weigh accurately about 75 mg of Ethyl Aminobenzoate Reference Standard, previously dried in a desiccator (silica gel) for 3 hours, dissolve in 25 mL of 1 mol/L hydrochloric acid TS, and add water to make exactly 100 mL. Pipet 5 mL of this solution, add water to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and standard solution, add 10 mL of 1 mol/L hydrochloric acid TS to each, then add 1 mL of a solution of sodium nitrite (1 in 200) prepared before use, and allow to stand for 5 minutes with occasional shaking. Add 5 mL of ammonium amidosulfate TS, shake well, and allow to stand for 10 minutes. Add 2 mL of *N-N*-diethyl-*N'*-1-naphthylethylenediamine oxalate-acetone TS, mix immediately, and add water to make exactly 50 mL. Allow to stand for 2 hours, and determine the absorbances, A_T and A_S , of these solutions at 550 nm as directed under Ultraviolet-visible Spectrophotometry <2.24> using a blank prepared in the same manner with 5 mL of water in place of the sample solution.

$$\begin{aligned} &\text{Amount (mg) of ethyl aminobenzoate (C}_9\text{H}_{11}\text{NO}_2) \\ &= W_S \times (A_T/A_S) \end{aligned}$$

W_S : Amount (mg) of Ethyl Aminobenzoate Reference Standard

Containers and storage Containers—Well-closed containers.

Scopolia Extract and Tannic Acid Suppositories

ロートエキス・タンニン坐剤

Method of preparation

Scopolia Extract	0.5 g
Tannic Acid	1 g
Cacao Butter or a suitable base	a sufficient quantity

Prepare 10 suppositories as directed under Suppositories, with the above ingredients.

Description Scopolia Extract and Tannic Acid Suppositories are light brown in color.

Identification (1) To 2 Scopolia Extract and Tannic Acid Suppositories add 20 mL of diethyl ether, and dissolve the base of suppositories with shaking for 10 minutes. Shake thoroughly the mixture with 15 mL of water, separate the water layer, and filter. To the filtrate add 10 mL of chloroform, shake well, and separate the chloroform layer. Take 5 mL of the chloroform solution, add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

(2) To 1 mL of the aqueous layer obtained in (1) after extraction with diethyl ether, add 2 drops of iron (III) chloride TS: a bluish-black color develops. Allow to stand: a bluish-black precipitate is formed (tannic acid).

Containers and storage Containers—Well-closed containers.

Scopolia Rhizome

Scopoliae Rhizoma

ロートコン

Scopolia Rhizome is the rhizome and root of *Scopolia japonica* Maximowicz, *Scopolia carniolica* Jacquin or *Scopolia parviflora* Nakai (*Solanaceae*).

When dried, it contains not less than 0.29% of total alkaloids [hyoscyamine (C₁₇H₂₃NO₃: 289.37) and scopolamine (C₁₇H₂₁NO₄: 303.35)].

Description Chiefly irregularly branched, slightly curved rhizome, about 15 cm in length, about 3 cm in diameter, occasionally longitudinally cut; externally grayish brown, with wrinkles; constrictions make the rhizome appear nodular; rarely, stem base at one end; stem scars at upper side of each node; roots or root scars on both sides and lower surface of rhizome; fractured surface granular, grayish white to light brown in color, with lighter colored cortex.

Odor characteristic; taste sweet, later slightly bitter.

Under a microscope <5.01>, xylem reveals groups of vessels arranged stepwise, and accompanied with xylem sieve tubes in medullary rays; parenchyma cells contain starch grains, and sometimes sand crystals of calcium oxalate.

Identification (1) To 1 g of pulverized Scopolia Rhizome add 10 mL of diethyl ether and 0.5 mL of ammonia TS, shake for 30 minutes, and filter. Wash the residue with 10 mL of diethyl ether, transfer the filtrate and the washing to a separator, add 20 mL of diluted sulfuric acid (1 in 50), shake well, and drain off the acid extract into another separator. Render the solution slightly alkaline with ammonia TS, add 10 mL of diethyl ether, shake well, transfer the diethyl ether layer to a porcelain dish, and evaporate the diethyl ether on a water bath. To the residue add 5 drops of fuming nitric acid, and evaporate the mixture on a water bath to dryness. Cool, dissolve the residue in 1 mL of *N,N*-dimethylformamide, and add 5 to 6 drops of tetraethylammonium hydroxide TS: a

red-purple to purple color develops.

(2) Place 2.0 g of pulverized *Scopolia Rhizome* in a glass-stoppered centrifuge tube, add 30 mL of ammonia TS, and centrifuge after irradiation of ultrasonic waves for 5 minutes. Transfer the supernatant liquid to a separator, add 40 mL of ethyl acetate, and shake. Drain off the ethyl acetate layer, add 3 g of anhydrous sodium sulfate to the ethyl acetate, shake, and filter after the ethyl acetate becomes clear. Evaporate the filtrate to dryness under reduced pressure, dissolve the residue in 1 mL of ethanol (95), and use this solution as the sample solution. Separately, dissolve 2 mg of Atropine Sulfate Reference Standard and 1 mg of Scopolamine Hydrobromide Reference Standard in 1 mL each of ethanol (95), and use these solutions as standard solution (1) and standard solution (2). Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of acetone, water and ammonia water (28) (90:7:3) to a distance of about 10 cm, and dry the plate at 80°C for 10 minutes. After cooling, spray evenly Dragendorff's TS for spraying on the plate: two principal spots from the sample solution and each yellow-red spot from the standard solutions show the same color tone and the same *Rf* value.

(3) To 3 g of pulverized *Scopolia Rhizome* add 10 mL of chloroform, shake thoroughly, and filter. To 5 mL of the filtrate add 5 mL of ammonia TS, shake, and allow to stand: the ammonia layer shows a blue-green fluorescence.

Total ash <5.01> Not more than 7.0%.

Assay Weigh accurately about 0.7 g of pulverized *Scopolia Rhizome*, previously dried at 60°C for 8 hours, in a glass-stoppered, centrifuge tube, and moisten with 15 mL of ammonia TS. To this add 25 mL of diethyl ether, stopper the centrifuge tube tightly, shake for 15 minutes, centrifuge, and separate the diethyl ether layer. Repeat this procedure twice with the residue using 25-mL portions of diethyl ether. Combine all the extracts, and evaporate the diethyl ether on a water bath. Dissolve the residue in 5 mL of the mobile phase, add exactly 3 mL of the internal standard solution, and add the mobile phase to make 25 mL. Filter this solution through a filter of a porosity of not more than 0.8 μ m, discard the first 2 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, weigh accurately about 25 mg of Atropine Sulfate Reference Standard (previously determine the loss on drying <2.41> in the same manner as Atropine Sulfate Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution A. Weigh accurately about 25 mg of Scopolamine Hydrobromide Reference Standard (previously determine the loss on drying <2.41> in the same manner as Scopolamine Hydrobromide Hydrate), dissolve in the mobile phase to make exactly 25 mL, and use this solution as standard stock solution B. Pipet 5 mL of standard stock solution A and 1 mL of standard stock solution B, add exactly 3 mL of the internal standard solution, then add 25 mL of the mobile phase, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratios, Q_{TA} and Q_{SA} , of the peak area of hyoscyamine (atropine), and the ratios, Q_{TS} and Q_{SS} , of the peak area of scopolamine to that of the internal standard in each solution,

calculate the amounts of hyoscyamine and scopolamine by the following equation, and designate the total as the amount of total alkaloids.

$$\begin{aligned} \text{Amount (mg) of hyoscyamine (C}_{17}\text{H}_{23}\text{NO}_3) \\ = W_{SA} \times (Q_{TA}/Q_{SA}) \times (1/5) \times 0.8551 \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of scopolamine (C}_{17}\text{H}_{21}\text{NO}_4) \\ = W_{SS} \times (Q_{TS}/Q_{SS}) \times (1/25) \times 0.7894 \end{aligned}$$

W_{SA} : Amount (mg) of Atropine Sulfate Reference Standard, calculated on the dried basis

W_{SS} : amount (mg) of Scopolamine Hydrobromide Reference Standard, calculated on the dried basis

Internal standard solution—A solution of brucine dihydrate in the mobile phase (1 in 2500).

Operating conditions—

Detector: An ultraviolet absorption spectrometer (wavelength: 210 nm).

Column: A stainless steel column 4 mm in inside diameter and 15 cm in length, packed with octadecylsilylanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 20°C.

Mobile phase: Dissolve 6.8 g of potassium dihydrogenphosphate in 900 mL of water, add 10 mL of triethylamine, adjust with phosphoric acid to pH 3.5, and add water to make 1000 mL. To 9 parts of this solution add 1 part of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of scopolamine is about 8 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, scopolamine, atropine and the internal standard are eluted in this order with the resolution between the peaks of scopolamine and atropine being not less than 11, and with the resolution between the peaks of atropine and the internal standard being not less than 4.

Scutellaria Root

Scutellariae Radix

オウゴン

Scutellaria Root is the root of *Scutellaria baicalensis* Georgi (*Labiatae*), from which the periderm has been removed.

It contains not less than 10.0% of baicalin (C₂₁H₁₈O₁₁: 446.36), calculated on the basis of dried material.

Description Cone-shaped, semitubular or flattened root, 5 – 20 cm in length, 0.5 – 3 cm in diameter; externally yellow-brown, with coarse and marked longitudinal wrinkles, and with scattered scars of lateral root and remains of brown periderm; scars of stem or remains of stem at the crown; xylem rotted in old roots, often forming a hollow; hard in texture and easily broken; fractured surface fibrous and yellow in color.

Almost odorless; taste, slightly bitter.

Identification (1) Boil gently 0.5 g of pulverized Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown.

(2) To 2 g of pulverized Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one spot among the spots from the sample solution and a dark green spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Assay Weigh accurately about 0.5 g of pulverized Scutellaria Root, add 30 mL of the mobile phase, heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture of the pulverized Scutellaria Root and the mobile phase into a glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of the mobile phase, transfer the washings to the glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. To the residue add 30 mL of the mobile phase, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add the mobile phase to make exactly 100 mL, then pipet 2 mL of the extract, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (separately determine the water) dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Pipet 10 μ L of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, *A_T* and *A_S*, of baicalin in each solution.

$$\begin{aligned} & \text{Amount (mg) of baicalin (C}_{21}\text{H}_{18}\text{O}_{11}) \\ & = W_S \times (A_T/A_S) \times 5 \end{aligned}$$

W_S: Amount (mg) of Baicalin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilylated silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

Selection of column: Dissolve 1 mg of Baicalin Reference Standard and 2 mg of methyl parahydroxybenzoate in methanol to make 100 mL. Perform the test with 10 μ L of this solution under the above operating conditions and calculate the resolution. Use a column giving elution of baicalin and methyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 3.

System repeatability: When the test is repeated 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Powdered Scutellaria Root

Scutellariae Radix Pulverata

オウゴン末

Powdered Scutellaria Root is the powder of Scutellaria Root.

It contains not less than 10.0% of baicalin (C₂₁H₁₈O₁₁: 446.36), calculated on the basis of dried material.

Description Powdered Scutellaria Root occurs as a yellow-brown powder. It is almost odorless, and has a slight, bitter taste.

Under a microscope <5.01>, Powdered Scutellaria Root reveals fragments of parenchyma cells containing small amount of starch grains, fragments of reticulate vessels, tracheids and elongated stone cells; also a few fragments of spiral vessels and xylem fibers are observed.

Identification (1) Boil gently 0.5 g of Powdered Scutellaria Root with 20 mL of diethyl ether under a reflux condenser on a water bath for 5 minutes, cool, and filter. Evaporate the filtrate, dissolve the residue in 10 mL of ethanol (95), and to 3 mL of the solution add 1 to 2 drops of dilute iron (III) chloride TS: a grayish green color develops, and it changes to purple-brown later.

(2) To 2 g of Powdered Scutellaria Root add 10 mL of methanol, warm on a water bath for 3 minutes, cool, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Baicalin Reference Standard in 1 mL of methanol, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-butanol, water and acetic acid (100) (4:2:1) to a distance of

about 10 cm, and air-dry the plate. Spray evenly iron (III) chloride-methanol TS on the plate: one spot among the spots from the sample solution and dark green spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of Powdered Scutellaria Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of Powdered Scutellaria Root according to Method 4, and perform the test (not more than 5 ppm).

(3) Foreign matter—Under a microscope <5.01>, Powdered Scutellaria Root does not show crystals of calcium oxalate.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Assay Weigh accurately about 0.5 g of Powdered Scutellaria Root, add 30 mL of the mobile phase, heat under a reflux condenser on a water bath for 30 minutes, and cool. Transfer the mixture to a glass-stoppered centrifuge tube, centrifuge, and separate the supernatant liquid. Wash the vessel for the reflux extraction with 30 mL of the mobile phase, transfer the washings to the glass-stoppered centrifuge tube, centrifuge after shaking for 5 minutes, and separate the supernatant liquid. To the residue add 30 mL of the mobile phase, shake for 5 minutes, centrifuge, and separate the supernatant liquid. Combine all the extracts, add the mobile phase to make exactly 100 mL, then pipet 2 mL of the extract, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Baicalin Reference Standard (previously determine the water) and dissolve in methanol to make exactly 20 mL. Pipet 2 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Pipet 10 μ L of the sample solution and standard solution, and perform the test as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, *A_T* and *A_S*, of baicalin in each solution.

$$\begin{aligned} &\text{Amount (mg) of baicalin (C}_{21}\text{H}_{18}\text{O}_{11}) \\ &= W_S \times (A_T/A_S) \times 5 \end{aligned}$$

W_S: Amount (mg) of Baicalin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 277 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 146) and acetonitrile (18:7).

Flow rate: Adjust the flow rate so that the retention time of baicalin is about 6 minutes.

Selection of column: Dissolve 1 mg of Baicalin Reference Standard and 2 mg of methyl parahydroxybenzoate in

methanol to make 100 mL. Perform the test with 10 μ L of this solution under the above operating conditions and calculate the resolution. Use a column giving elution of baicalin and methyl parahydroxybenzoate in this order with the resolution between these peaks being not less than 3.

System repeatability: When repeat the test 6 times with the standard solution under the above operating conditions, the relative standard deviation of the peak area of baicalin is not more than 1.5%.

Senega

Senegae Radix

セネガ

Senega is the root of *Polygala senega* Linné or *Polygala senega* Linné var. *latifolia* Torrey et Gray (*Polygalaceae*).

Description Slender, conical root often branched, 3 – 10 cm in length; main root 0.5 – 1.5 cm in diameter; externally light grayish brown to grayish brown; with many longitudinal wrinkles and sometimes with twisted protruding lines; tuberously enlarged crown, with remains of stems and red buds; branched rootlets twisted; a transverse section reveals grayish brown cortex and yellowish white xylem; usually round, and sometimes cuneate to semicircular; cortex on the opposite side is thickened.

Odor, characteristic, resembling the aroma of methyl salicylate; taste, sweet at first but leaving an acrid taste.

Under a microscope <5.01>, a transverse section of the main root reveals a cork layer consisting of several rows of light brown cork cells; secondary cortex composed of parenchyma cells and sieve tubes, traversed by medullary rays, 1 to 3 cells wide; medullary rays on xylem not distinct. Its parenchyma cells contain oil droplets, but starch grains and calcium oxalate crystals are absent.

Identification (1) Shake vigorously 0.5 g of pulverized Senega with 10 mL of water: a lasting fine foam is produced.

(2) Shake 0.5 g of pulverized Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum at about 317 nm.

Purity (1) Stem—The amount of stems contained in Senega is not more than 2.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than stems contained in Senega is not more than 1.0%.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Powdered Senega

Senegae Radix Pulverata

セネガ末

Powdered Senega is the powder of Senega.

Description Powdered Senega occurs as a light brown powder, and has a characteristic odor resembling the aroma of methyl salicylate; taste, sweet at first, but later acrid.

Under a microscope <5.01>, Powdered Senega reveals fragments of pitted vessels, reticulate vessels and tracheids; fragments of xylem fibers with oblique pits; fragments of xylem parenchyma cells with simple pits; fragments of phloem parenchyma containing oily droplets; fragments of exodermis often composed of cells suberized and divided into daughter cells; oily droplets stained red by sudan III TS. The parenchyma cells of Powdered Senega do not contain starch grains and crystals of calcium oxalate.

Identification (1) Shake vigorously 0.5 g of Powdered Senega with 10 mL of water: a lasting fine foam is produced.

(2) Shake 0.5 g of Powdered Senega with 30 mL of water for 15 minutes, and filter. Take 1 mL of the filtrate, mix with 50 mL of water, and determine the absorption spectrum of the solution as directed under Ultraviolet-visible Spectrophotometry <2.24>: it exhibits a maximum at about 317 nm.

Purity Foreign matter <5.01>—Under a microscope, stone cells, starch grains or crystals of calcium oxalate are not observable.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 30.0%.

Senega Syrup

Syrupus Senegae

セネガシロップ

Method of preparation

Senega, in medium cutting	40 g
Sucrose	780 g
10 vol% Ethanol	a sufficient quantity
Purified Water	a sufficient quantity
To make 1000 mL	

Add 400 mL of 10 vol% ethanol to Senega, and macerate for one or two days. Filter the extract, wash the residue with a small amount of 10 vol% Ethanol, filter, and combine the filtrate of the extracts and washings until total volume measures about 500 mL. Dissolve Sucrose in the mixture, by warming if necessary, and dilute to 1000 mL with Purified

Water. May be prepared with an appropriate quantity of Ethanol and Purified Water in place of 10 vol% Ethanol.

Description Senega Syrup is a yellow-brown, viscous liquid. It has a characteristic odor resembling methyl salicylate and a sweet taste.

Identification Add 5 mL of water to 1 mL of Senega Syrup, and shake: lasting small bubbles are produced.

Containers and storage Containers—Tight containers.

Senna Leaf

Sennae Folium

センナ

Senna Leaf is the leaflets of *Cassia angustifolia* Vahl or *Cassia acutifolia* Delile (*Leguminosae*).

It contains not less than 1.0% of total sennosides [sennoside A ($C_{42}H_{38}O_{20}$: 862.74) and sennoside B ($C_{42}H_{38}O_{20}$: 862.74)], calculated on the basis of dried material.

Description Lanceolate to narrow lanceolate leaflets, 1.5 – 5 cm in length, 0.5 – 1.5 cm in width, light grayish yellow to light grayish yellow-green in color; margin entire, apex acute, base asymmetric, petiole short; under a magnifying glass, vein marked, primary lateral veins running toward the apex along the margin and joining the lateral vein above; lower surface having slight hairs.

Odor slight; taste, bitter.

Under a microscope <5.01>, a transverse section of Senna Leaf reveals epidermis with thick cuticle, with numerous stomata, and with thick-walled, warty unicellular hairs; epidermal cells are often separated into two loculi by a septum which is in parallel with the surface of the leaf, and contain mucilage in the inner loculus; palisade of a single layer under each epidermis; spongy tissue, consisting of 3 to 4 layers, and containing clustered or solitary crystals of calcium oxalate; cells adjacent to vascular bundle, forming crystal cell rows.

Identification (1) Macerate 0.5 g of pulverized Senna Leaf in 10 mL of diethyl ether for 2 minutes, and filter. Add 5 mL of ammonia TS to the filtrate: a yellow-red color is produced in the water layer. To the residue of maceration add 10 mL of water, and macerate for 2 minutes. Filter, and add 5 mL of ammonia TS: a yellow-red color is produced in the water layer.

(2) To 2 g of pulverized Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 by adding 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 1 mL of a mixture of tetrahydrofuran and water (7:3), and use this solution as the standard solution. Perform the test as directed under Thin-layer Chro-

matography <2.03> with the sample solution and standard solution. Spot 10 μL each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm): one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same R_f value.

Purity (1) Rachis and fruit—The amount of rachis and fruits contained in Senna Leaf does not exceed 5.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than rachis and fruits contained in Senna Leaf does not exceed 1.0%.

(3) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 12.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of pulverized Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard (separately determine the water), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B Reference Standard (separately determine the water), dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μL each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa} , of sennoside A, and the peak areas, A_{Tb} and A_{Sb} , of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennosides.

$$\begin{aligned} \text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}\text{)} \\ = W_{\text{Sa}} \times (A_{\text{Ta}}/A_{\text{Sa}}) \times (1/4) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of sennoside B (C}_{42}\text{H}_{38}\text{O}_{20}\text{)} \\ = W_{\text{Sb}} \times (A_{\text{Tb}}/A_{\text{Sb}}) \times (1/2) \end{aligned}$$

W_{Sa} : Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

W_{Sb} : Amount (mg) of Sennoside B Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside di-

ameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μm in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μL of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μL of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Powdered Senna Leaf

Sennae Folium Pulveratum

センナ末

Powdered Senna Leaf is the powder of Senna Leaf.

It contains not less than 1.0% of total sennosides [sennoside A (C₄₂H₃₈O₂₀: 862.74) and sennoside B (C₄₂H₃₈O₂₀: 862.74)], calculated on the basis of dried material.

Description Powdered Senna Leaf occurs as a light yellow to light grayish yellow-green powder. It has a slight odor and a bitter taste.

Under a microscope <5.01>, Powdered Senna Leaf reveals fragments of vessels and vein tissue accompanied with crystal cell rows; fragments of thick-walled, bent, unicellular hairs; fragments of palisade and spongy tissue; clustered and solitary crystals of calcium oxalate, 10 to 20 μm in diameter.

Identification (1) Macerate 0.5 g of Powdered Senna Leaf in 10 mL of diethyl ether for 2 minutes, and filter. Add 5 mL of ammonia TS to the filtrate: a yellow-red color is produced in the water layer. To the residue of maceration add 10 mL of water, and macerate for 2 minutes. Filter, and add 5 mL of ammonia TS: a yellow-red color is produced in the water layer.

(2) To 2 g of Powdered Senna Leaf add 40 mL of a mixture of tetrahydrofuran and water (7:3), shake for 30 minutes, and centrifuge. Transfer the supernatant liquid to a separator, add 13 g of sodium chloride, and shake for 30 minutes. Separate the water layer with undissolved sodium chloride, and adjust the pH to 1.5 with 1 mol/L hydrochloric acid TS. Transfer this solution to another separator, shake with 30 mL of tetrahydrofuran for 10 minutes, separate the tetrahydrofuran layer, and use this solution as the sample solution. Separately, dissolve 1 mg of Sennoside A Reference Standard in 1 mL of a mixture of tetrahydrofuran and water

(7:3), and use this solution as the standard solution. Perform the test as directed under Thin-layer Chromatography <2.03> with the sample solution and standard solution. Spot 10 μ L each of these solutions on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of 1-propanol, ethyl acetate, water and acetic acid (100) (40:40:30:1) to a distance of about 15 cm, and air-dry the plate. Examine under ultraviolet light (main wavelength: 365 nm); one spot among the spots from the sample solution and a red fluorescent spot from the standard solution show the same color tone and the same *R_f* value.

Purity (1) Foreign matter <5.01>—Under a microscope, stone cells and thick fibers are not observable.

(2) Total BHC's and total DDT's <5.01>—Not more than 0.2 ppm, respectively.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 12.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Assay Weigh accurately about 0.5 g of Powdered Senna Leaf in a glass-stoppered centrifuge tube, add 25 mL of diluted methanol (7 in 10), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add 10 mL of diluted methanol (7 in 10), shake for 10 minutes, centrifuge, and separate the supernatant liquid. Repeat this procedure once more, combine all the extracts, add diluted methanol (7 in 10) to make exactly 50 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Sennoside A Reference Standard (separately determine the water) dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (1). Weigh accurately about 10 mg of Sennoside B Reference Standard (separately determine the water) dissolve in a solution of sodium hydrogen carbonate (1 in 100) to make exactly 20 mL, and use this solution as standard stock solution (2). Pipet 5 mL of the standard stock solution (1) and 10 mL of the standard stock solution (2), add methanol to make exactly 50 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the peak areas, A_{Ta} and A_{Sa} , of sennoside A, and the peak areas, A_{Tb} and A_{Sb} , of sennoside B in each solution, calculate the amounts of sennoside A and sennoside B by the following equations, and designate the total as the amount of total sennoside.

$$\begin{aligned} \text{Amount (mg) of sennoside A (C}_{42}\text{H}_{38}\text{O}_{20}) \\ = W_{Sa} \times (A_{Ta}/A_{Sa}) \times (1/4) \end{aligned}$$

$$\begin{aligned} \text{Amount (mg) of sennoside B (C}_{42}\text{H}_{38}\text{O}_{20}) \\ = W_{Sb} \times (A_{Tb}/A_{Sb}) \times (1/2) \end{aligned}$$

W_{Sa} : Amount (mg) of Sennoside A Reference Standard, calculated on the anhydrous basis

W_{Sb} : Amount (mg) of Sennoside B Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 340 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized

silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: Dissolve 2.45 g of tetra-*n*-heptylammonium bromide in 1000 mL of a mixture of diluted 1 mol/L acetic acid-sodium acetate buffer solution, pH 5.0 (1 in 10) and acetonitrile (17:8).

Flow rate: Adjust the flow rate so that the retention time of sennoside A is about 26 minutes.

System suitability—

System performance: When the procedure is run with 10 μ L of the standard solution under the above operating conditions, sennoside B and sennoside A are eluted in this order with the resolution between these peaks being not less than 15, and the number of theoretical plates of the peak of sennoside A being not less than 8000.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak area of sennoside A is not more than 1.5%.

Sinomenium Stem

Sinomeni Caulis et Rhizoma

ボウイ

Sinomenium Stem is the climbing stem and rhizome of *Sinomenium acutum* Rehder et Wilson (*Menispermaceae*).

Description Round or elliptic sections, 0.2–0.4 cm in thickness, 1–4.5 cm in diameter; cortex on both fractured surfaces, light brown to dark brown; in xylem, grayish brown vessel portions and dark brown medullary rays lined alternately and radially; flank, dark gray, with longitudinal wrinkles and warty protrusions.

Almost odorless; taste, bitter.

Under a microscope <5.01>, a transverse section reveals extremely thick-walled stone cells in primary cortex and pericycle; irregular-sized vessels lined nearly stepwise in the vessel portion; cells of medullary ray mostly not lignified, and extremely thick-walled and large stone cells scattered here and there; primary cortex containing needle crystals of calcium oxalate; medullary rays containing starch grains, simple grain, 3–10 μ m in diameter, and small needle crystals of calcium oxalate.

Identification To 0.5 g of pulverized Sinomenium Stem add 10 mL of dilute acetic acid, heat for 2 minutes on a water bath with frequent shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is immediately produced.

Total ash <5.01> Not more than 7.0%.

Acid-insoluble ash <5.01> Not more than 0.5%.

Smilax Rhizome

Smilacis Rhizoma

サンキライ

Smilax Rhizome is the tuber of *Smilax glabra* Roxburgh (*Liliaceae*).

Description Flattened and irregular cylindrical tuber, often with node-like branches; usually 5 – 15 cm in length, 2 – 5 cm in diameter; the outer surface grayish yellow-brown to yellow-brown, and the upper surface scattered with knotty remains of stem; cross section irregular elliptical to obtuse triangular, consisting of extremely thin cortical layer and mostly of stele.

Odor, slight; almost tasteless.

Under a microscope <5.01>, a transverse section reveals a 2- to 3-cell-wide cork layer, with extremely narrow cortical layer, usually consisting of a 2- to 4-cell-wide, thick-walled parenchyma cells, showing large mucilage cells here and there; mucilage cell containing raphides of calcium oxalate; stele consisting chiefly of parenchyma cells, and scattered with vascular bundles; parenchyma cells containing starch grains composed mostly of simple grains, 12 – 36 μm in diameter, and sometimes mixed with 2- to 4-compound grains.

Total ash <5.01> Not more than 5.0%.

Powdered Smilax Rhizome

Smilacis Rhizoma Pulveratum

サンキライ末

Powdered Smilax Rhizome is the powder of Smilax Rhizome.

Description Powdered Smilax Rhizome occurs as a light yellow-brown powder, and has a slight odor, and is practically tasteless.

Under a microscope <5.01>, Powdered Smilax Rhizome reveals starch grains and fragments of parenchyma cells containing them; fragments of raphides of calcium oxalate contained in mucilage masses; fragments of lignified parenchyma cells of cortical layer; fragments of cork cells and scalariform vessels; starch grains composed mostly of simple grains, and mixed with a few 2- to 4-compound grains 12 – 36 μm in diameter.

Purity Foreign matter—Under a microscope <5.01>, Powdered Smilax Rhizome does not show a large quantity of stone cells or thick-walled fibers.

Total ash <5.01> Not more than 5.0%.

Sodium Bicarbonate and Bitter Tincture Mixture

苦味重曹水

Method of preparation

Sodium Bicarbonate	30 g
Bitter Tincture	20 mL
Water or Purified Water	a sufficient quantity
<hr/>	
	To make 1000 mL

Prepare before use, with the above ingredients.

Description Sodium Bicarbonate and Bitter Tincture Mixture is a clear, yellowish liquid, having a bitter taste.

Containers and storage Containers—Tight containers.

Sophora Root

Sophorae Radix

クジン

Sophora Root is the root of *Sophora flavescens* Aiton (*Leguminosae*) or often such root from which the periderm has been removed.

Description Cylindrical root, 5 – 20 cm in length, 2 – 3 cm in diameter; externally dark brown to yellow-brown, with distinct longitudinal wrinkles, and with laterally extended lenticels; root without periderm, externally yellowish white, with somewhat fibrous surface; the transversely cut surface, light yellow-brown; cortex, 0.1 – 0.2 cm in thickness, slightly tinged with dark color near cambium, forming a crack between xylem.

Odor, slight; taste, extremely bitter and lasting.

Identification To 0.5 g of powdered Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes with occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is produced immediately.

Purity (1) Stem—The amount of its stems contained in Sophora Root does not exceed 10.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than stems contained in Sophora Root does not exceed 1.0%.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Powdered Sophora Root

Sophorae Radix Pulverata

クジン末

Powdered Sophora Root is the powder of Sophora

Root.

Description Powdered Sophora Root occurs as a light brown powder. It has a slight odor, and an extremely bitter and lasting taste.

Under a microscope <5.01>, Powdered Sophora Root reveals mainly starch grains and fragments of parenchyma cells containing them, fibers, bordered pitted vessels, reticulate vessels; a few fragments of corky tissue and solitary crystals of calcium oxalate. Starch grains usually composed of 2- to 4-compound grains 15 – 20 μm in diameter, and simple grains 2 – 5 μm in diameter.

Identification To 0.5 g of Powdered Sophora Root add 10 mL of dilute acetic acid, heat on a water bath for 3 minutes while occasional shaking, cool, and filter. To 5 mL of the filtrate add 2 drops of Dragendorff's TS: an orange-yellow precipitate is produced immediately.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Sweet Hydrangea Leaf

Hydrangeae Dulcis Folium

アマチャ

Sweet Hydrangea Leaf is the leaf and twig of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (*Saxifragaceae*).

Description Usually wrinkled and contracted leaf, dark green to dark yellow-green in color. When soaked in water and smoothed out, it is lanceolate to acuminate ovate, about 12 cm in length, about 5 cm in width; margin serrated, base slightly wedged; coarse hair on both surfaces, especially on the veins; lateral veins not reaching the margin but curving upwards and connecting with each other; petiole short and less than one-fifth of the length of lamina.

Odor, slight; taste, characteristically sweet.

Identification Mix 0.5 g of pulverized Sweet Hydrangea Leaf with 8 mL of a mixture of diethyl ether and petroleum ether (1:1), shake well, filter, and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of dilute ethanol, and add 1 drop of dilute iron (III) chloride TS: a red-purple color develops, which disappears on the addition of 2 to 3 drops of dilute sulfuric acid.

Purity (1) Stem—The amount of stems contained in Sweet Hydrangea Leaf does not exceed 3.0%.

(2) Foreign matter <5.01>—The amount of foreign matter other than stems contained in Sweet Hydrangea Leaf does not exceed 1.0%.

Loss on drying <5.01> Not more than 13.0% (6 hours).

Total ash <5.01> Not more than 12.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Powdered Sweet Hydrangea Leaf

Hydrangeae Dulcis Folium Pulveratum

アマチャ末

Powdered Sweet Hydrangea Leaf is the powder of Sweet Hydrangea Leaf.

Description Powdered Sweet Hydrangea Leaf occurs as a dark yellow-green powder, and has a faint odor and a characteristic, sweet taste.

Under a microscope <5.01>, Powdered Sweet Hydrangea Leaf reveals fragments of epidermis with wavy lateral membrane; stomata with two subsidiary cells; unicellular and thin-walled hair with numerous protrusions of the surface, 150 – 300 μm in length; fragments of palisade tissue and spongy tissue; fragments of vascular bundle and mucilage cells containing raphides of calcium oxalate 50 – 70 μm in length.

Identification Mix 0.5 g of Powdered Sweet Hydrangea Leaf with 8 mL of a mixture of diethyl ether and petroleum ether (1:1), shake well, filter, and evaporate the filtrate to dryness. Dissolve the residue in 1 mL of dilute ethanol, and add 1 drop of dilute iron (III) chloride TS: a red-purple color develops, which disappears on the addition of 2 to 3 drops of dilute sulfuric acid.

Purity Foreign matter <5.01>—Under a microscope, Powdered Sweet Hydrangea Leaf does not show stone cells, a large quantity of fibers or starch grains.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 12.0%.

Acid-insoluble ash <5.01> Not more than 2.5%.

Swertia Herb

Swertiae Herba

センブリ

Swertia Herb is the whole herb of *Swertia japonica* Makino (*Gentianaceae*) collected during the blooming season.

It contains not less than 2.0% of swertiamarin ($\text{C}_{16}\text{H}_{22}\text{O}_{10}$: 374.34), calculated on the basis of dried material.

Description Herb, 20 cm in length, having flowers, opposite leaves, stems, and, usually, with short, lignified roots; stems square, about 0.2 cm in diameter, often with branches; the leaves and stems dark green to dark purple or yellow-brown in color; the flowers white to whitish, and the roots yellow-brown. When smoothed by immersing in water, leaves, linear or narrow lanceolate, 1 – 4 cm in length, 0.1 – 0.5 cm in width, entire, and sessile; corolla split deeply as five lobes; the lobes narrow, elongated ellipse shape, and under a magnifying glass, with two elliptical nectaries juxtaposed at the base of the inner surface; the margin of lobe resembles eye-

lashes; the five stamens grow on the tube of the corolla and stand alternately in a row with corolla-lobes; peduncle distinct. Odor, slight; taste, extremely bitter and persisting.

Identification To 2 g of pulverized Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same Rf value.

Purity Foreign matter <5.01>—The amount of straw and other foreign matters contained in Swertia Herb is not more than 1.0%.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 20.0%.

Assay Weigh accurately about 1 g of medium powder of Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin Reference Standard (separately determine the water), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of swertiamarin in each solution.

$$\begin{aligned} &\text{Amount (mg) of swertiamarin (C}_{16}\text{H}_{22}\text{O}_{10}) \\ &= W_S \times (A_T/A_S) \times 5 \end{aligned}$$

W_S : amount (mg) of Swertiamarin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (91:9).

Flow rate: Adjust the flow rate so that the retention time of swertiamarin is about 12 minutes.

System suitability—

System performance: Dissolve 1 mg each of Swertiamarin Reference Standard and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

Powdered Swertia Herb

Swertiae Herba Pulverata

センブリ末

Powdered Swertia Herb is the powder of Swertia Herb.

It contains not less than 2.0% of swertiamarin (C₁₆H₂₂O₁₀: 374.34), calculated on the basis of dried material.

Description Powdered Swertia Herb occurs as a grayish yellow-green to yellow-brown powder. It has a slight odor, and extremely bitter, persistent taste.

Under a microscope <5.01>, Powdered Swertia Herb reveals xylem tissues with fibers (components of stems and roots); assimilation tissues (components of leaves and calyces); striated epidermis (components of stems and peduncles); tissues of corollas and filaments with spiral vessels; cells of anthers and their inner walls; spherical pollen grains with granular patterns (components of flowers), about 30 μ m in diameter; starch grains are simple grain, about 6 μ m in diameter, and very few.

Identification To 2 g of Powdered Swertia Herb add 10 mL of ethanol (95), shake for 5 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 2 mg of Swertiamarin Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, 1-propanol and water (6:4:3) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot among several spots from the sample solution and a red spot from the standard solution show the same color tone and the same Rf value.

Purity Foreign matter—Under a microscope <5.01>, crystals of calcium oxalate, a large quantity of starch grains and groups of stone cells are not observable.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 6.5%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 20.0%.

Assay Weigh accurately about 1 g of Powdered Swertia Herb in a glass-stoppered centrifuge tube, add 40 mL of methanol, shake for 15 minutes, centrifuge, and separate the supernatant liquid. To the residue add 40 mL of methanol, and proceed in the same manner. Combine the extracts, and add methanol to make exactly 100 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg of Swertiamarin Reference Standard (separately determine the water), dissolve in methanol to make exactly 20 mL. Pipet 5 mL of the solution, add the mobile phase to make exactly 20 mL, and use this solution as the standard solution. Perform the test with exactly 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_T and A_S , of swertiamarin in each solution.

$$\begin{aligned} & \text{Amount (mg) of swertiamarin (C}_{16}\text{H}_{22}\text{O}_{10}) \\ & = W_S \times (A_T/A_S) \times 5 \end{aligned}$$

W_S : Amount (mg) of Swertiamarin Reference Standard, calculated on the anhydrous basis

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 238 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 50°C.

Mobile phase: A mixture of water and acetonitrile (91:9).

Flow rate: Adjust the flow rate so that the retention time of swertiamarin is about 12 minutes.

System suitability—

System performance: Dissolve 1 mg each of Swertiamarin Reference Standard and theophylline in the mobile phase to make 10 mL. When the procedure is run with 10 μ L of this solution under the above operating conditions, theophylline and swertiamarin are eluted in this order with the resolution of these peaks being not less than 10.

System repeatability: When the test is repeated 6 times with 10 μ L of the standard solution under the above operating conditions, the relative standard deviation of the peak areas of swertiamarin is not more than 1.5%.

Swertia and Sodium Bicarbonate Powder

センブリ・重曹散

Method of preparation

Powdered Swertia Herb	30 g
Sodium Bicarbonate	700 g
Starch, Lactose Hydrate or their mixture	a sufficient quantity
To make 1000 g	

Prepare as directed under Powders, with the above in-

gredients.

Description Swertia and Sodium Bicarbonate Powder occurs as a light grayish yellow powder, having a bitter taste.

Identification (1) To 10 g of Swertia and Sodium Bicarbonate Powder add 10 mL of ethanol (95), shake for 15 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 1 mg of Swertiamarin Reference Standard in 1 mL of ethanol (95), and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 30 μ L each of the sample solution and standard solution on a plate of silica gel with fluorescent indicator for thin-layer chromatography. Proceed as directed in the Identification under Powdered Swertia Herb.

(2) To 0.5 g of Swertia and Sodium Bicarbonate Powder add 10 mL of water. After stirring, centrifuge the mixture with 500 revolutions per minute. Smear, using a small glass rod, the slide glass with a small amount of the precipitate, add 1 drop of a mixture of water and glycerin (1:1), and put a cover glass on it so that the tissue section spreads evenly without overlapping each other, taking precaution against inclusion of bubbles, and use this as the preparation for microscopic examination. If the precipitate separates into two layers, proceed with the upper layer in the same manner, and use as the preparation for microscopic examination. Heat the preparation for microscopic examination in a short time: the preparation reveals the yellow-green to yellow-brown, approximately spherical pollen grains with granular patterns under a microscope <5.01>. The pollen grains are 25 – 34 μ m in diameter.

(3) The supernatant liquid obtained in (2) by centrifuging responds to the Qualitative Tests <1.09> (1) for bicarbonate.

Containers and storage Containers—Well-closed containers.

Termeric

Curcumae Rhizoma

ウコン

Termeric is the rhizome or being removed the cork layer from it of *Curcuma longa* Linné (*Zingiberaceae*), usually after being passed through hot water.

Description Termeric is a main rhizome or a lateral rhizome; main rhizome, nearly ovoid, about 3 cm in diameter, about 4 cm in length; lateral rhizome, cylindrical, with round tips, curved, about 1 cm in diameter, 2 - 6 cm in length; both main and lateral rhizomes with cyclic nodes; rhizome with cork layer, yellowish brown, lustrous; rhizome without cork layer, dark yellowish red, with yellowish red powders on surface; hard in texture, not easily broken; transversely cut surface yellowish brown to reddish brown, lustrous like wax.

Odor, characteristic; taste, slightly bitter and stimulant, it colors a saliva yellow on chewing.

Under a microscope <5.01>, a transverse section reveals the outermost layer to be composed of a cork layer 4 - 10 cells thick; sometimes a cork layer partly remains; cortex and stele, divided by a single-layered endodermis, composed of

parenchyma, vascular bundles scattered; oil cells scattered in parenchyma; parenchymatous cells contain yellow substances, sandy and solitary crystals of calcium oxalate, and gelatinized starch.

Identification To 0.5 g of pulverized Termeric add 20 mL of methanol, shake for 15 minutes, filter and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 5 μ L of the sample solution on a plate of silica gel for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, hexane and acetic acid (100) (70:30:1) to a distance of about 10 cm, and air-dry the plate: a yellow spot appears at around R_f 0.4.

Loss on drying <5.01> Not more than 17.0% (6 hours).

Total ash <5.01> Not more than 7.5%.

Acid-insoluble ash <5.01> Not more than 1.0%.

Extract content <5.01> Not less than 9.0% (dilute ethanol-soluble extract).

Toad Venom

Bufois Venenum

センソ

Toad Venom is the venomous secretion of *Bufo bufo gargarizans* Cantor or *Bufo melanostictus* Schneider (*Bufo*idae).

When dried, it contains not less than 5.8% of bufo steroid.

Description A round disk with slightly dented bottom and protuberant surface, about 8 cm in diameter, about 1.5 cm in thickness, the mass of one disk being about 80 to 90 g; or a round disk with almost flattened surfaces on both sides, about 3 cm in diameter, and about 0.5 cm in thickness, the mass of one disk being about 8 g; externally red-brown to blackish brown, somewhat lustrous, approximately uniform and horny, hard in texture, and difficult to break; fractured surface nearly flat, and edges of broken pieces red-brown and translucent.

Odorless; taste, bitter and irritating, followed a little later by a lasting sensation of numbness.

Identification To 1 g of pulverized Toad Venom add 10 mL of acetone, shake for 10 minutes, filter, and use the filtrate as the sample solution. Separately, dissolve 5 mg of resibufogenin for thin-layer chromatography in 5 mL of acetone, and use this solution as the standard solution. Perform the test with these solutions as directed under Thin-layer Chromatography <2.03>. Spot 10 μ L each of the sample solution and standard solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of cyclohexane and acetone (3:2) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, and heat at 105°C for 5 minutes: one of several spots obtained from the sample solution has the same color tone and the same R_f value with the blue-green spot obtained from the standard solution.

Total ash <5.01> Not more than 5.0%.

Acid-insoluble ash <5.01> Not more than 2.0%.

Component determination Weigh accurately about 0.5 g of pulverized Toad Venom, previously dried in a desiccator (silica gel) for 24 hours, add 50 mL of methanol, heat under a reflux condenser on a water bath for 1 hour, cool, and filter. Wash the residue with 30 mL of methanol, and combine the washing and filtrate. To this solution add methanol to make exactly 100 mL. Pipet 10 mL of this solution, add exactly 5 mL of the internal standard solution, add methanol to make exactly 25 mL, and use this solution as the sample solution. Separately, weigh accurately about 10 mg, about 20 mg and about 20 mg of bufalin for component determination, cinobufagin for component determination and resibufogenin for component determination, respectively, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in methanol to make exactly 100 mL. Pipet 10 mL of this solution, proceed in the same manner as the sample solution, and use this solution as the standard solution. Perform the test with 10 μ L each of the sample solution and standard solution as directed under Liquid Chromatography <2.01> according to the following conditions. Determine the ratios, Q_{TB} and Q_{SB} , of the peak area of bufalin, Q_{TC} and Q_{SC} , of the peak area of cinobufagin, and Q_{TR} and Q_{SR} , of the peak area of resibufogenin, respectively, to that of the internal standard in each solution, and designate the total amount as an amount of bufosteroid.

$$\text{Amount (mg) of bufalin} = W_{SB} \times (Q_{TB}/Q_{SB})$$

$$\text{Amount (mg) of cinobufagin} = W_{SC} \times (Q_{TC}/Q_{SC})$$

$$\text{Amount (mg) of resibufogenin} = W_{SR} \times (Q_{TR}/Q_{SR})$$

W_{SB} : Amount (mg) of bufalin for component determination

W_{SC} : Amount (mg) of cinobufagin for component determination

W_{SR} : Amount (mg) of resibufogenin for component determination

Internal standard solution—A solution of indometacin in methanol (1 in 4000).

Operating conditions—

Detector: An ultraviolet spectrophotometer (wavelength: 300 nm).

Column: A stainless steel column 4 to 6 mm in inside diameter and 15 to 30 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 to 10 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of diluted phosphoric acid (1 in 1000) and acetonitrile (11:9).

Flow rate: Adjust the flow rate so that the retention time of the internal standard is 16 to 19 minutes.

Selection of column: Proceed with 10 μ L of the standard solution under the above operating conditions. Use a column giving elution of bufalin, cinobufagin, resibufogenin and the internal standard in this order, and clearly dividing each peak.

Tragacanth

Tragacantha

トラガント

Tragacanth is the exudation obtained from the trunks of *Astragalus gummifer* Labillardière or other species of the same genus (*Leguminosae*).

Description Tragacanth occurs as curved, flattened or lamellate fragments, 0.5 to 3 mm in thickness. It is white to light yellow in color, translucent, and horny in texture. It is easily broken, and swells in water.

Odorless; tasteless and mucilaginous.

Identification (1) To 1 g of powdered Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To pulverized Tragacanth add dilute iodine TS, and examine the mixture microscopically <5.01>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red to red color develops.

Total ash <5.01> Not more than 4.0%.

Powdered Tragacanth

Tragacantha Pulverata

トラガント末

Powdered Tragacanth is the powder of Tragacanth.

Description Powdered Tragacanth occurs as a white to yellowish white powder. It is odorless, tasteless and mucilaginous.

Under a microscope <5.01>, it, immersed in olive oil or liquid paraffin, reveals numerous angular fragments with a small amount of the circular or irregular lamellae or of starch grains. Starch grains are spherical to elliptical, mostly simple and occasionally 2- to 4-compound grains, simple grain, 3 – 25 μm in diameter. The fragments are swollen and altered with water.

Identification (1) To 1 g of Powdered Tragacanth add 50 mL of water: a nearly uniform, slightly turbid mucilage is formed.

(2) To Powdered Tragacanth add dilute iodine TS, and examine the mixture microscopically <5.01>: a few blue-colored starch grains are observable.

Purity Karaya gum—Boil 1 g of Powdered Tragacanth with 20 mL of water until a mucilage is formed, add 5 mL of hydrochloric acid, and again boil the mixture for 5 minutes: no light red to red color develops.

Total ash <5.01> Not more than 4.0%.

Containers and storage Containers—Tight containers.

Tribulus Fruit

Tribuli Fructus

シツリシ

Tribulus Fruit is the fruit of *Tribulus terrestris* Linné (*Zygophyllaceae*).

Description Pentagonal star shaped fruit, composed of five mericarps, 7 – 12 mm in diameter, often each mericarp separated; externally grayish green to grayish brown; a pair of longer and shorter spines on surface of each mericarp, the longer spine 3 – 7 mm in length, the shorter one 2 – 5 mm in length, numerous small processes on midrib; pericarp hard in texture, cut surface light yellow; each mericarp contains 1 – 3 seeds.

Almost odorless; taste, mild at first, followed by bitterness.

Under a microscope <5.01>, a transverse section reveals epicarp composed of a single-layered epidermis; mesocarp composed of parenchyma and sclerenchyma layer; endocarp composed of several-layered fiber cells; a single-layer of cell between mesocarp and endocarp contain solitary crystals of calcium oxalate; cotyledons of seed contain oil drops and aleurone grains, and occasionally starch grains.

Identification To 2 g of pulverized Tribulus Fruit add 5 mL of methanol, shake for 10 minutes, filter, and use the filtrate as the sample solution. Perform the test with this solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel for thin-layer chromatography, develop the plate with a mixture of ethyl acetate and water (40:1) to a distance of about 10 cm, and air-dry the plate. Spray evenly dilute sulfuric acid on the plate, heat at 105°C for 5 minutes, and examine under ultraviolet light (main wavelength: 365 nm): a blue-white fluorescent spot appears at around R_f 0.4.

Purity (1) Peduncle—Not more than 4.0%.

(2) Foreign matters <5.01>—Not more than 1.0% of foreign matters other than peduncle.

Loss on drying <5.01> Not more than 11.0% (6 hours).

Total ash <5.01> Not more than 13.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Extract content <5.01> Dilute ethanol-soluble extract: not less than 8.5%.

Trichosanthes Root

Trichosanthis Radix

カロコン

Trichosanthes Root is the root of *Trichosanthes kirilowii* Maximowicz, *Trichosanthes kirilowii* Maximowicz var. *Japonicum* Kitamura or *Trichosanthes bracteata* Voigt (*Cucurbitaceae*), from which the cortical layer has been removed.

Description Irregular cylindrical root 5–10 cm in length, 3–5 cm in diameter, often cut lengthwise; externally light yellowish white, and with irregular pattern of vascular bundles appearing as brownish yellow lines; fractured surface somewhat fibrous and light yellow in color; under a magnifying glass, the transverse section reveals wide medullary rays and brownish yellow spots or small holes formed by vessels.

Odorless; taste, slightly bitter.

Purity (1) Heavy metals <1.07>—Proceed with 3.0 g of pulverized *Trichosanthes* Root according to Method 3, and perform the test. Prepare the control solution with 3.0 mL of Standard Lead Solution (not more than 10 ppm).

(2) Arsenic <1.11>—Prepare the test solution with 0.40 g of pulverized *Trichosanthes* Root according to Method 4, and perform the test (not more than 5 ppm).

Total ash <5.01> Not more than 4.0%.

Uncaria Hook

Uncariae Uncis Cum Ramulus

チヨウトウコウ

Uncaria Hook is, hook or the hook-bearing stem, of *Uncaria rhynchophylla* Miquel, *Uncaria sinensis* Haviland or *Uncaria macrophylla* Wallich (*Rubiaceae*).

Uncaria Hook contains not less than 0.03% of total alkaloids (rhynchophylline and hirsutine), calculated on the dried basis.

Description *Uncaria Hook* is uncinat hook or short stem with opposite or single hook; the hook, 1 to 4 cm in length, curved and acuminate; externally red-brown to dark brown or yellow-brown, some one with hairs, the transverse section oblong to elliptical, light brown; stem thin and prismatic square to cylindrical, 2 to 5 mm in diameter, externally, red-brown to dark brown or yellow-brown; the transverse section, square to elliptical; the pith light brown, square to elliptical; hard in texture.

Odorless and practically tasteless.

Under a microscope <5.01>, a transverse section of the hook reveals vascular bundles in the cortex, unevenly distributed and arranged in a ring. Parenchyma cells in the secondary cortex containing sand crystals of calcium oxalate.

Identification To 1 g of pulverized *Uncaria Hook* add 20 mL of methanol, boil under a reflux condenser on a water bath for 5 minutes, and filter. Evaporate the filtrate to dryness, add 5 mL of dilute acetic acid to the residue, warm the mixture on a water bath for 1 minute, and filter after cooling. Spot 1 drop of the filtrate on a filter paper, air-dry, spray Dragendorff's TS for spraying on it, and allow to stand: a yellow-red color develops.

Loss on drying <5.01> Not more than 12.0% (6 hours).

Total ash <5.01> Not more than 4.0%.

Extract content <5.01> Dilute ethanol-souble extract: not less than 8.5%.

Component determination Weigh accurately about 0.2 g of medium powder of *Uncaria Hook*, transfer into a glass-stoppered centrifuge tube, add 30 mL of a mixture of methanol and dilute acetic acid (7:3), shake for 30 minutes, centrifuge, and separate the supernatant liquid. To the residue add two 10-mL portions of a mixture of methanol and dilute acetic acid (7:3), proceed in the same manner, and combine all of the supernatant liquid. To the combined liquid add a mixture of methanol and dilute acetic acid (7:3) to make exactly 50 mL, and use this as the sample solution. Separately, weigh accurately about 5 mg of rhynchophylline for component determination, previously dried in a desiccator (silica gel) for 24 hours, and dissolve in a mixture of methanol and dilute acetic acid (7:3) to make exactly 100 mL. Pipet 1 mL of this solution, add a mixture of methanol and dilute acetic acid (7:3) to make exactly 10 mL, and use this solution as the standard solution (1). Separately, dissolve 1 mg of hirsutine in 100 mL of a mixture of methanol and dilute acetic acid (7:3), and use this solution as the standard solution (2). Perform the test with exactly 20 μ L each of the sample solution and standard solutions (1) and (2) as directed under Liquid Chromatography <2.01> according to the following conditions, and determine the peak areas, A_{Ta} and A_{Tb} , of rhynchophylline and hirsutine obtained from the sample solution, and the peak area, A_S , of rhynchophylline from the standard solution (1).

Amount (mg) of total alkaloids (rhynchophylline and hirsutine)

$$= W_S \times \{(A_{Ta} + 1.405A_{Tb})/A_S\} \times (1/20)$$

W_S : Amount (mg) of rhynchophylline for component determination

Operating conditions—

Detector: An ultraviolet absorption photometer (wavelength: 245 nm).

Column: A stainless steel column 4.6 mm in inside diameter and 25 cm in length, packed with octadecylsilanized silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Dissolve 3.85 g of ammonium acetate in 200 mL of water, add 10 mL of acetic acid (100) and water to make 1000 mL, and add 350 mL of acetonitrile.

Flow rate: Adjust the flow rate so that the retention time of rhynchophylline is about 17 minutes.

System suitability—

System performance: Dissolve 5 mg of rhynchophylline for component determination in 100 mL of a mixture of methanol and dilute acetic acid (7:3). To 5 mL of this solution add 1 mL of ammonia solution (28), and reflux for 10 minutes or warm at about 50°C for 2 hours. After cooling, to 1 mL of the solution so obtained add a mixture of methanol and dilute acetic acid (7:3) to make 5 mL. When the procedure is run with 20 μ L of this solution under the above operating conditions, the peak of isorhynchophylline is appears in addition to the peak of rhynchophylline, and the resolution between these peaks is not less than 1.5.

System repeatability: When the test is repeated 6 times with 20 μ L of the standard solution (1) under the above operating conditions, the relative standard deviation of the peak areas of rhynchophylline is not more than 1.5%.

Uva Ursi Fluidextract

ウワウルシ流エキス

Uva Ursi Fluidextract contains not less than 3.0 w/v% of arbutin.

Method of preparation Prepare an infusion from Bearberry Leaf, in coarse powder, as directed under Fluidextracts, using hot Purified Water. Remove a part of the accompanying tannin, evaporate the mixture under reduced pressure, if necessary, and add Purified Water to adjust the percentage. It may contain an appropriate quantity of Ethanol.

Description Uva Ursi Fluidextract is a yellow-brown to dark red-brown liquid, and has a bitter and astringent taste.

It is miscible with water and with ethanol (95).

Identification To 1 mL of Uva Ursi Fluidextract add 30 mL of a mixture of ethanol (95) and water (7:3), shake, filter, and use the filtrate as the sample solution. Proceed as directed in the Identification (2) under Bearberry Leaf.

Component determination Pipet 1 mL of Uva Ursi Fluidextract, add water to make exactly 100 mL, and use this solution as the sample solution. Proceed as directed in the Component determination under Bearberry Leaf.

$$\text{Amount (mg) of arbutin} = W_S \times (A_T/A_S)$$

W_S : Amount (mg) of arbutin for component determination

Containers and storage Containers—Tight containers.

Zanthoxylum Fruit

Zanthoxyli Fructus

サンシヨウ

Zanthoxylum Fruit is the pericarps of the ripe fruit of *Zanthoxylum piperitum* De Candolle (*Rutaceae*), from which the seeds separated from the pericarps have been mostly removed.

Description Capsules of 2 or 3 flattened spheroidal mericarps, which are dehiscent in 2 pieces about 5 mm in diameter; the outer surface of pericarp, dark yellow-red to dark red-brown, with numerous dented spots originated from oil sacs; the inner surface, light yellowish white.

Odor, characteristically aromatic; taste, acrid, which gives numbing sensation to the tongue.

Under a microscope <5.01>, transverse section of Zanthoxylum Fruit reveals the external epidermis and the adjoined unicellular layer containing red-brown tannin; the pericarp holds oil sacs being up to approximately 500 μm in diameter and sporadically vascular bundles consisting mainly of spiral vessels; the endocarp consists of stone cell layers; inner epidermal cells very small.

Identification To 0.5 g of pulverized Zanthoxylum Fruit add 100 mL of diluted ethanol (7 in 10), stopper the vessel

tightly, shake for 30 minutes, filter, and use this filtrate as the sample solution. Perform the test with the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot showing a grayish red to red color at the R_f value of about 0.7 appears.

Purity (1) Seed—The amount of the seeds contained in Zanthoxylum Fruit does not exceed 20.0%.

(2) Peduncle and twig—The amount of the peduncles and twigs contained in Zanthoxylum Fruit does not exceed 5.0%.

(3) Foreign matter <5.01>—The amount of foreign matter other than peduncles and twigs contained in Zanthoxylum Fruit does not exceed 1.0%.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 30.0 g of pulverized Zanthoxylum Fruit: the volume of essential oil is not less than 1.0 mL.

Powdered Zanthoxylum Fruit

Zanthoxyli Fructus Pulveratus

サンシヨウ末

Powdered Zanthoxylum Fruit is the powder of Zanthoxylum Fruit.

Description Powdered Zanthoxylum Fruit occurs as a dark yellow-brown powder. It has a strong, characteristic aroma and an acrid taste leaving a sensation of numbness on the tongue.

Under a microscope <5.01>, Powdered Zanthoxylum Fruit reveals fragments of inner tissue of pericarp consisting of stone cells with membranes about 2.5 μm in thickness; fragments of spiral and annular vessels 10 to 15 μm in diameter; fragments of oil sacs containing essential oil or resin; fragments of epidermal cells, polygonal in surface view, containing tannin; numerous oil drops; masses of tannin, colored red by adding vanillin-hydrochloric acid TS.

Identification To 0.5 g of Powdered Zanthoxylum Fruit add 100 mL of diluted ethanol (7 in 10), stopper the vessel tightly, shake for 30 minutes, filter, and perform the test with the filtrate as the sample solution as directed under Thin-layer Chromatography <2.03>. Spot 10 μL of the sample solution on a plate of silica gel with complex fluorescent indicator for thin-layer chromatography. Develop the plate with a mixture of ethyl acetate, ethanol (95) and water (8:2:1) to a distance of about 10 cm, and air-dry the plate. Examine under ultraviolet light (broad spectrum wavelength): one spot showing a grayish red to red color at the R_f value of about 0.7 appears.

Total ash <5.01> Not more than 6.0%.

Acid-insoluble ash <5.01> Not more than 1.5%.

Essential oil content <5.01> Perform the test with 30.0 g of

Powdered Zanthoxylum Fruit: the volume of essential oil is not less than 0.8 mL.

Containers and storage Containers—Tight containers.

Zedoary

Zedoariae Rhizoma

ガジュツ

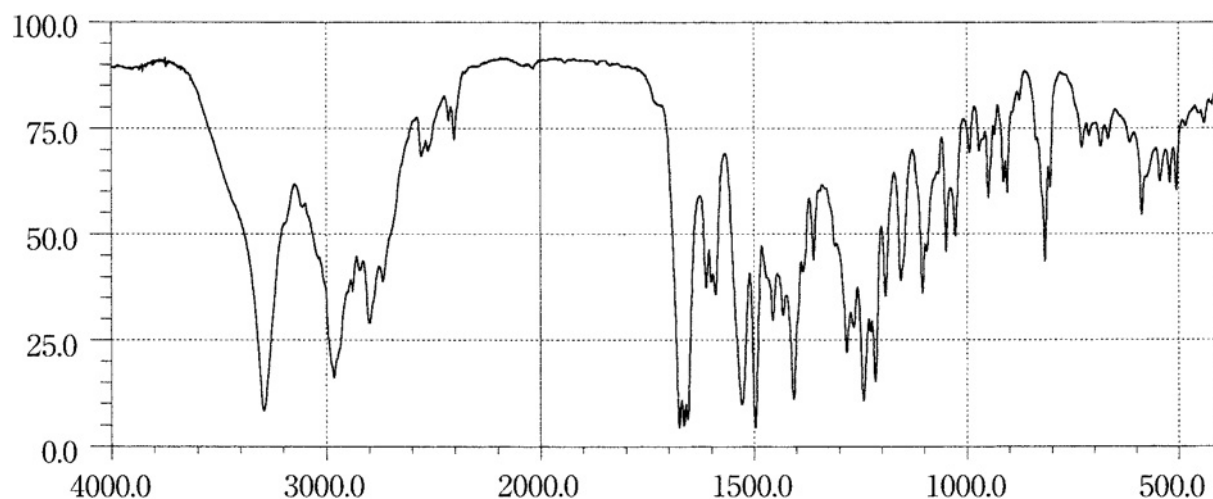
Zedoary is the rhizome of *Curcuma zedoaria* Roscoe (*Zingiberaceae*), usually after being passed through hot water.

Description Nearly ovoid rhizome, 4 – 6 cm in length, 2.5 – 4 cm in diameter; externally grayish yellow-brown to grayish brown; nodes protruded as rings; internode of 0.5 – 0.8 cm, with thin, longitudinal wrinkles, scars of removed roots, and small protrusions of branched rhizomes; under a magnifying glass, external surface covered with coarse hairs; horny in texture and difficult to cut; cross section grayish brown in color; cortex 2 – 5 mm in thickness, stele thick, a light grayish brown ring separating them.

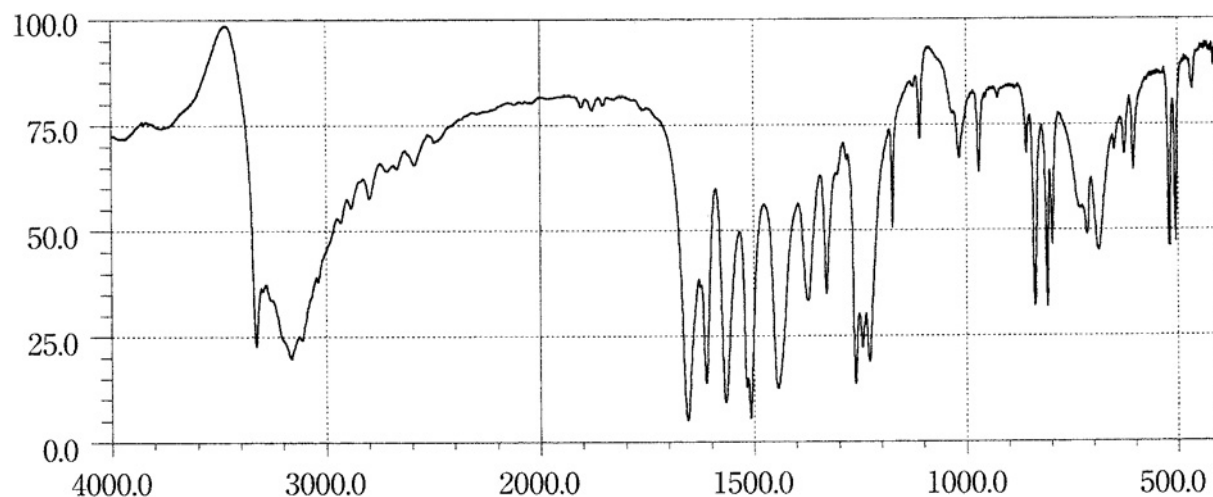
Odor, characteristic; taste, pungent, bitter and cooling.

Total ash <5.01> Not more than 7.0%.

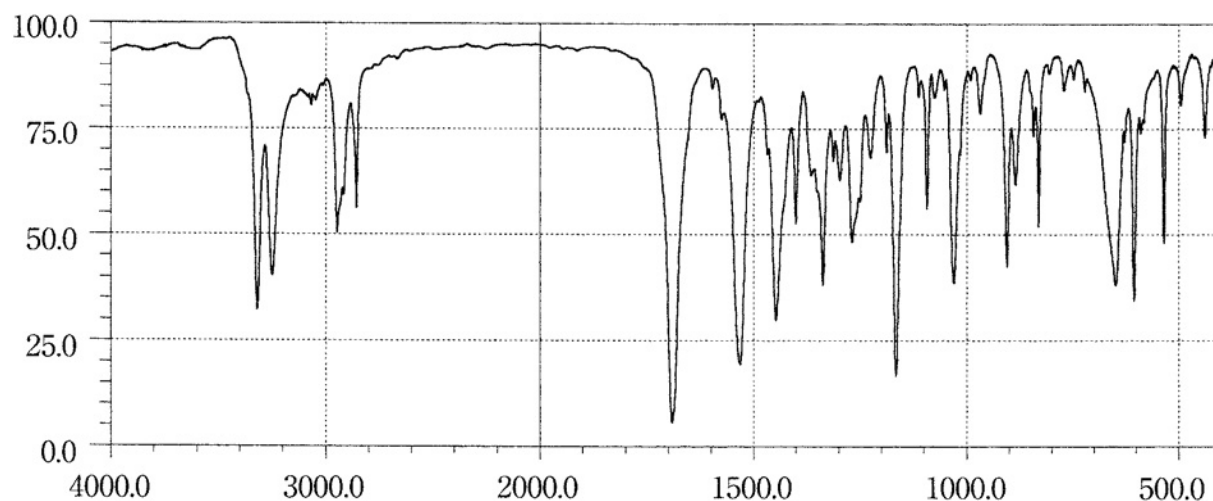
Essential oil content <5.01> Perform the test with 50.0 g of pulverized Zedoary, provided that 1 mL of silicon resin is previously added to the sample in the flask: the volume of essential oil is not less than 0.5 mL.

Acetubolol Hydrochloride

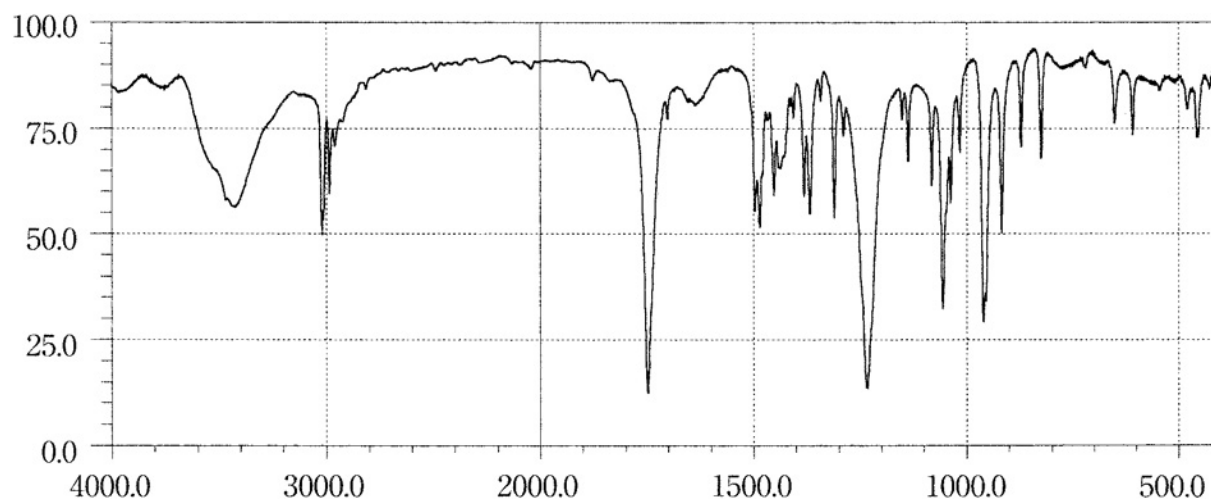
Preparation of sample: Potassium bromide disk method

Acetaminophen

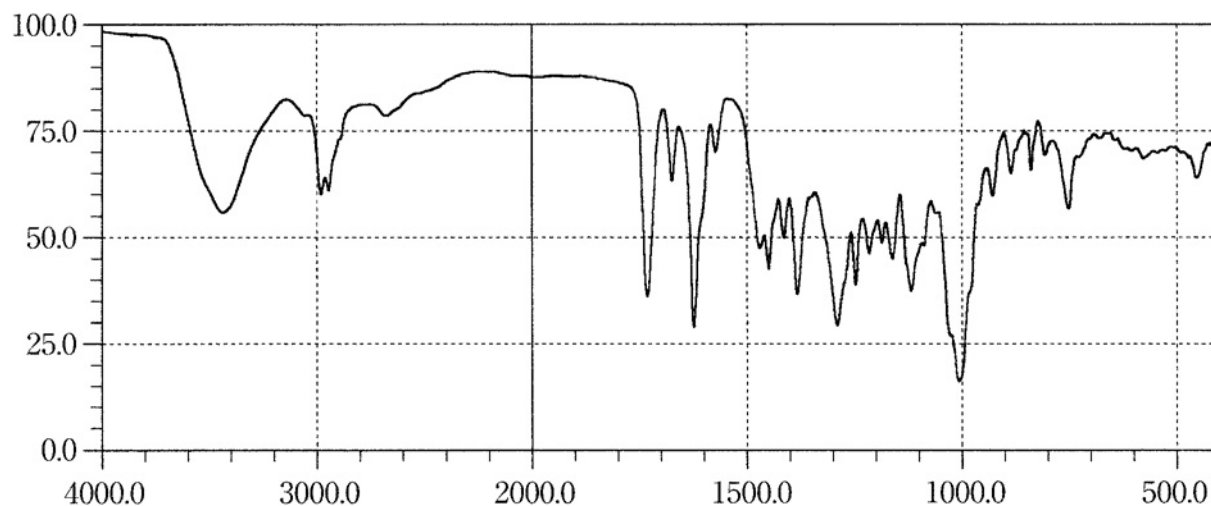
Preparation of sample: Potassium bromide disk method

Acetohexamide

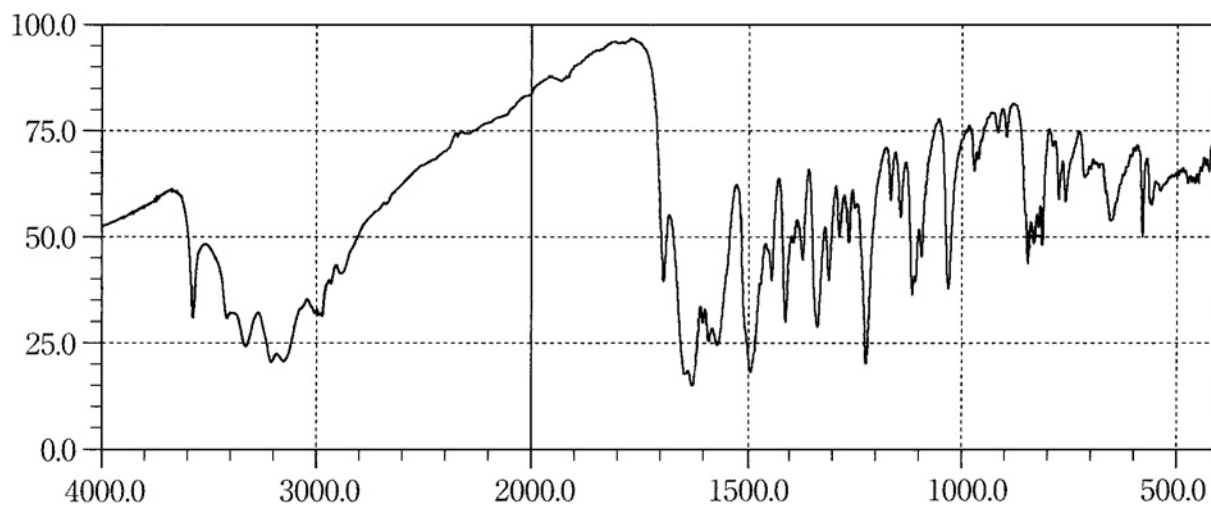
Preparation of sample: Potassium bromide disk method

Acetylcholine Chloride for Injection

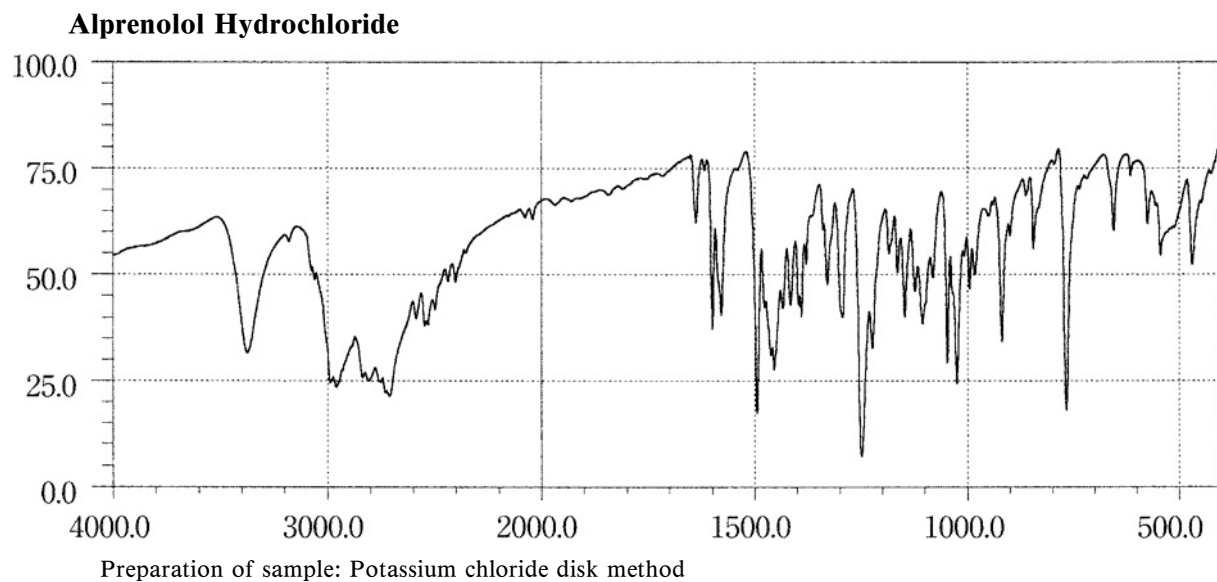
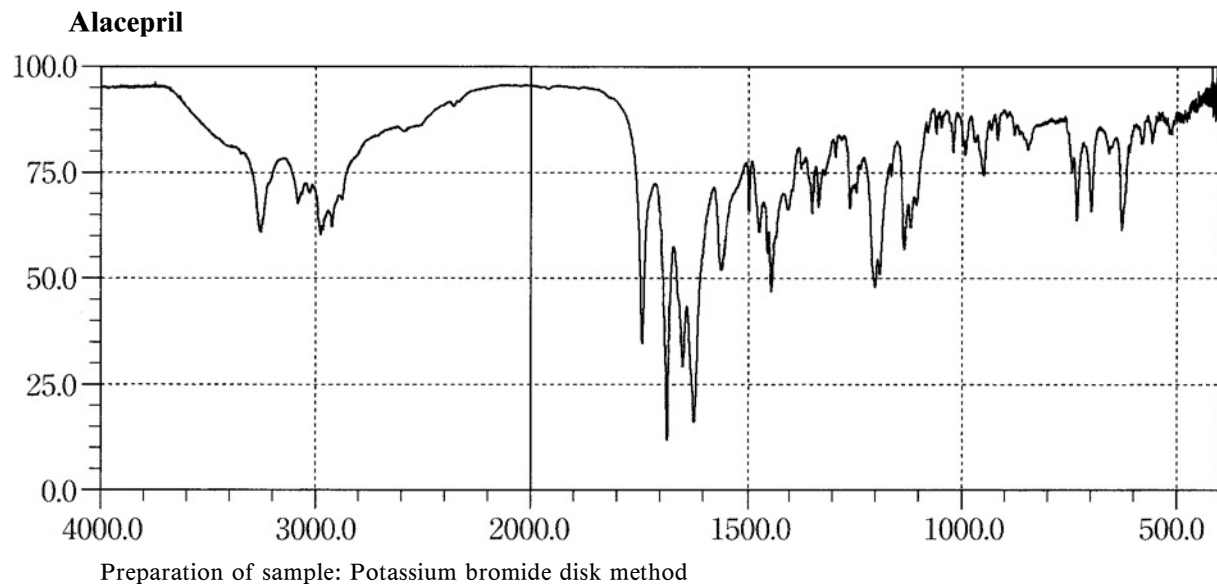
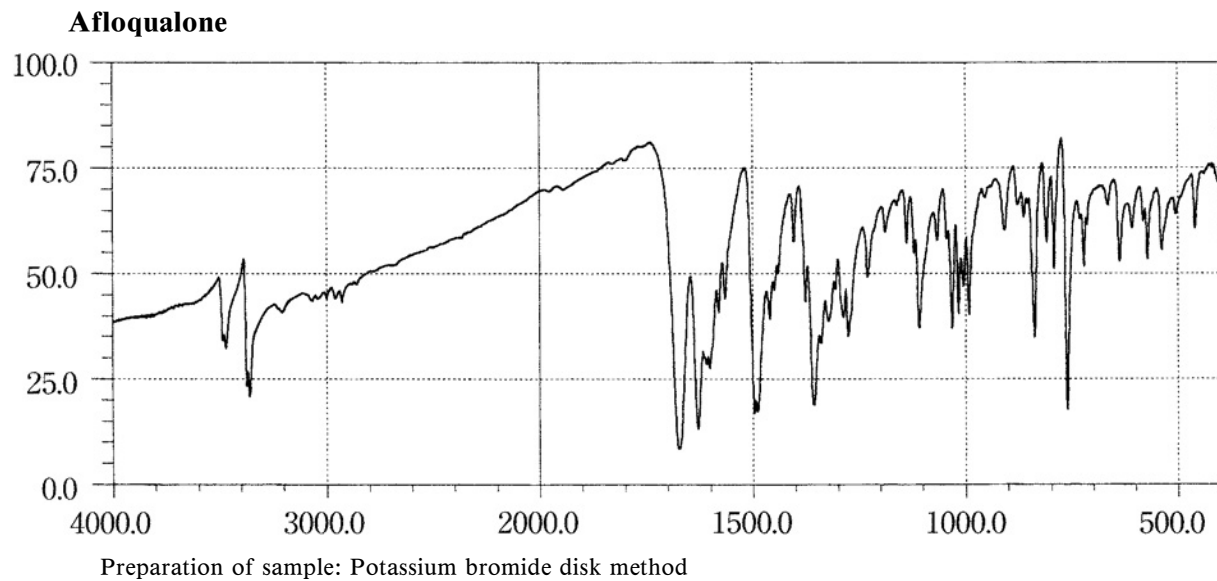
Preparation of sample: Potassium bromide disk method

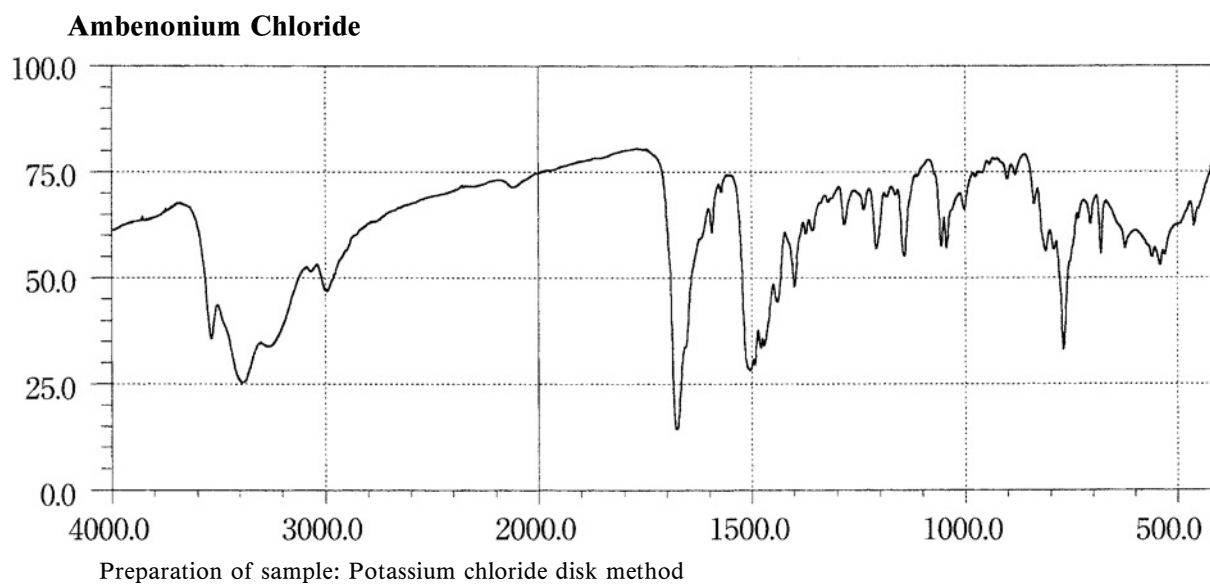
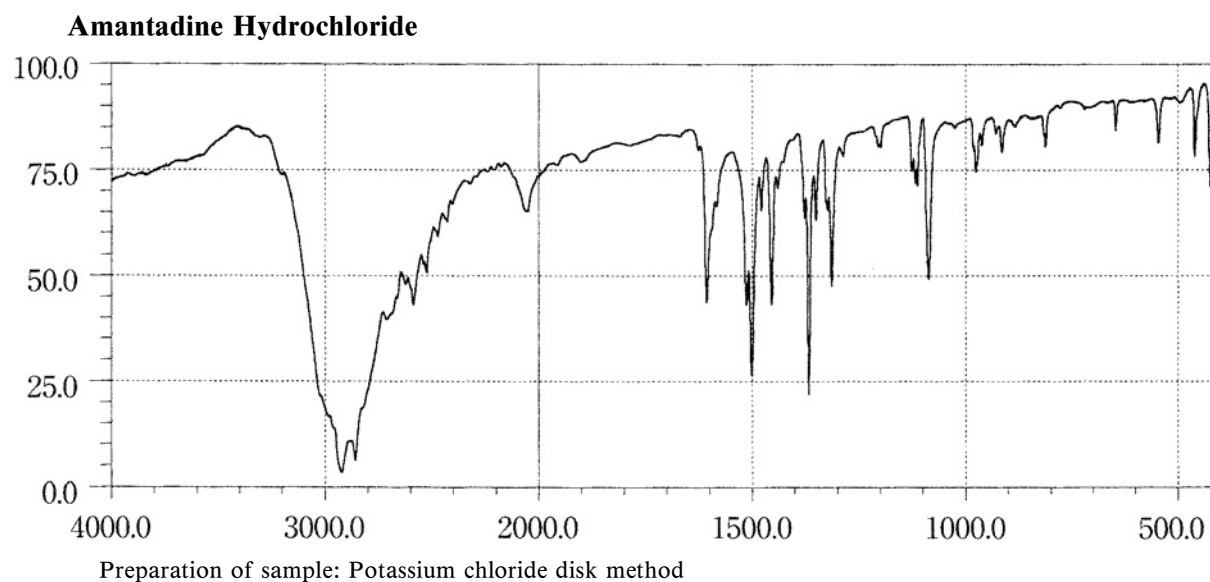
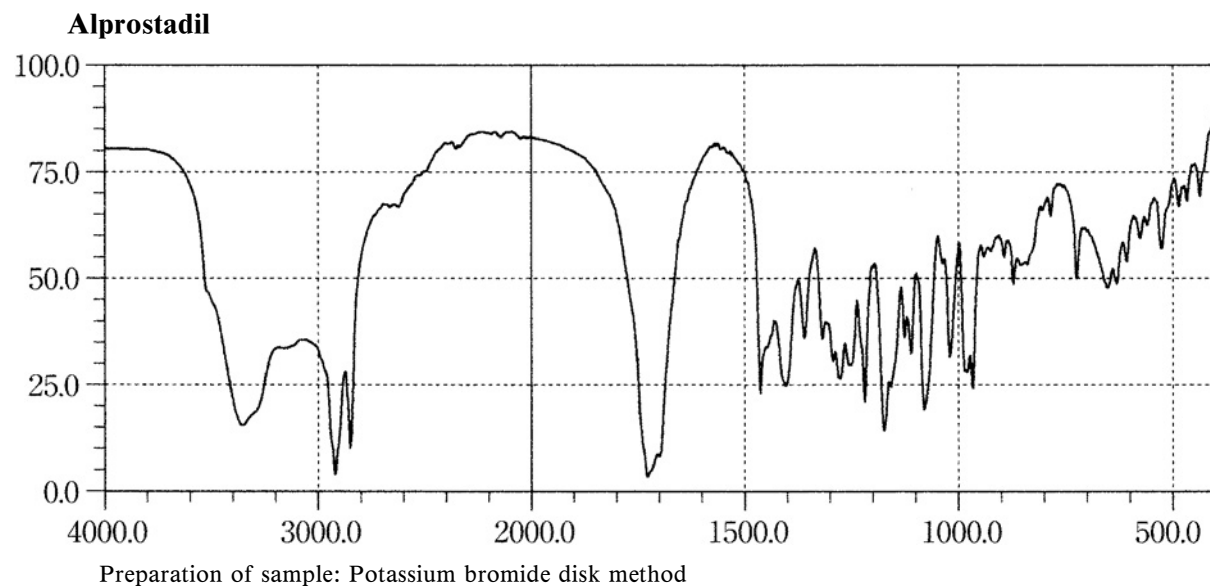
Aclarubicin Hydrochloride

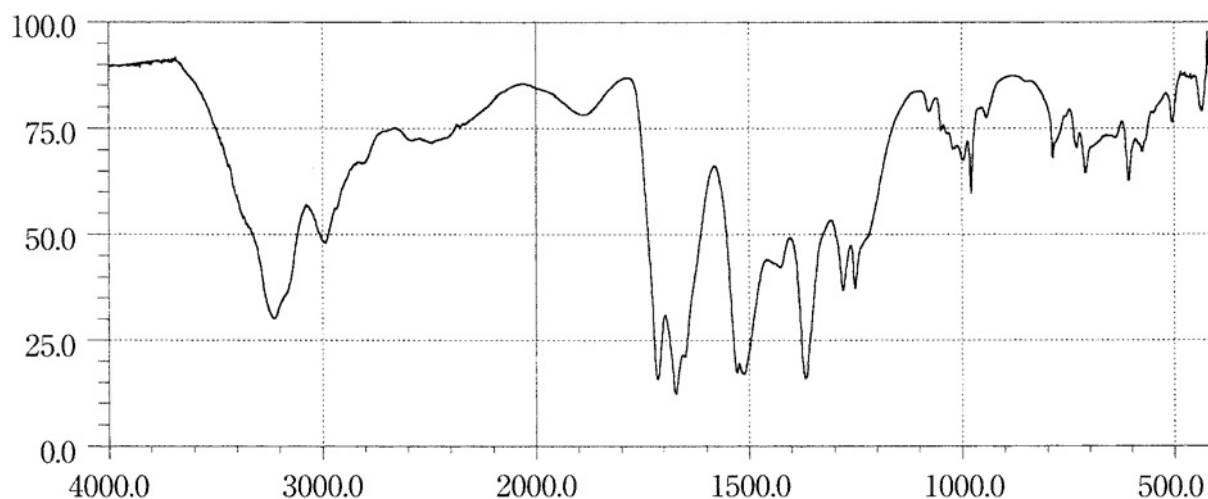
Preparation of sample: Potassium bromide disk method

Acrinol Hydrate

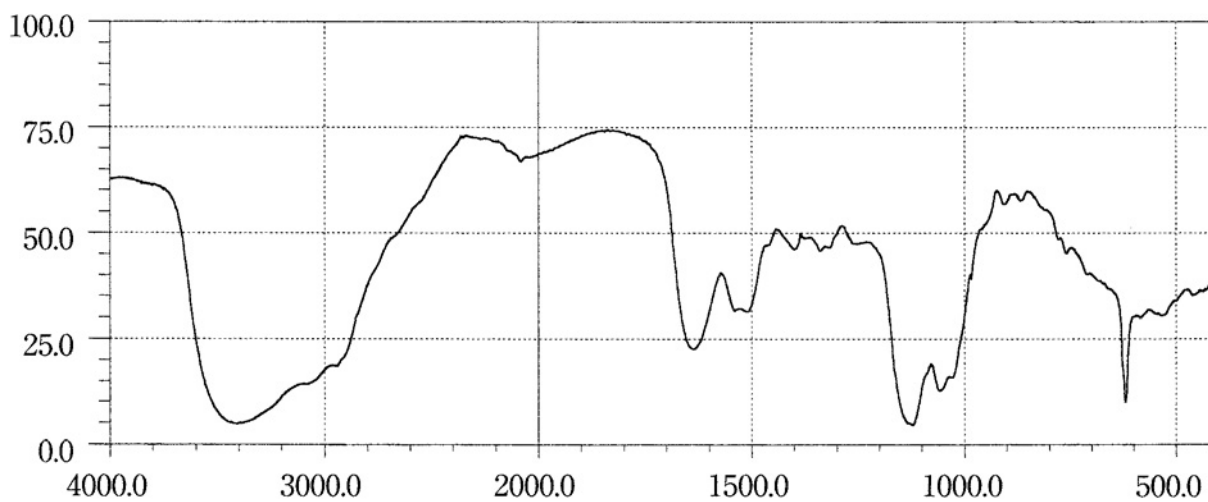
Preparation of sample: Potassium bromide disk method



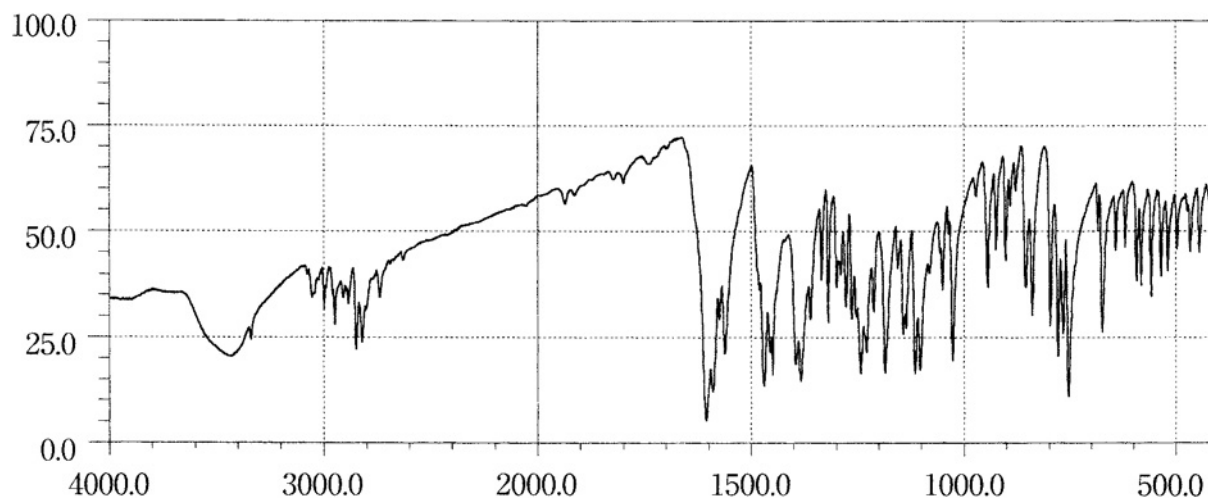


Amidotrizoic Acid

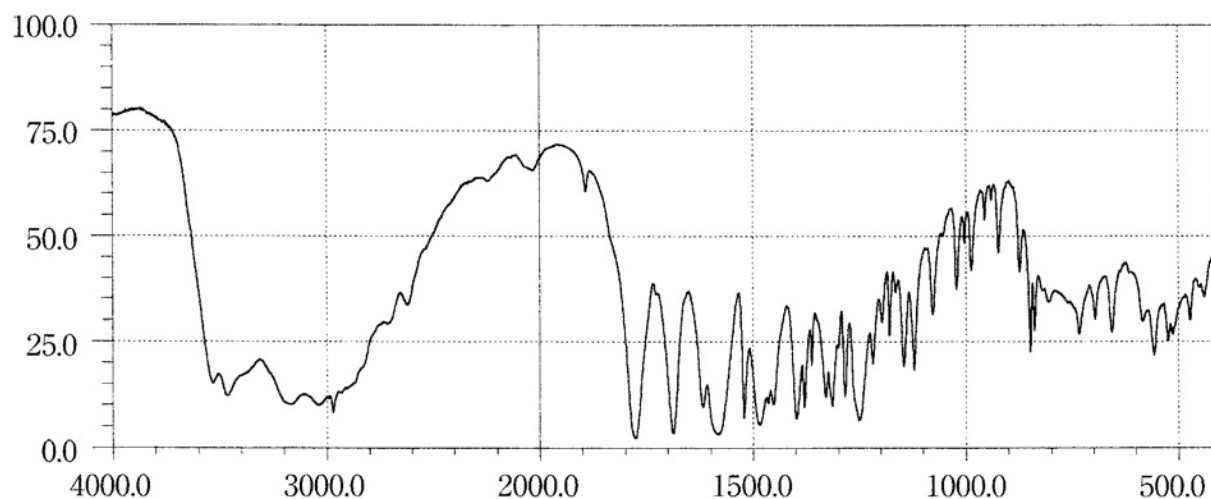
Preparation of sample: Potassium bromide disk method

Amikacin Sulfate

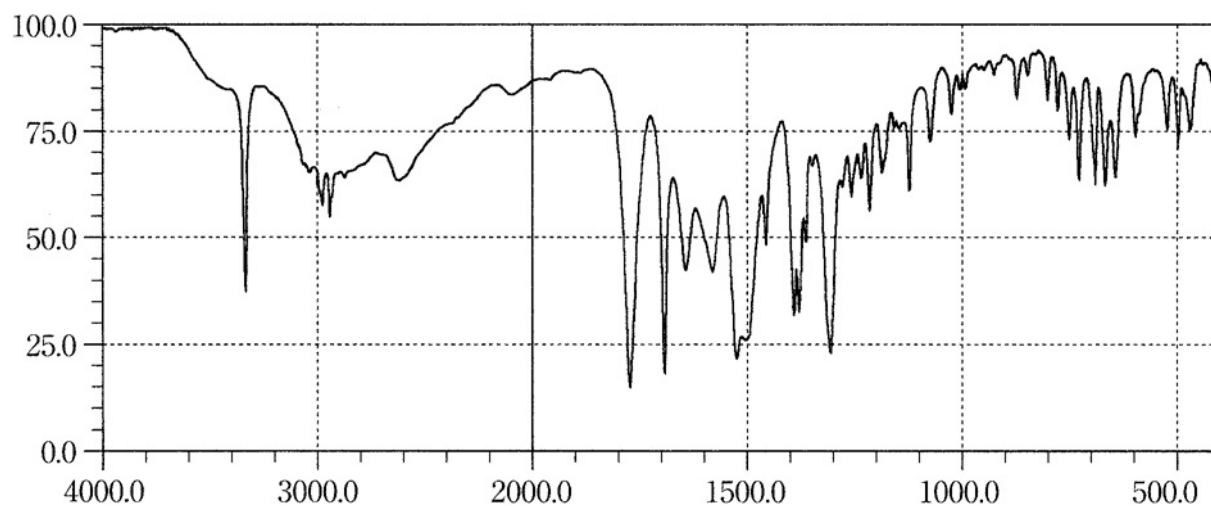
Preparation of sample: Potassium bromide disk method

Amoxapine

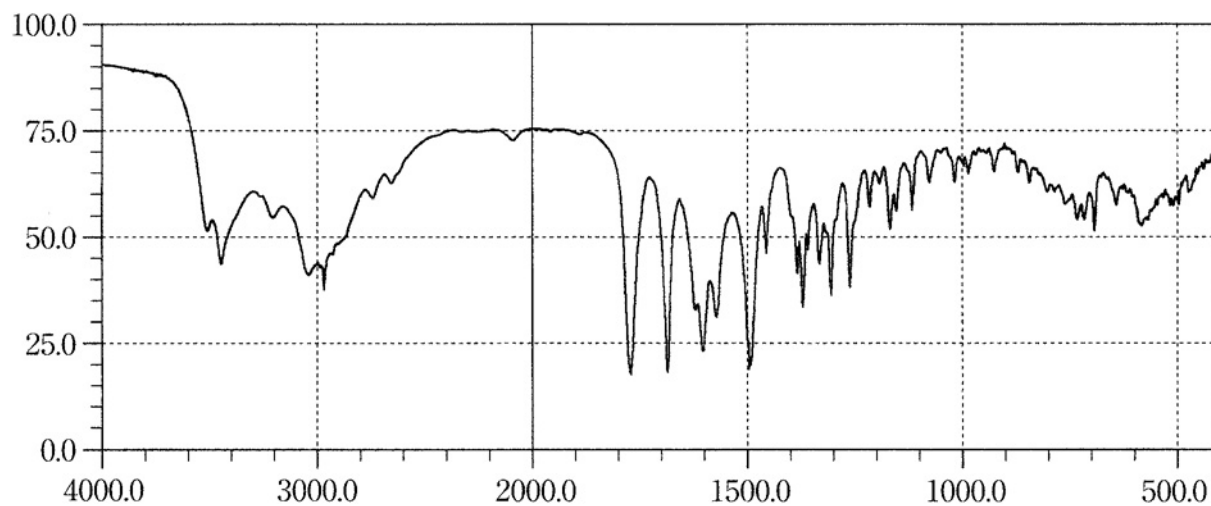
Preparation of sample: Potassium bromide disk method

Amoxicillin Hydrate

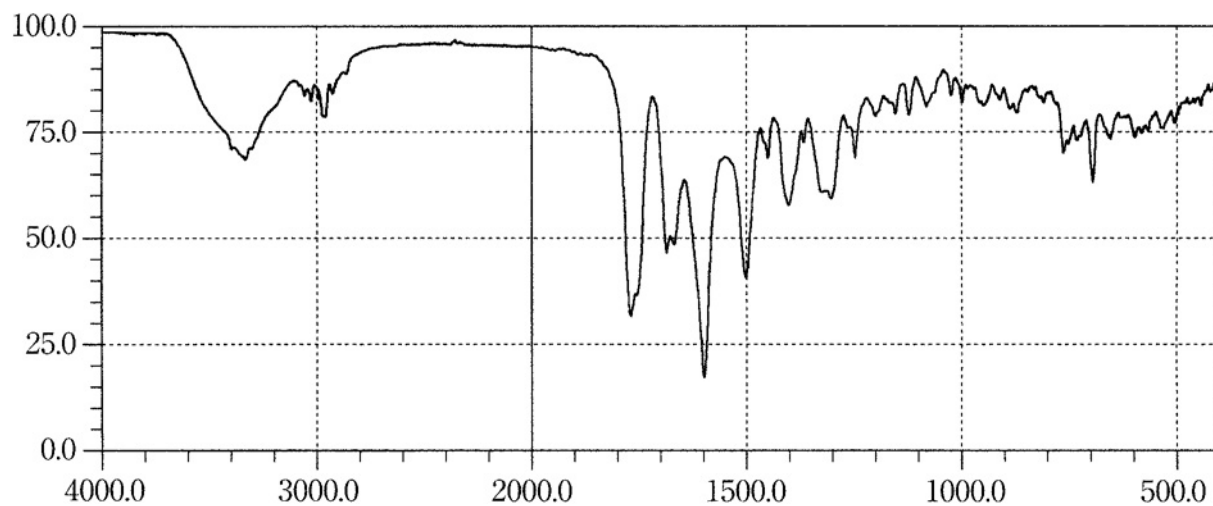
Preparation of sample: Potassium bromide disk method

Anhydrous Ampicillin

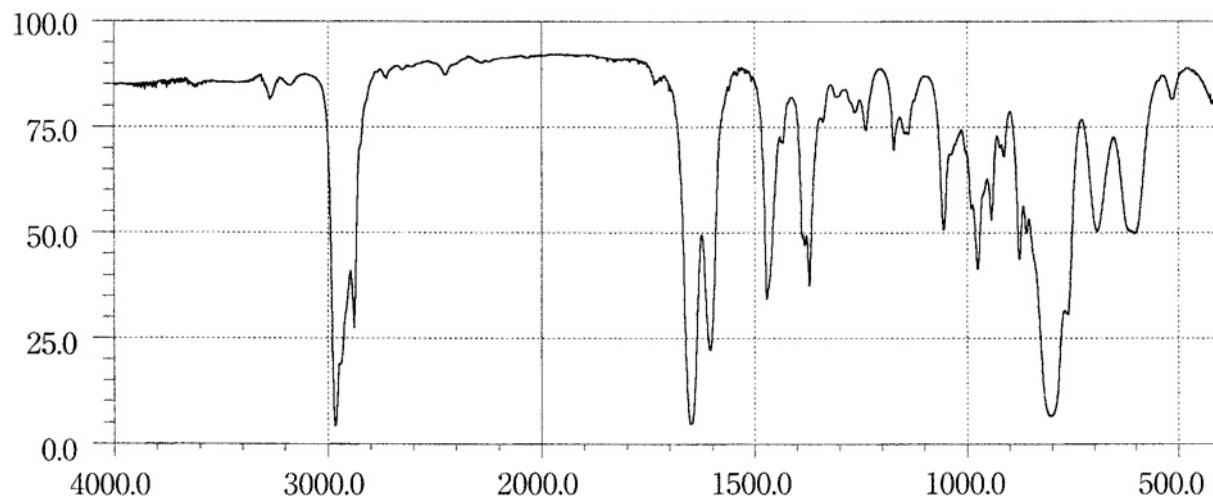
Preparation of sample: Potassium bromide disk method

Ampicillin Hydrate

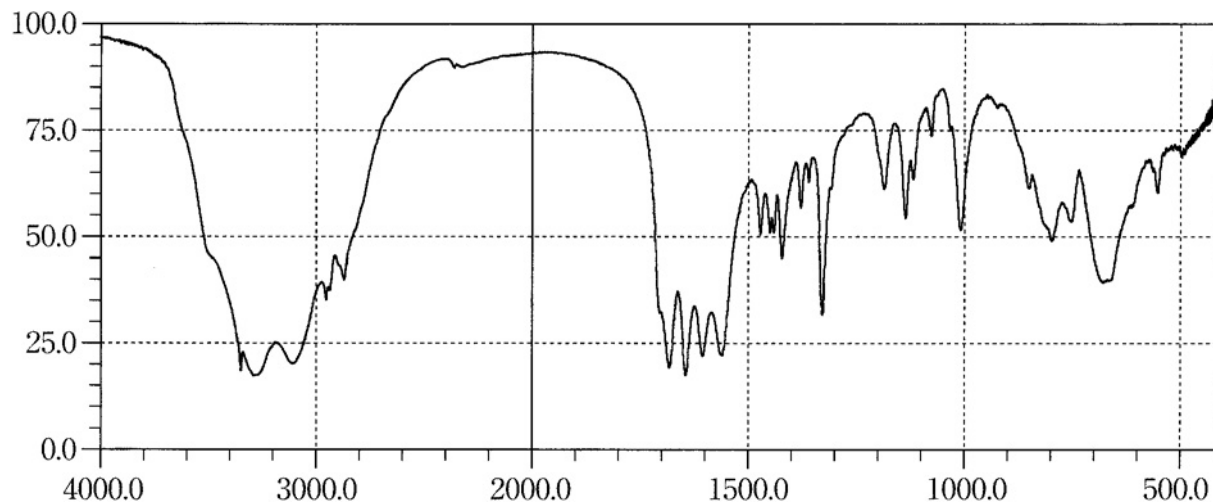
Preparation of sample: Potassium bromide disk method

Ampicillin Sodium

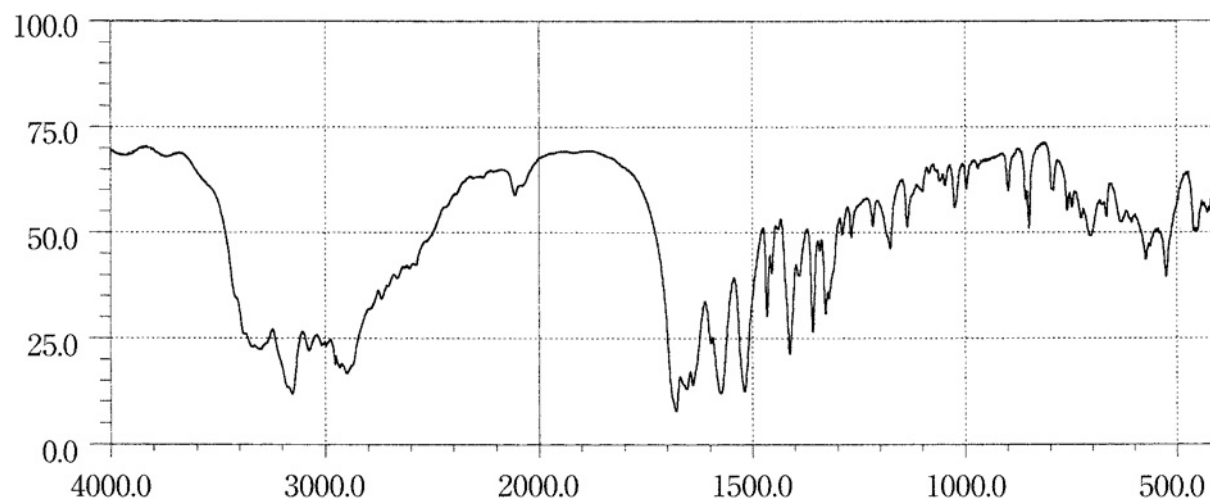
Preparation of sample: Potassium bromide disk method

Amyl Nitrite

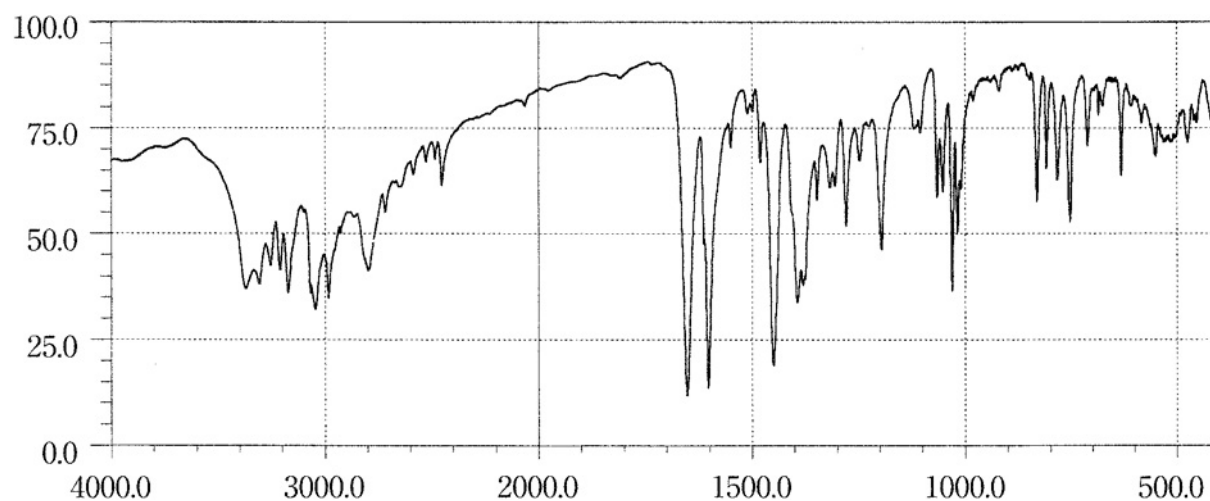
Preparation of sample: Liquid film method

L-Arginine

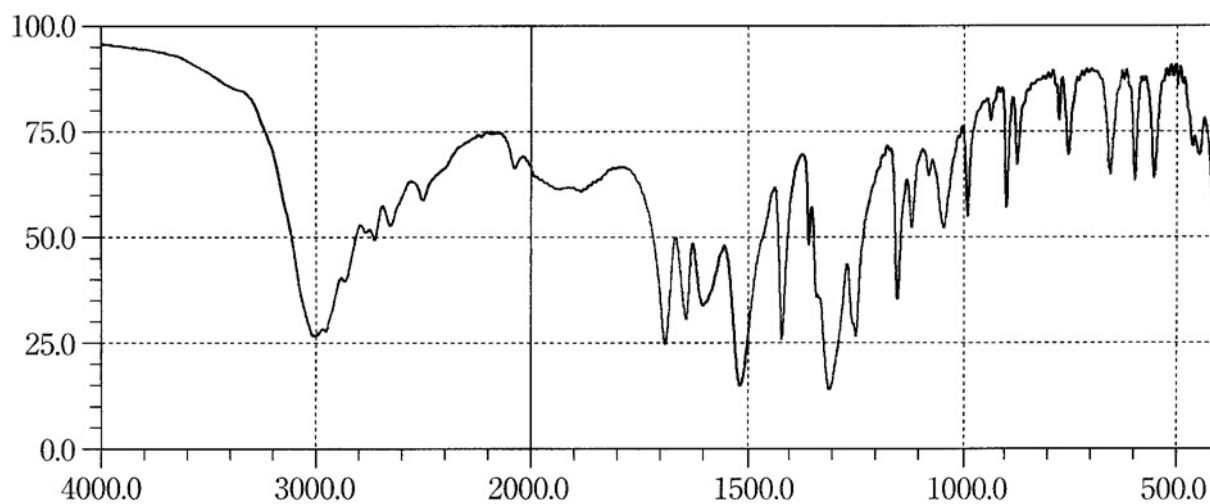
Preparation of sample: Potassium bromide disk method

L-Arginine Hydrochloride

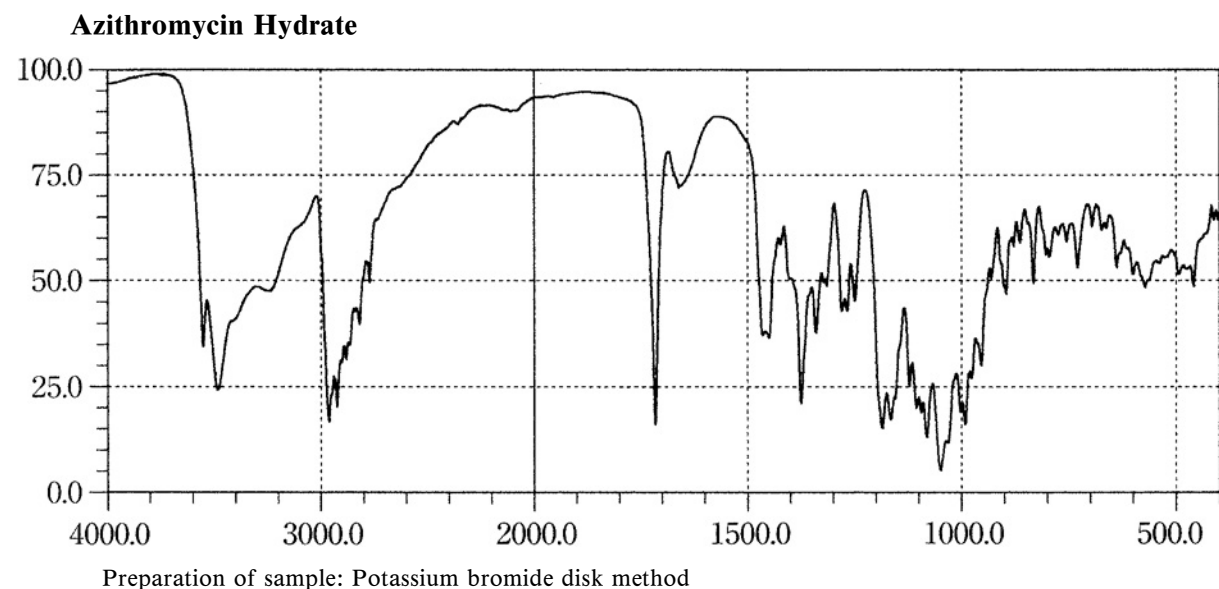
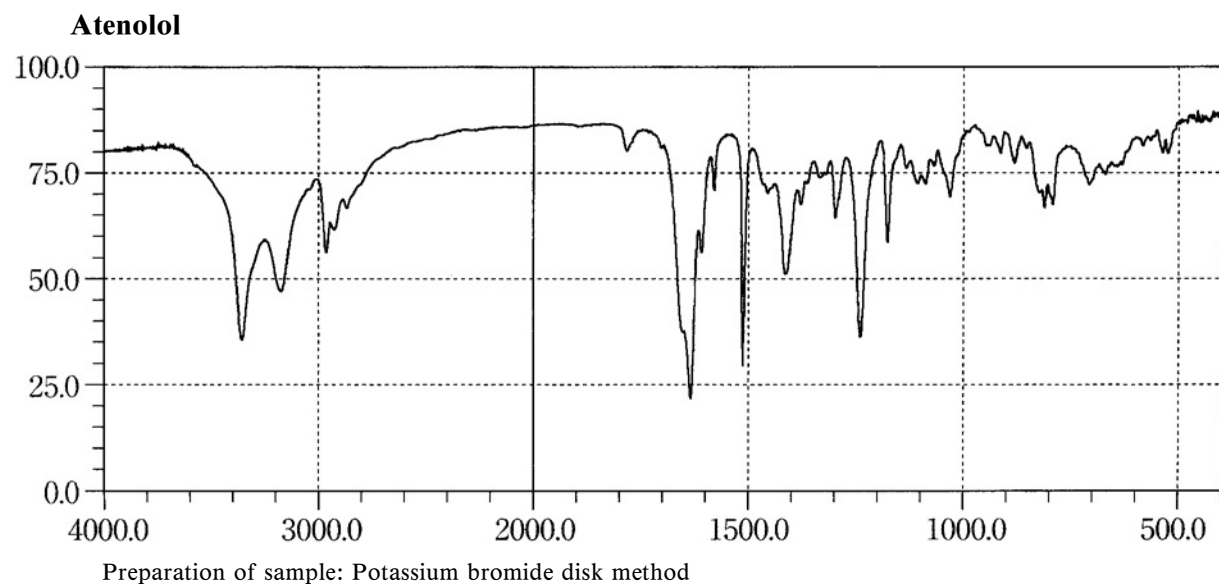
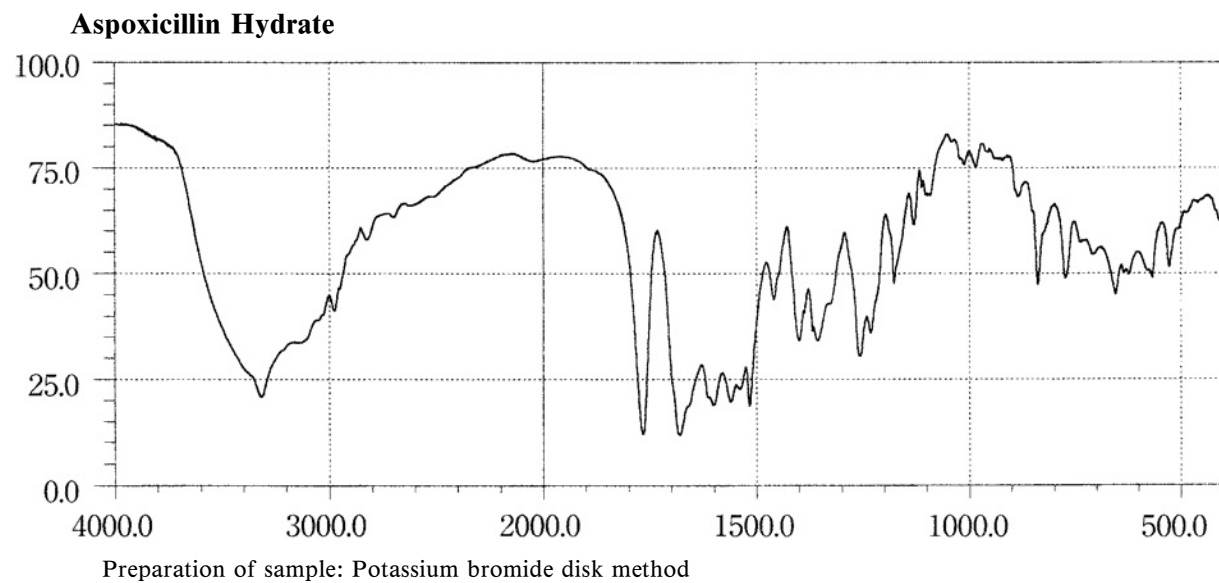
Preparation of sample: Potassium bromide disk method

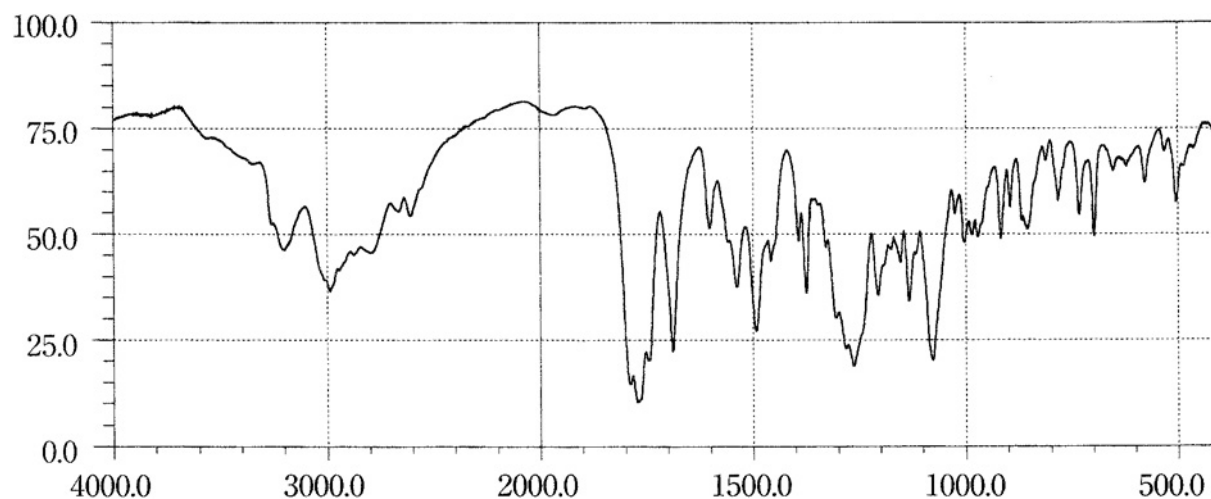
Arotinolol Hydrochloride

Preparation of sample: Potassium bromide disk method

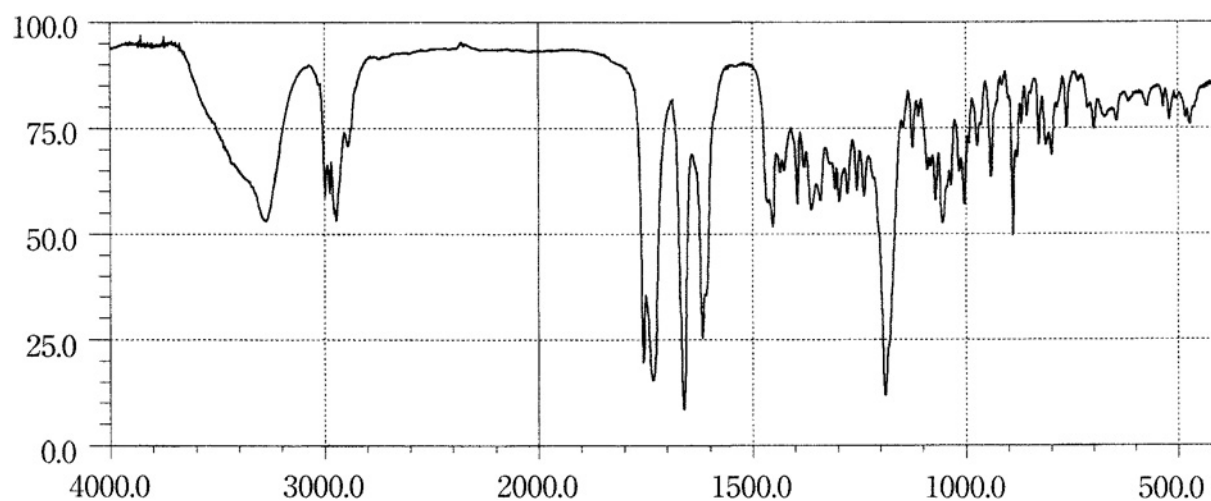
L-Aspartic Acid

Preparation of sample: Potassium bromide disk method

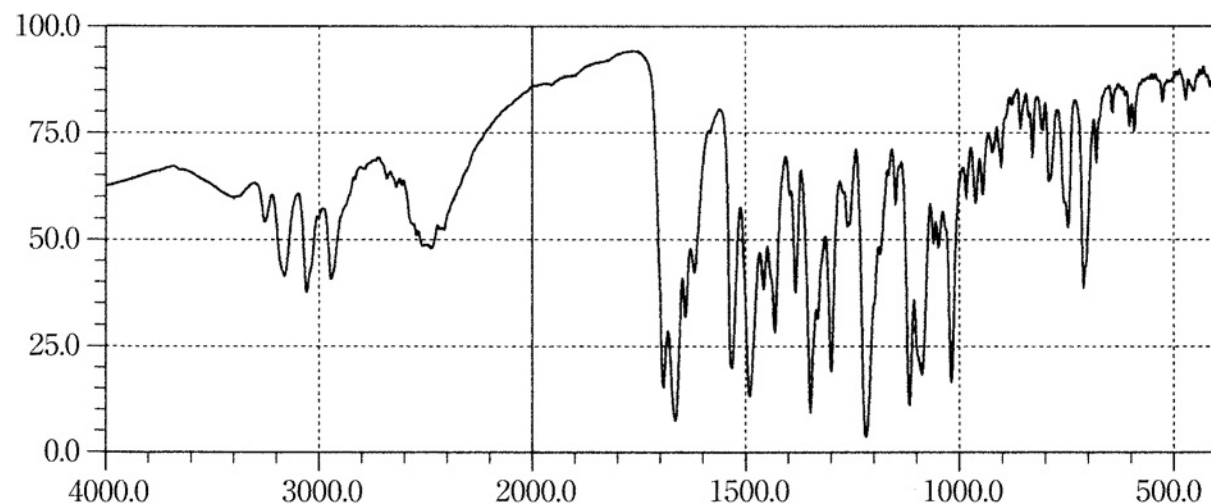


Bacampicillin Hydrochloride

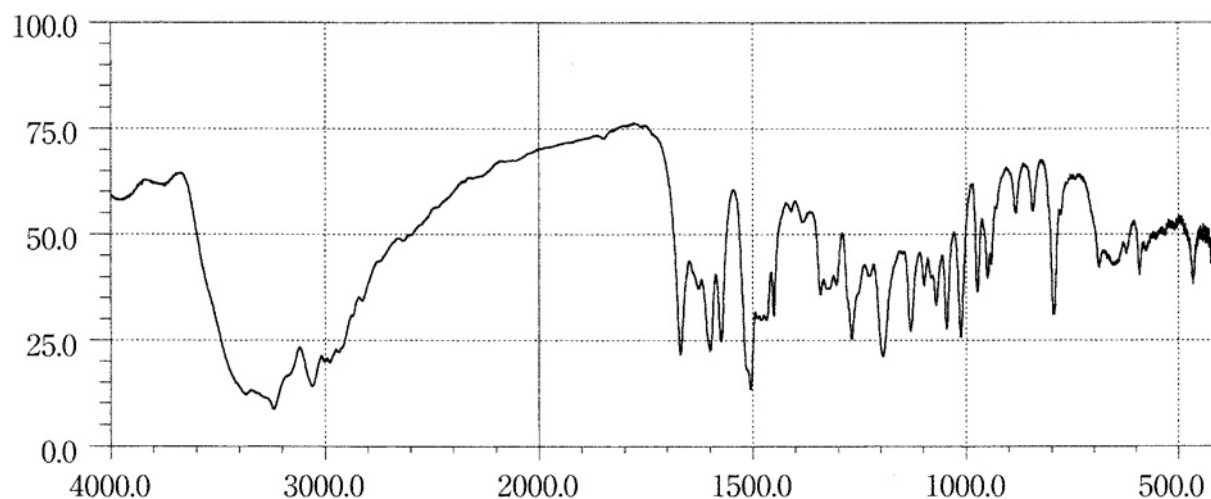
Preparation of sample: Potassium chloride disk method

Beclometasone Dipropionate

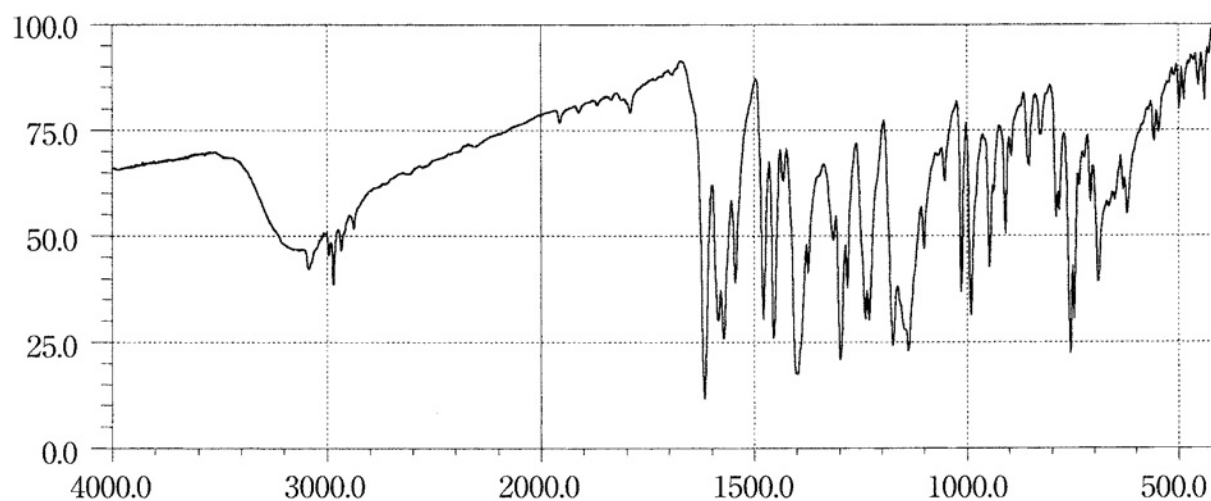
Preparation of sample: Potassium bromide disk method

Benidipine Hydrochloride

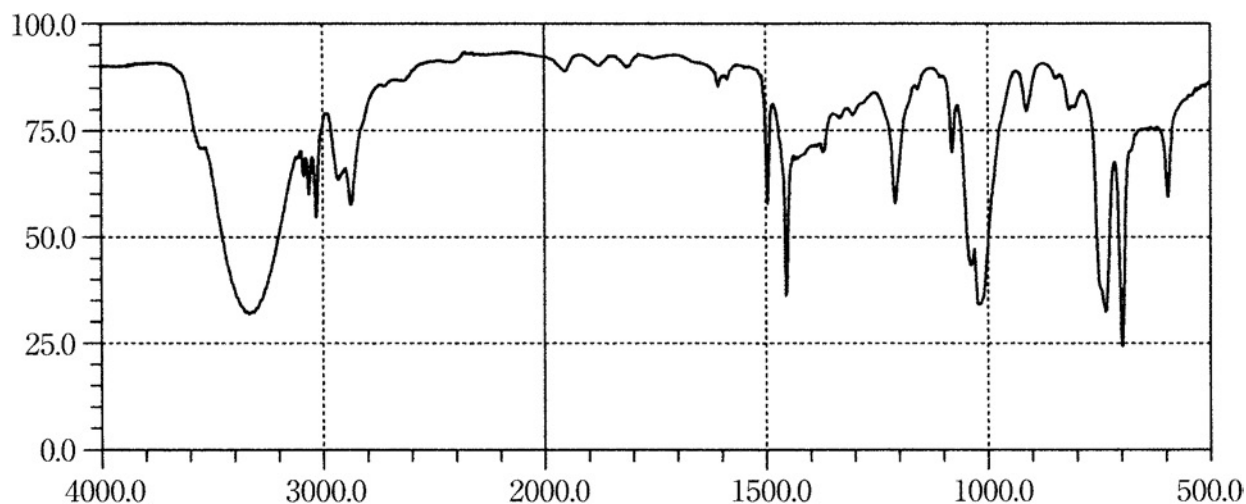
Preparation of sample: Potassium chloride disk method

Benserazide Hydrochloride

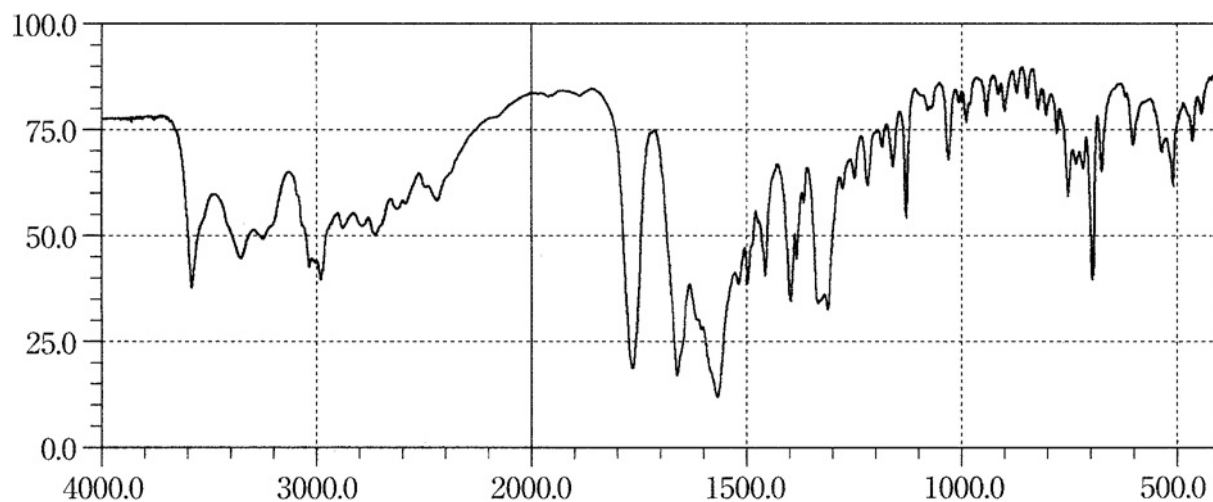
Preparation of sample: Potassium bromide disk method

Benzbromarone

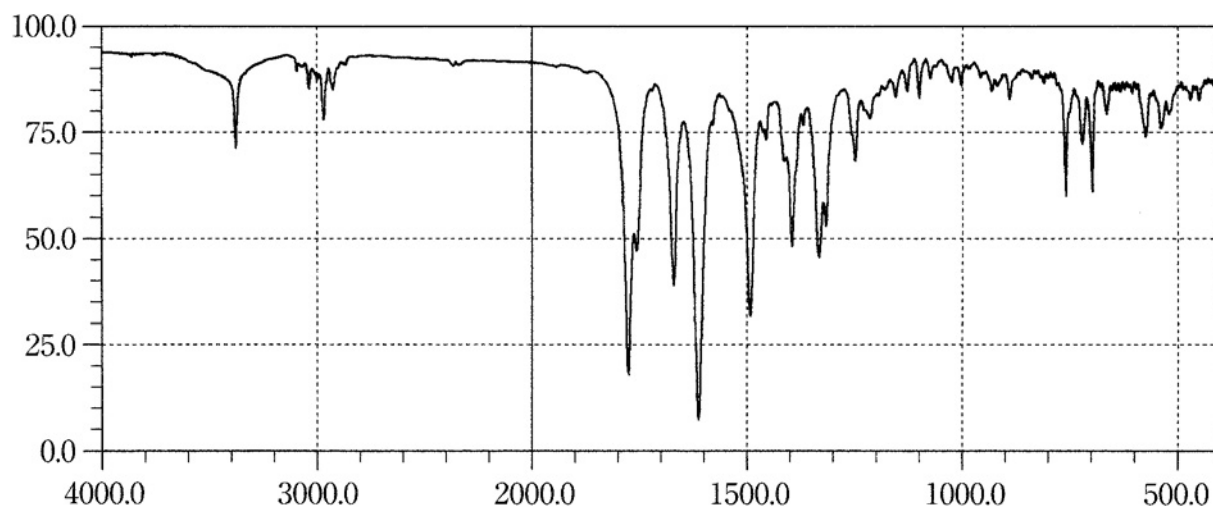
Preparation of sample: Potassium bromide disk method

Benzyl Alcohol

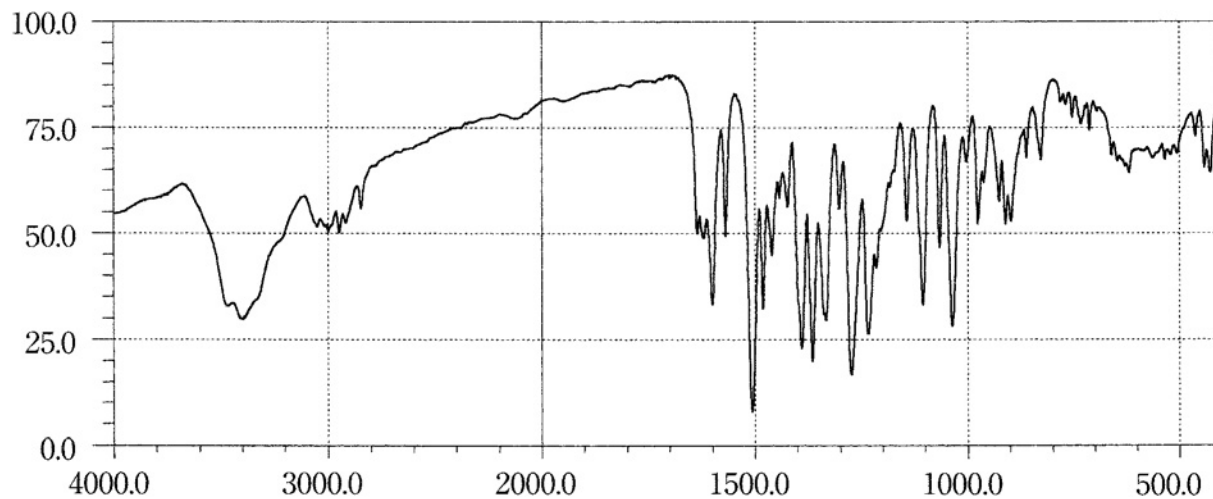
Preparation of sample: Liquid film method

Benzylpenicillin Benzathine Hydrate

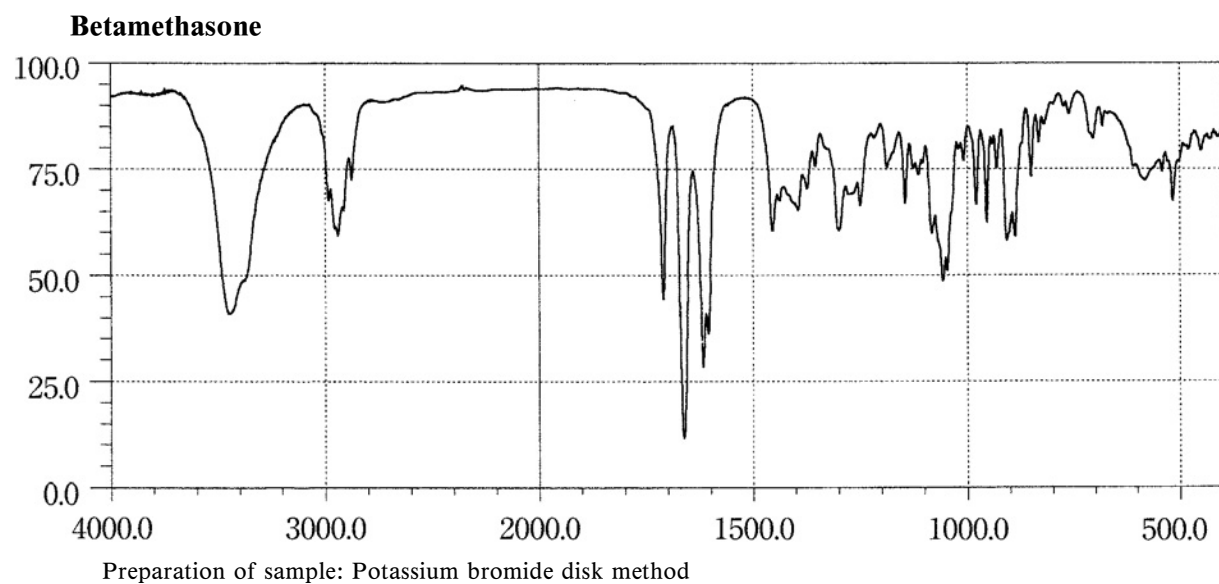
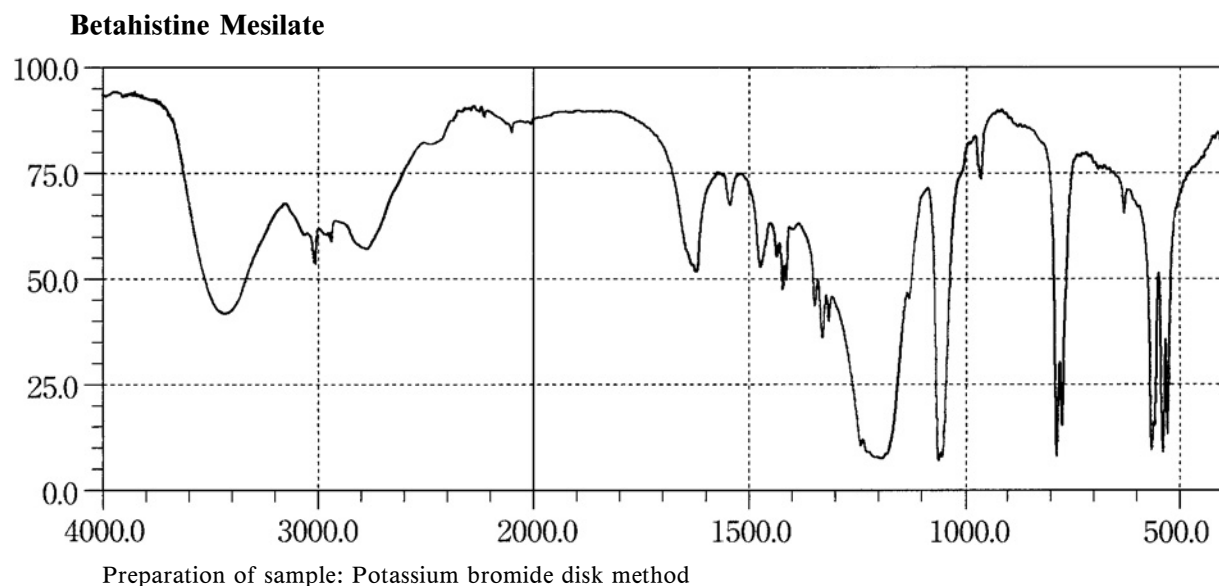
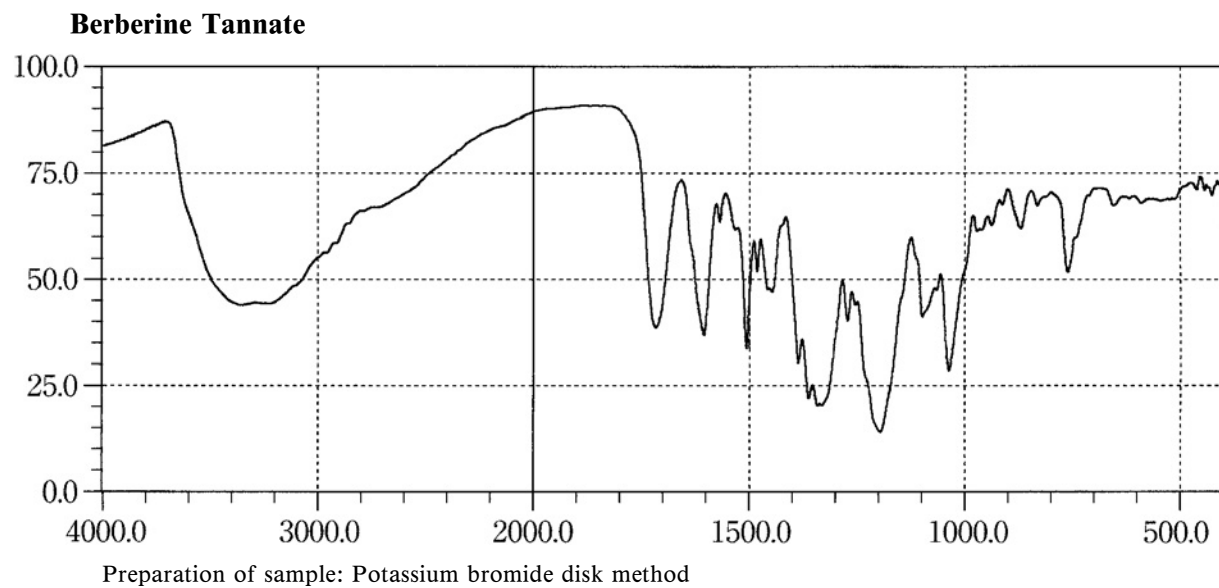
Preparation of sample: Potassium bromide disk method

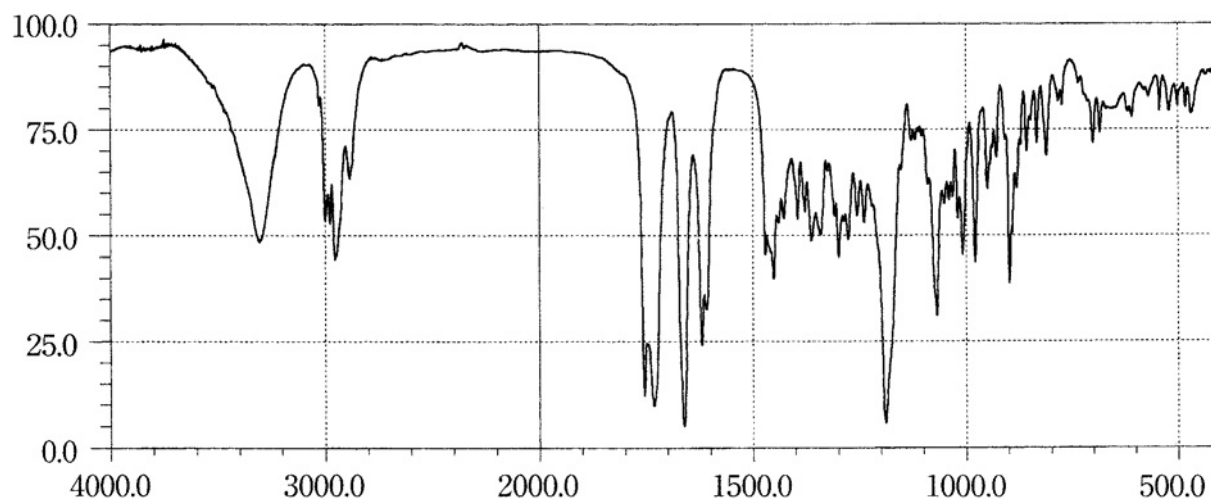
Benzylpenicillin Potassium

Preparation of sample: Potassium bromide disk method

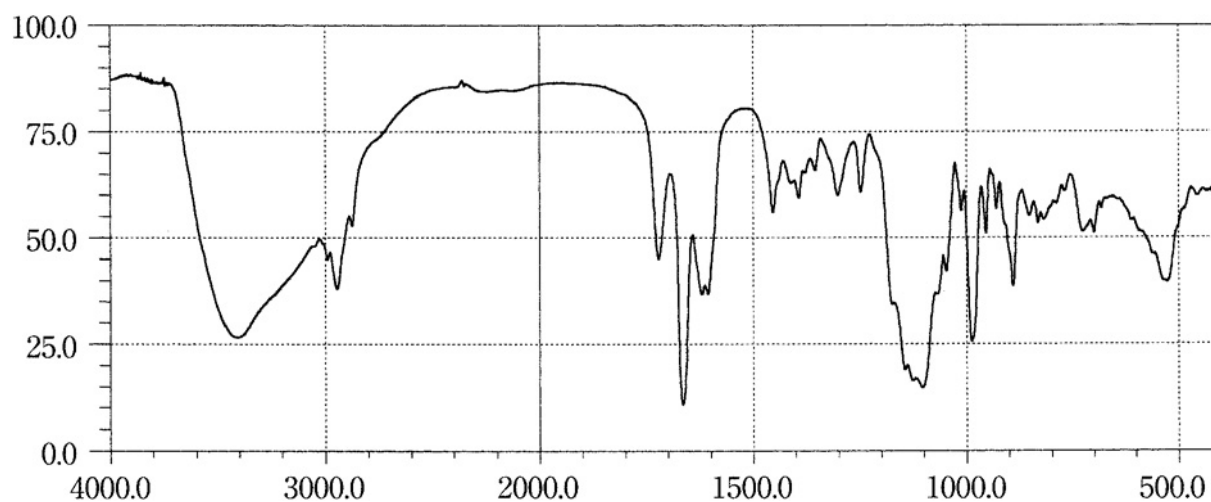
Berberine Chloride Hydrate

Preparation of sample: Potassium bromide disk method

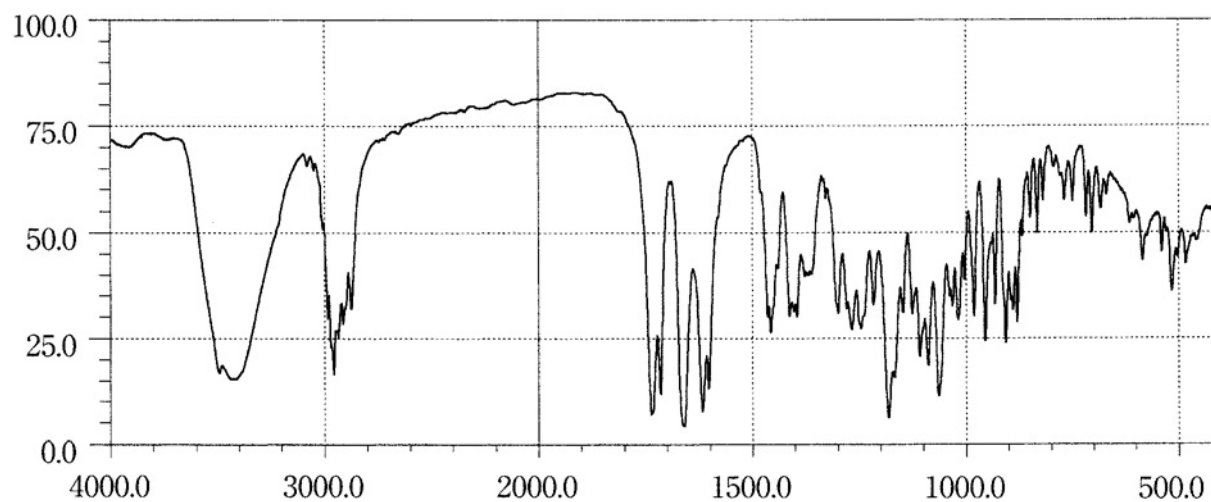


Betamethasone Dipropionate

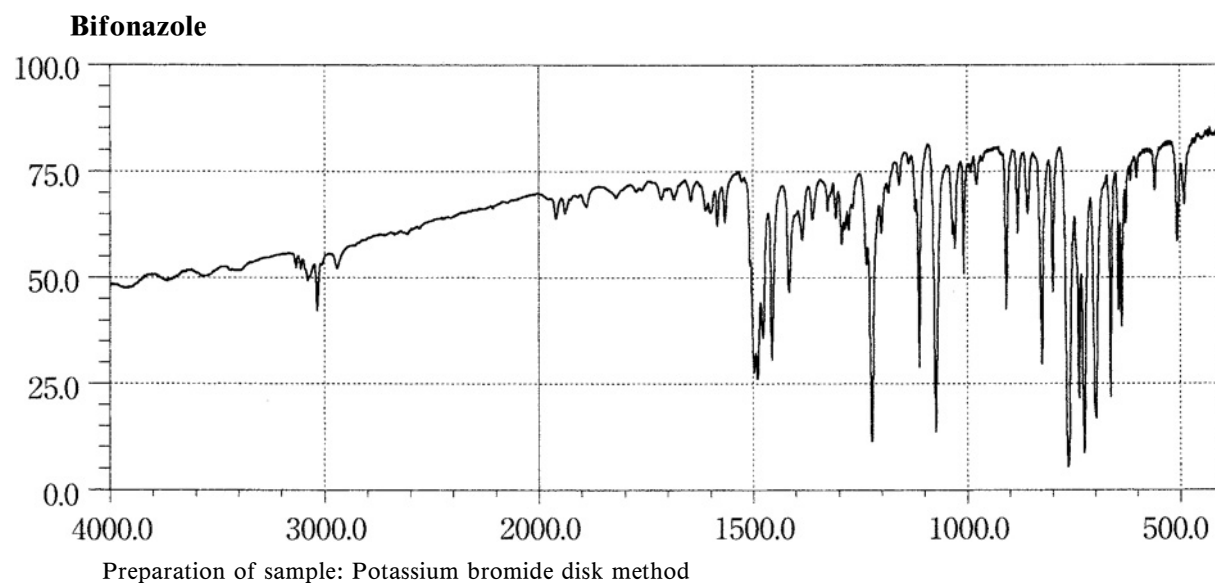
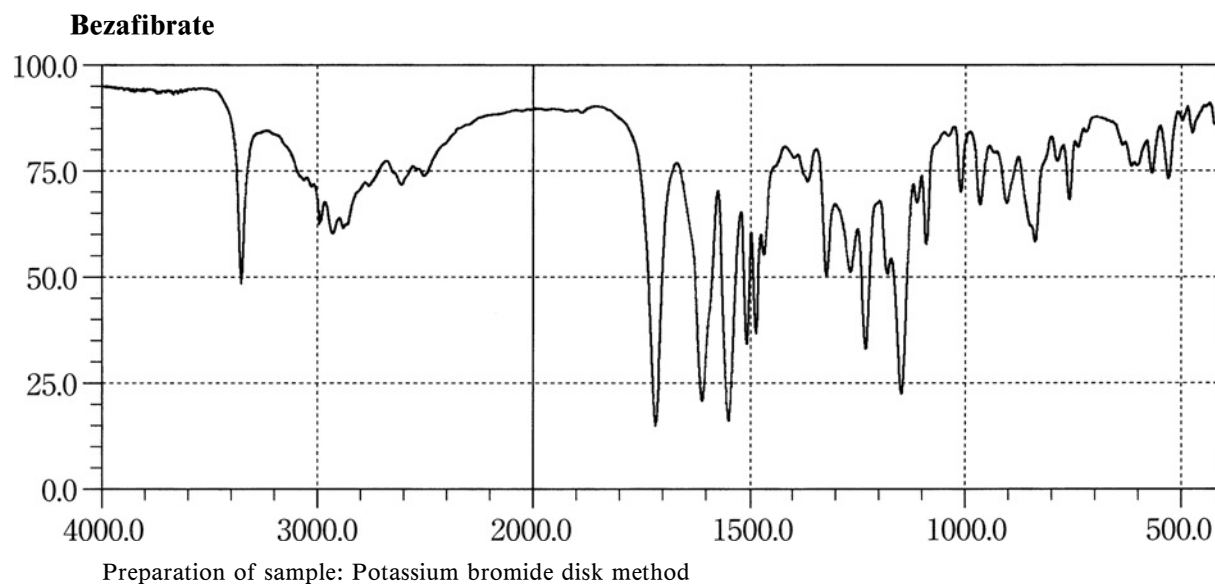
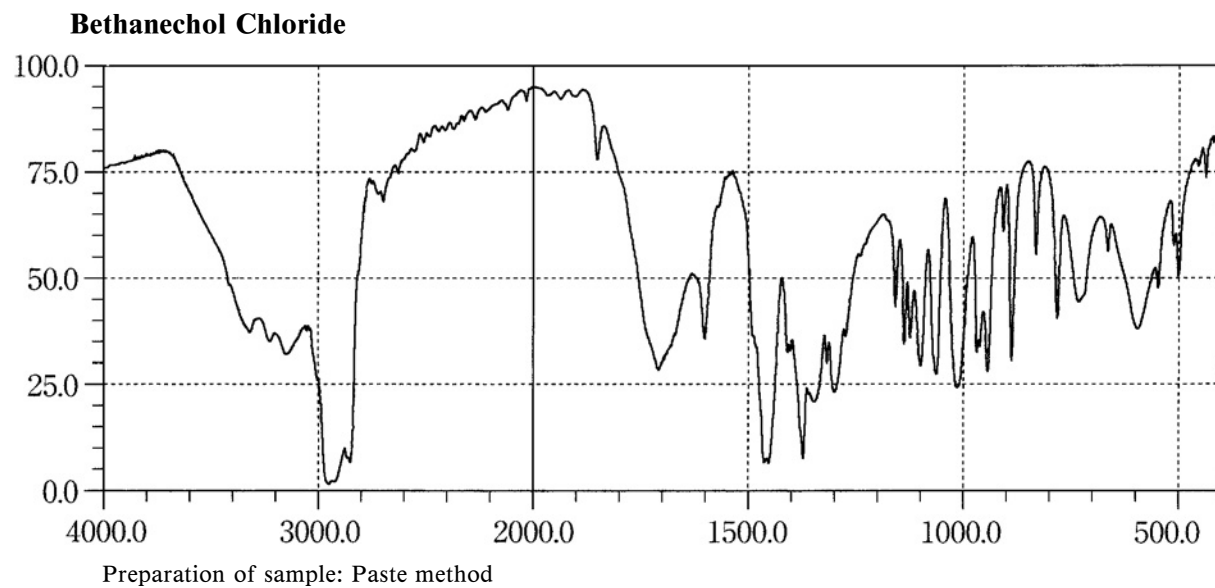
Preparation of sample: Potassium bromide disk method

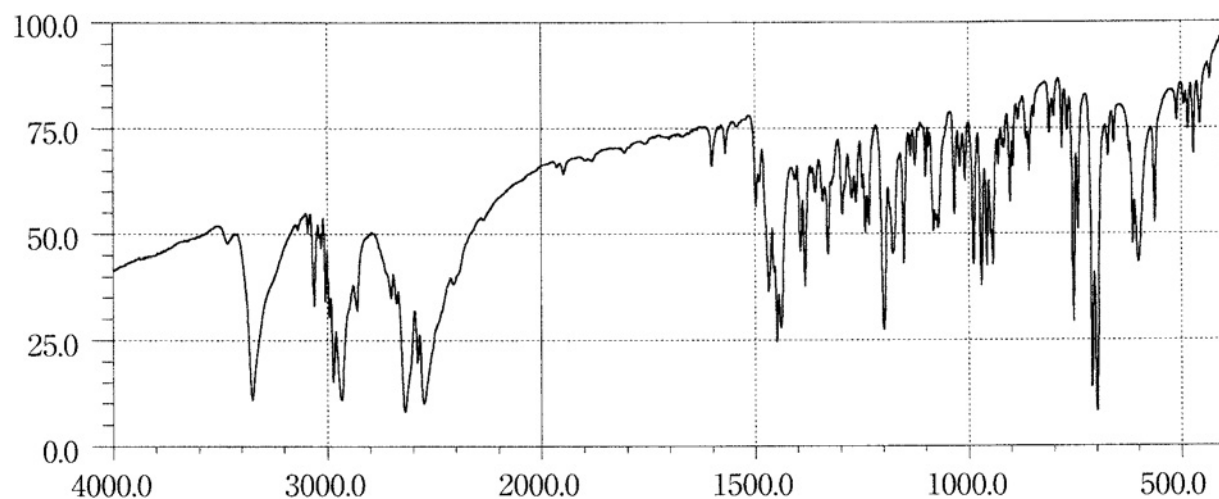
Betamethasone Sodium Phosphate

Preparation of sample: Potassium bromide disk method

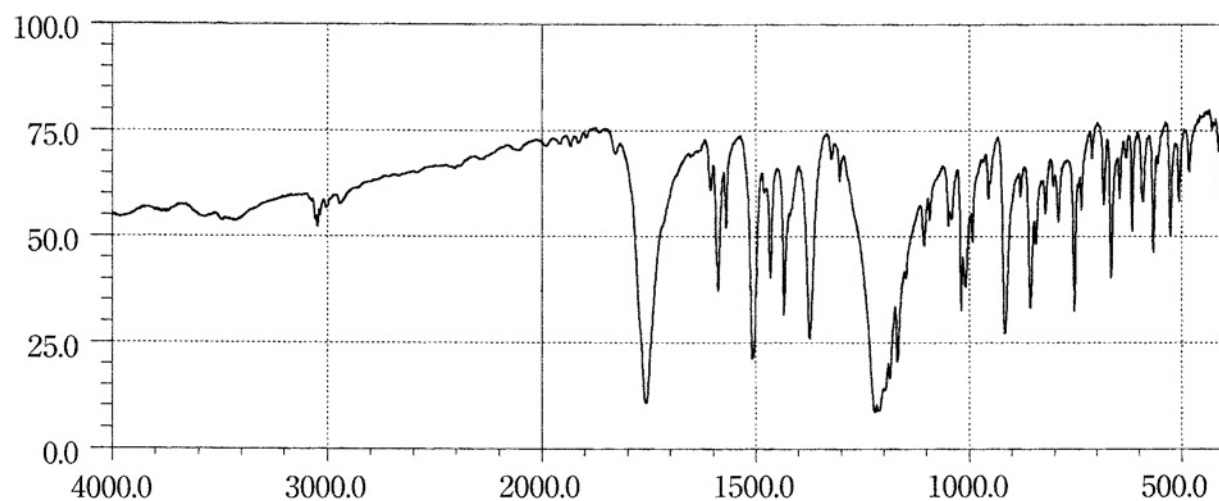
Betamethasone Valerate

Preparation of sample: Potassium bromide disk method

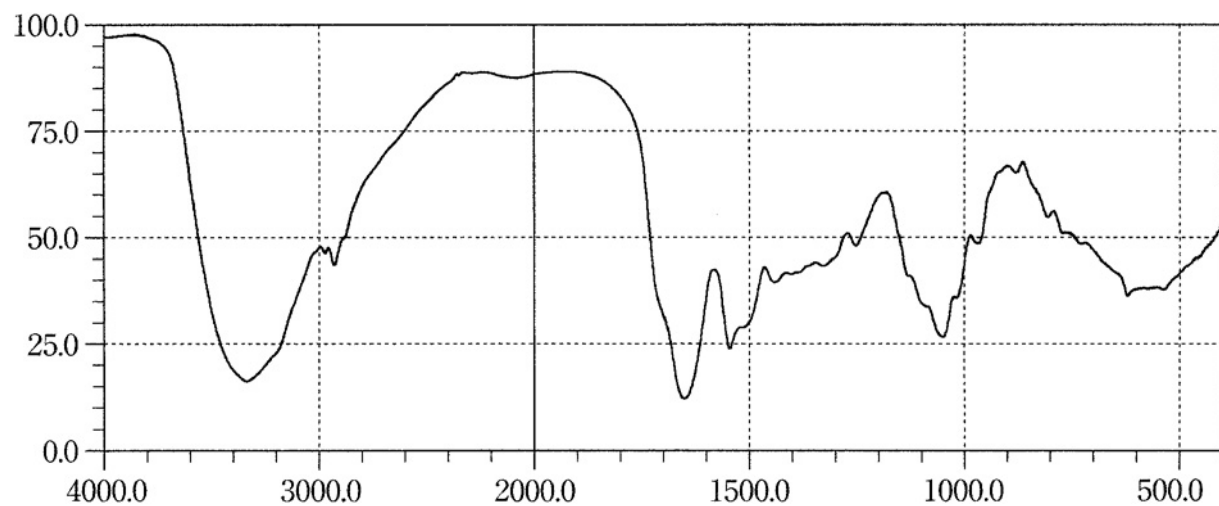


Biperiden Hydrochloride

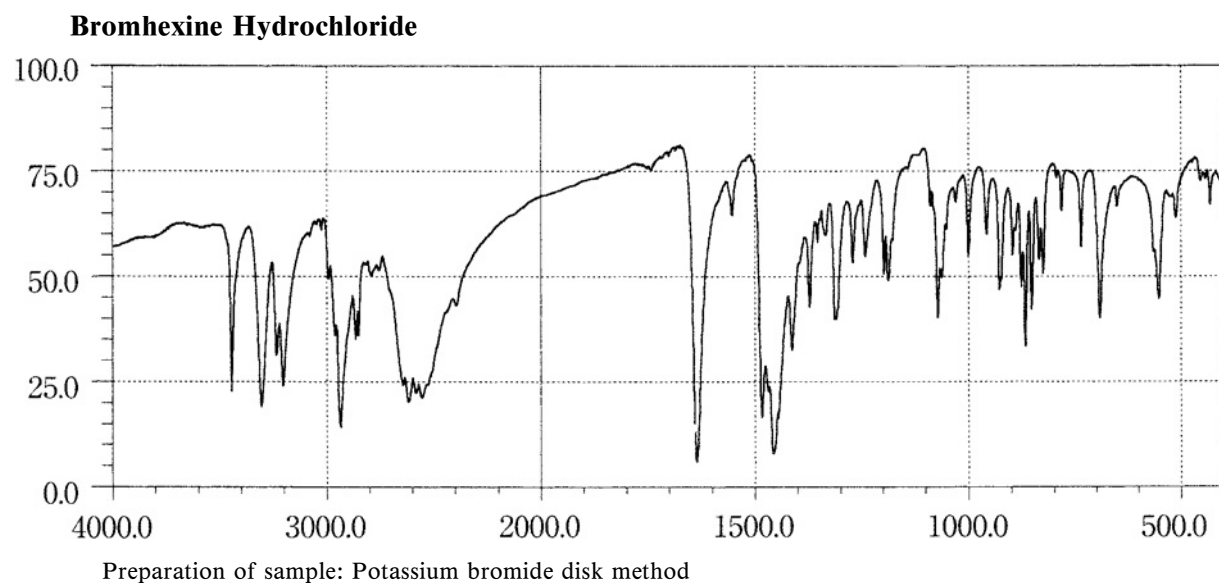
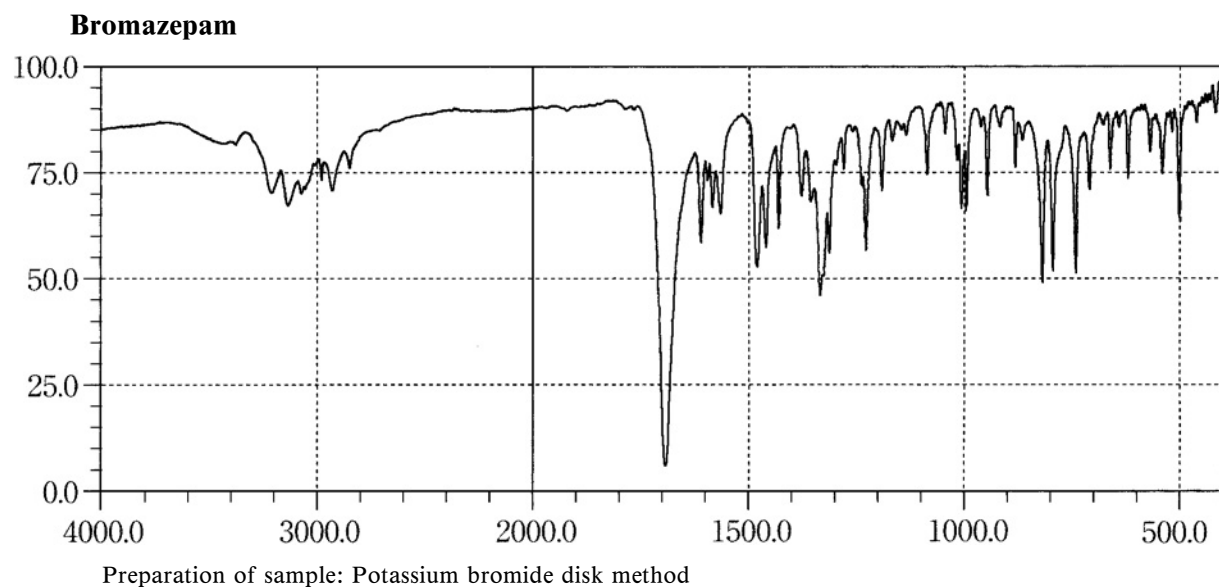
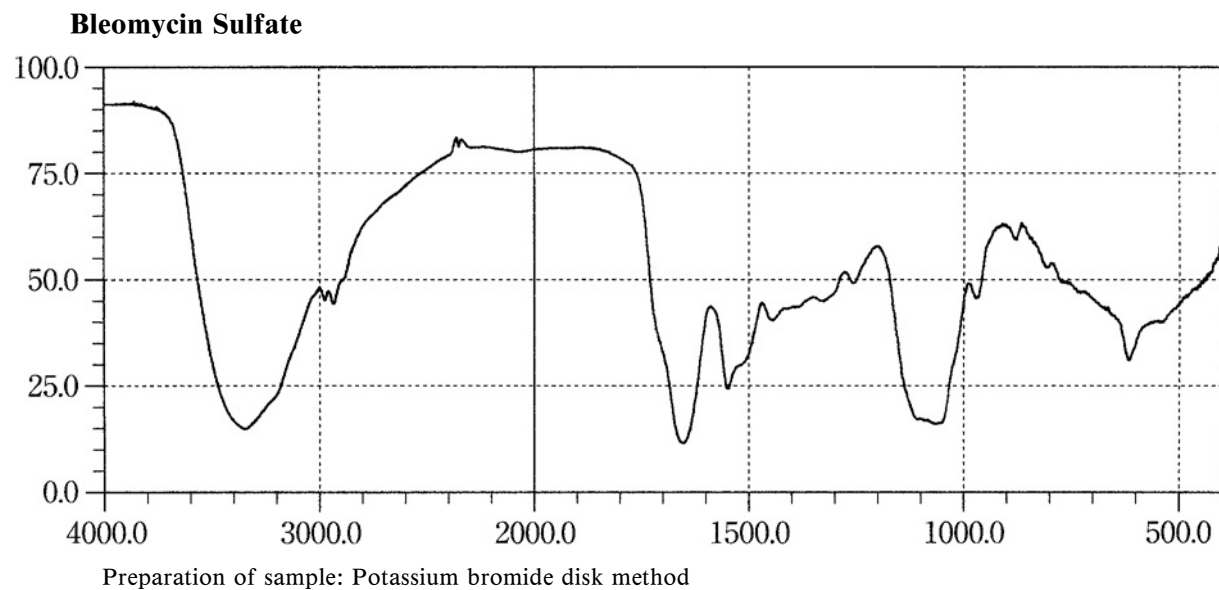
Preparation of sample: Potassium chloride disk method

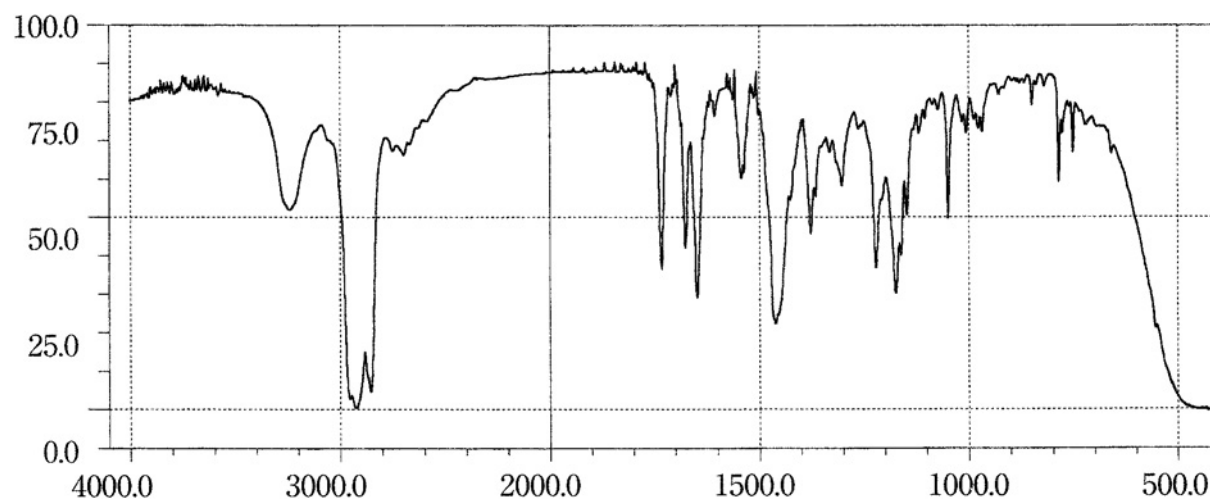
Bisacodyl

Preparation of sample: Potassium bromide disk method

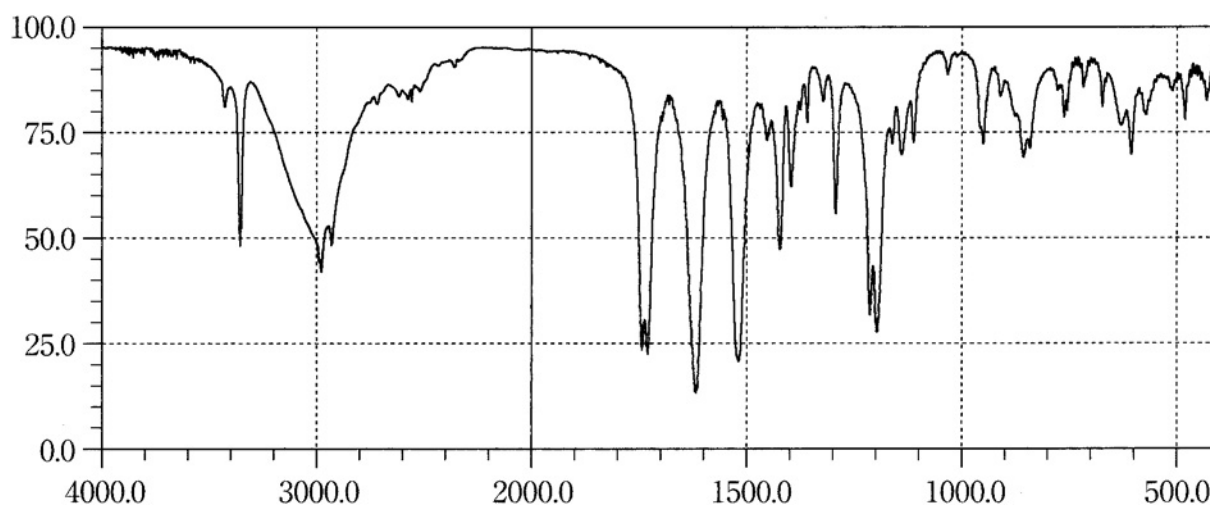
Bleomycin Hydrochloride

Preparation of sample: Potassium bromide disk method

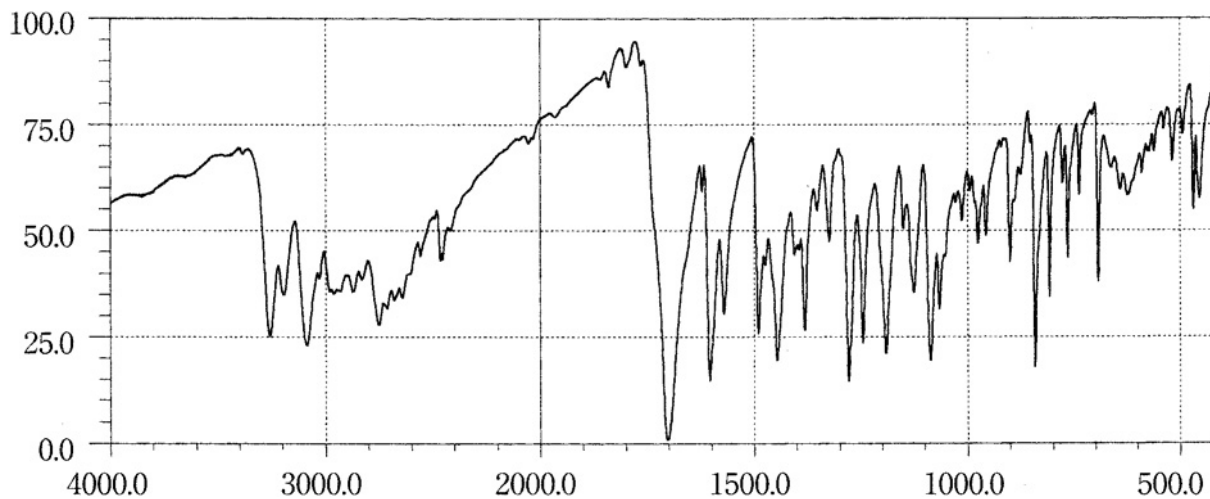


Bromocriptine Mesilate

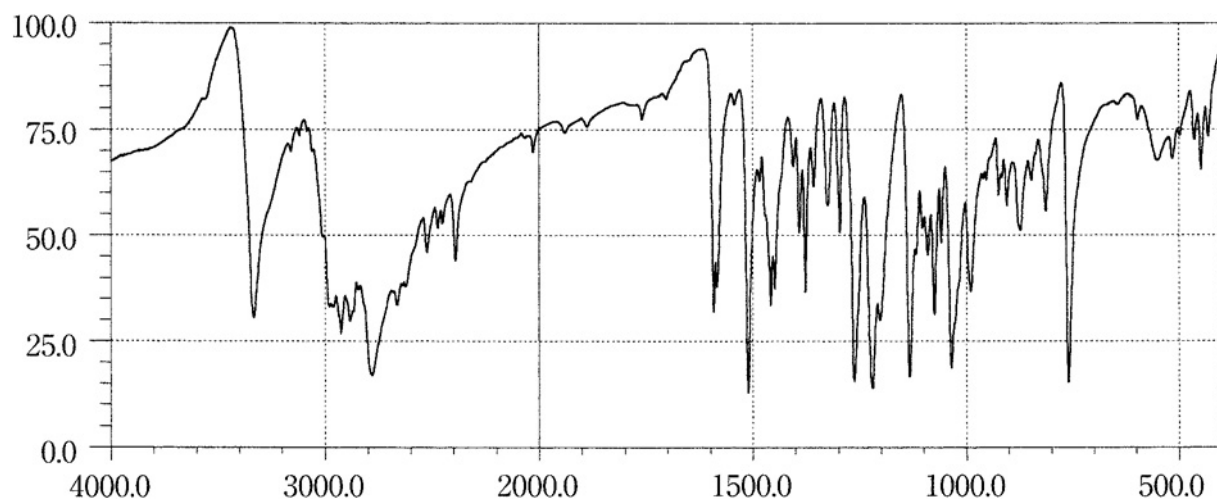
Preparation of sample: Paste method

Bucillamine

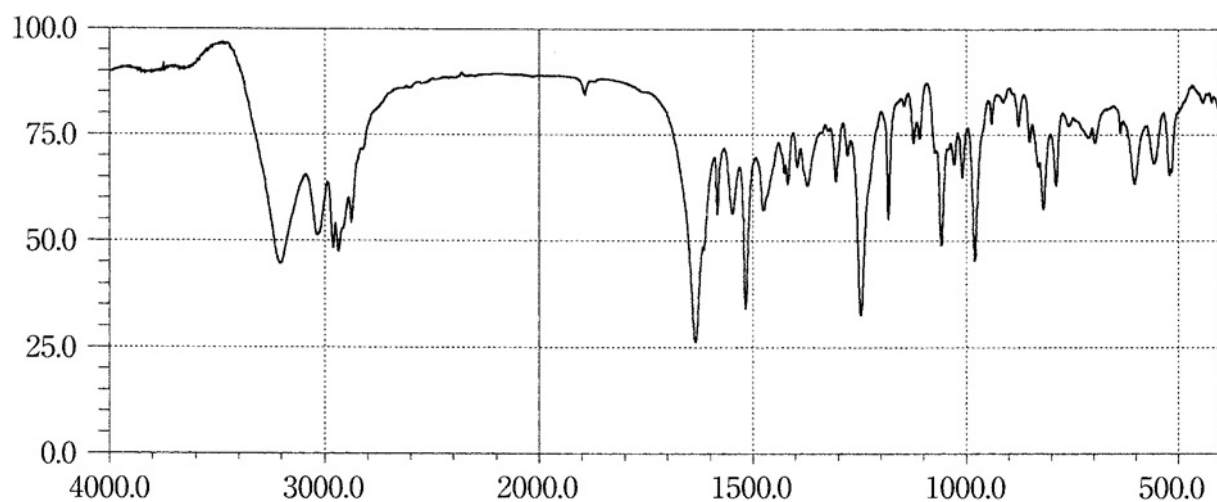
Preparation of sample: Potassium bromide disk method

Bucumolol Hydrochloride

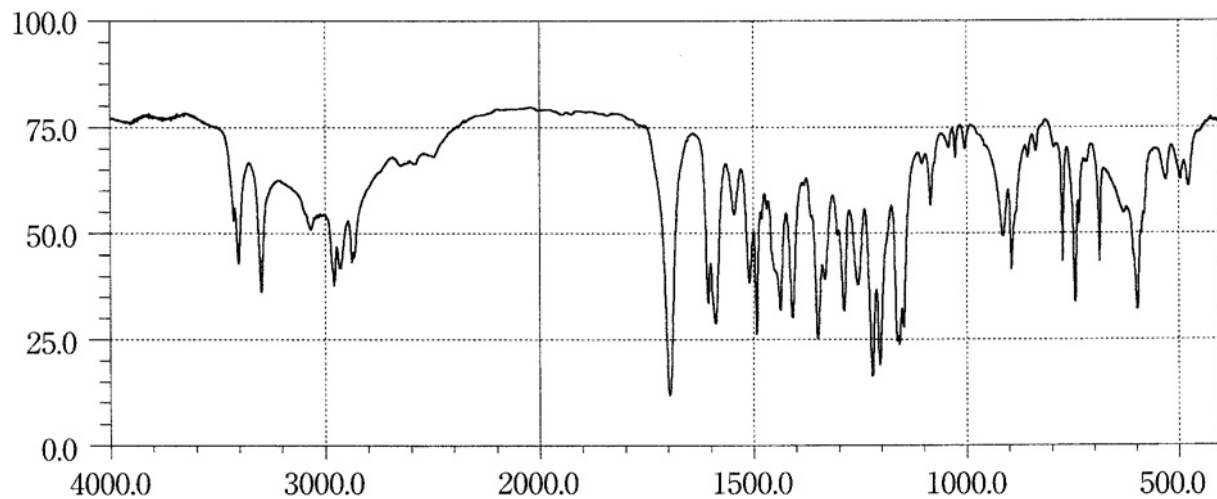
Preparation of sample: Potassium chloride disk method

Bufetolol Hydrochloride

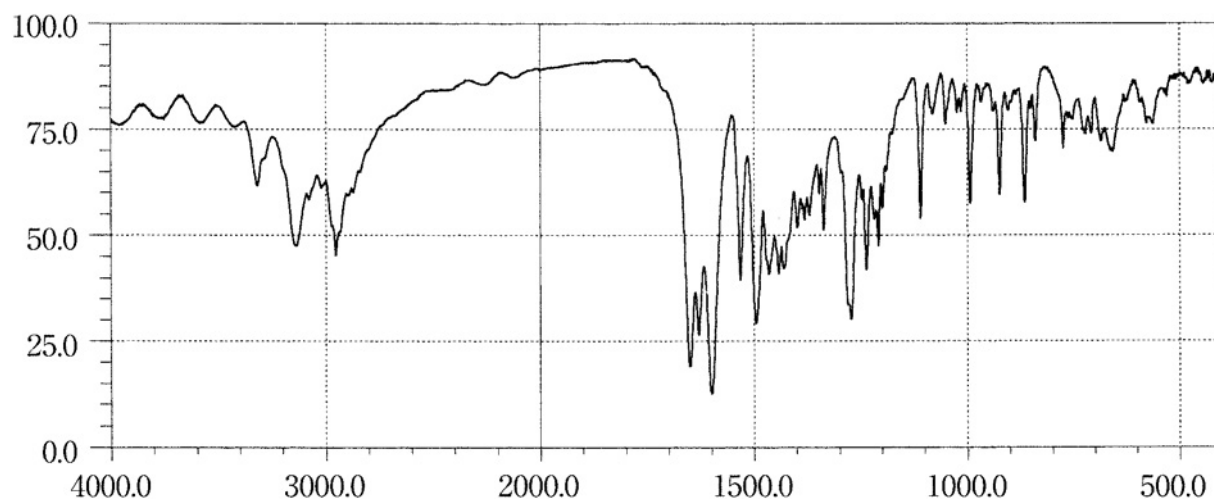
Preparation of sample: Potassium chloride disk method

Bufexamac

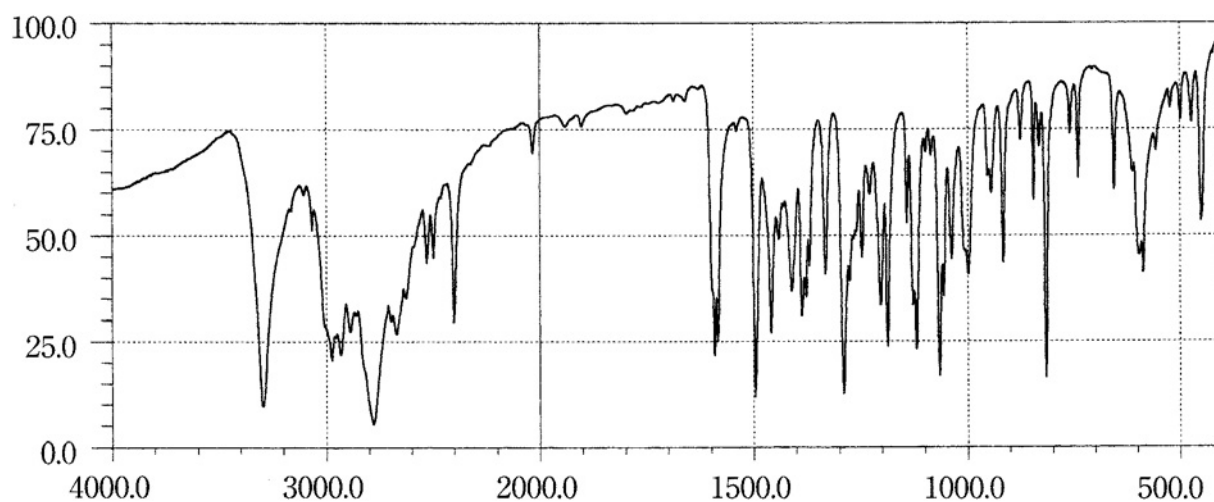
Preparation of sample: Potassium bromide disk method

Bumetanide

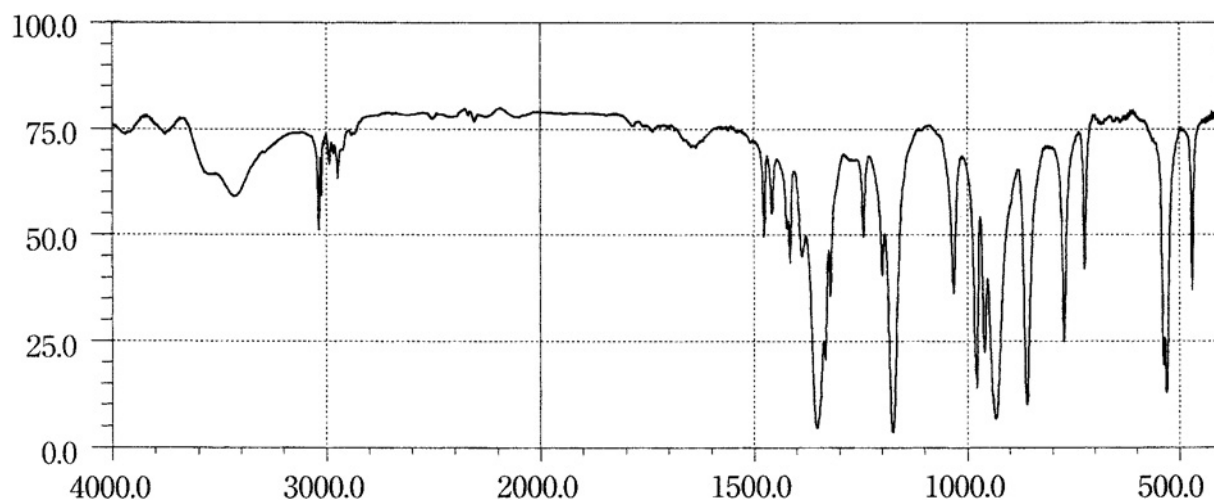
Preparation of sample: Potassium bromide disk method

Bunazosin Hydrochloride

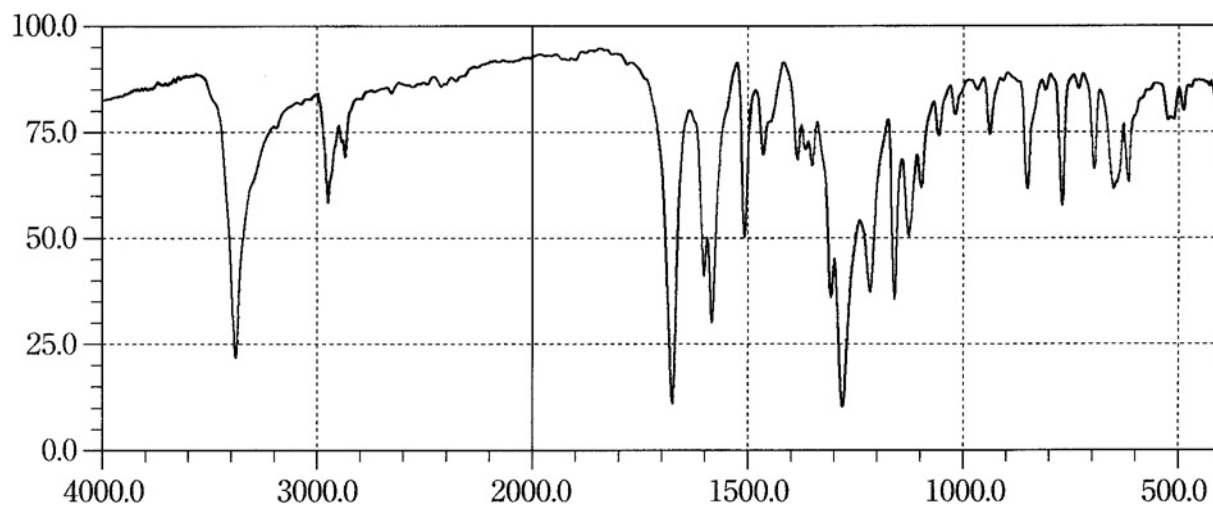
Preparation of sample: Potassium bromide disk method

Bupranolol Hydrochloride

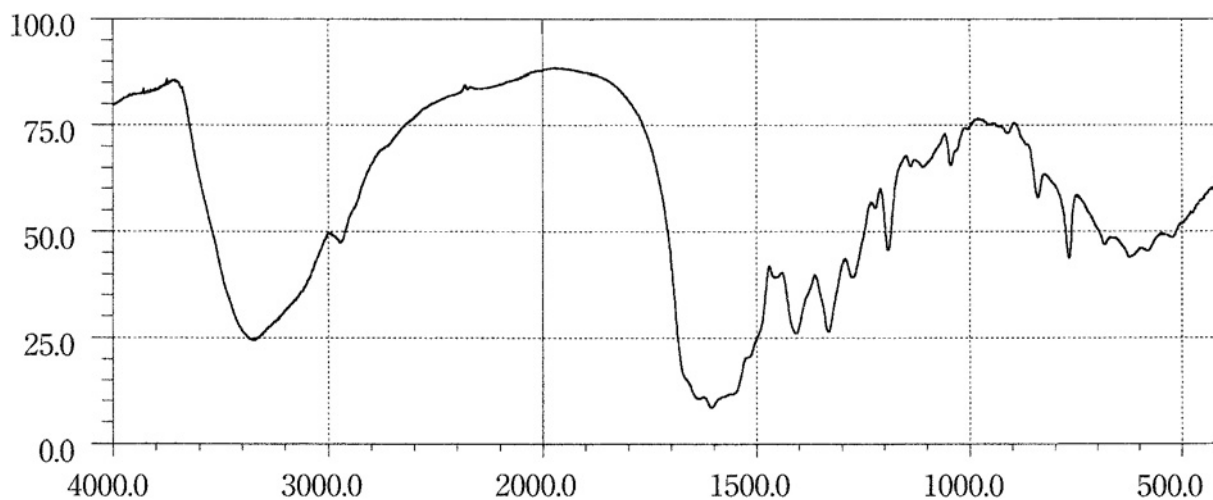
Preparation of sample: Potassium chloride disk method

Busulfan

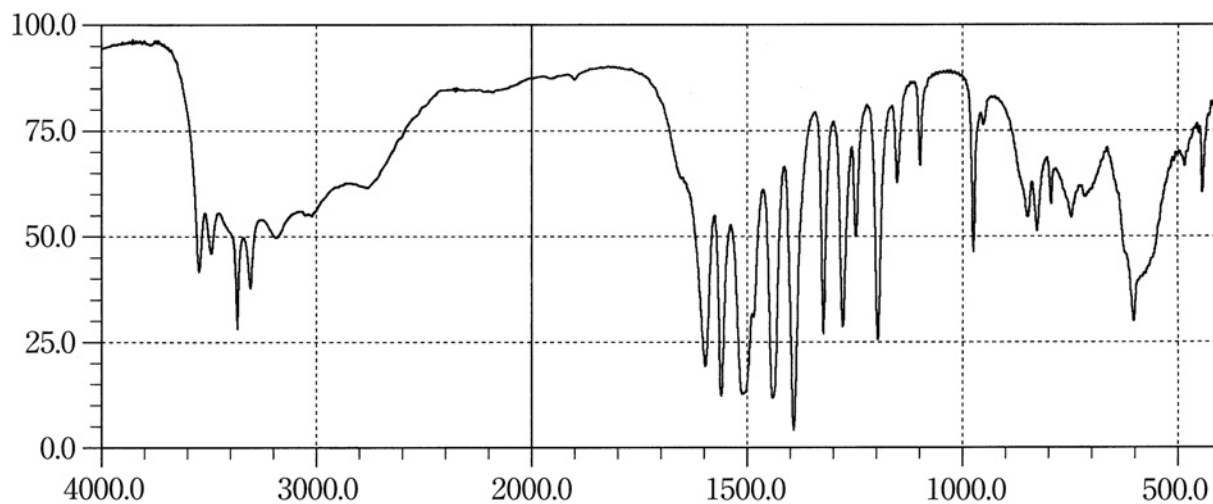
Preparation of sample: Potassium bromide disk method

Butyl Parahydroxybenzoate

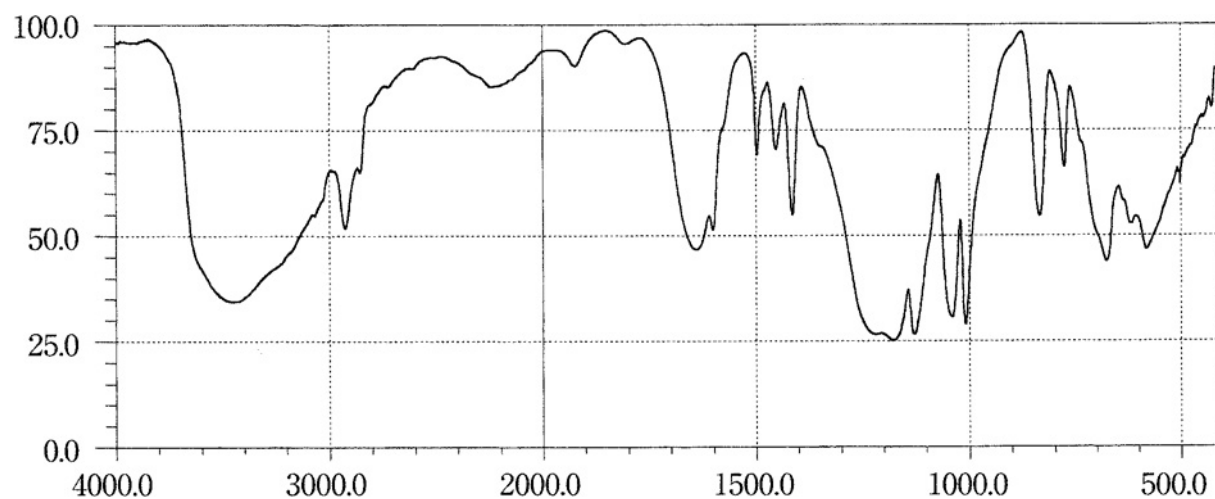
Preparation of sample: Potassium bromide disk method

Calcium Folate

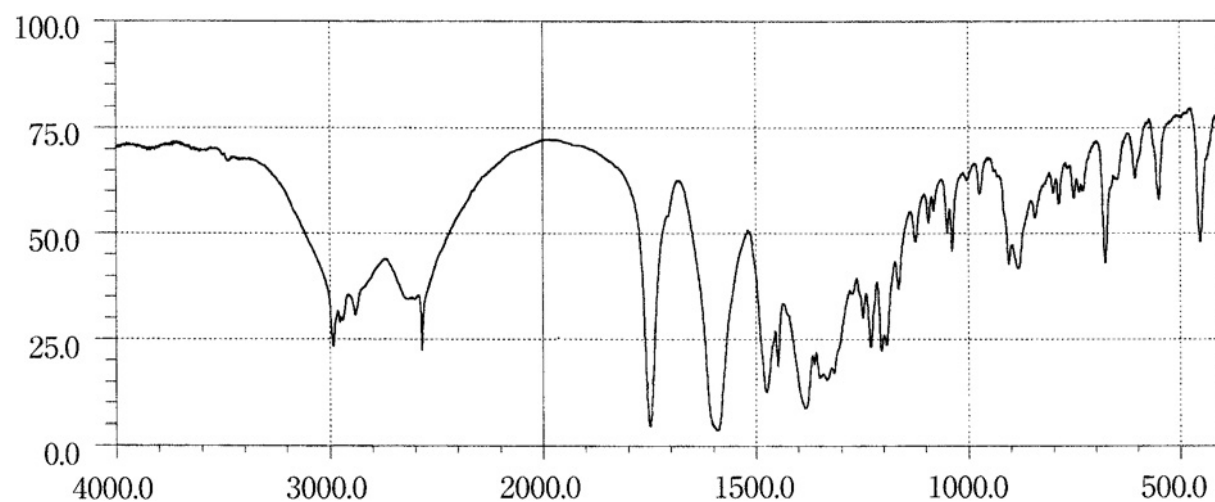
Preparation of sample: Potassium bromide disk method

Calcium Para-aminosalicylate Hydrate

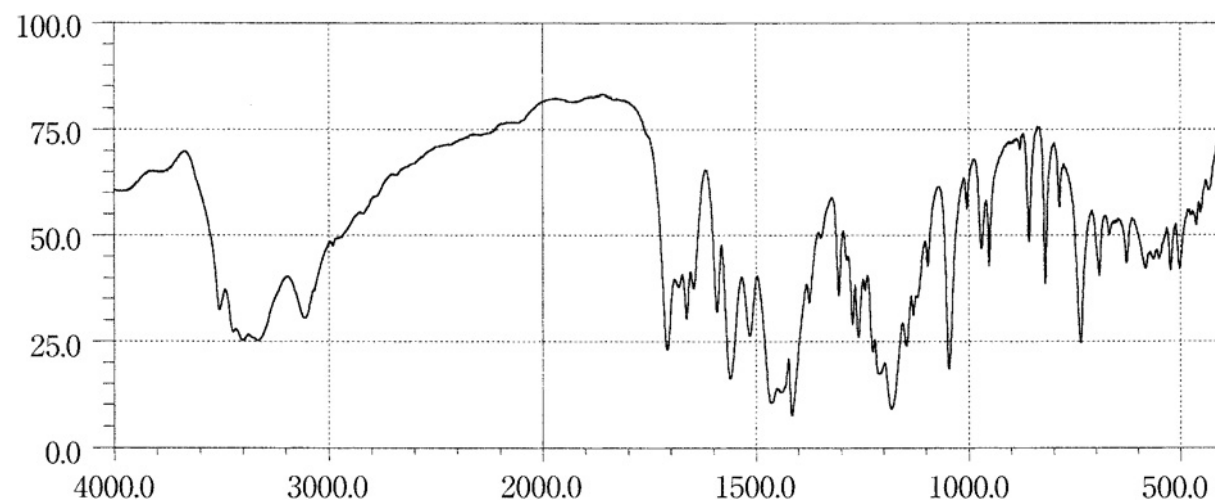
Preparation of sample: Potassium bromide disk method

Calcium Polystyrene Sulfonate

Preparation of sample: Potassium bromide disk method

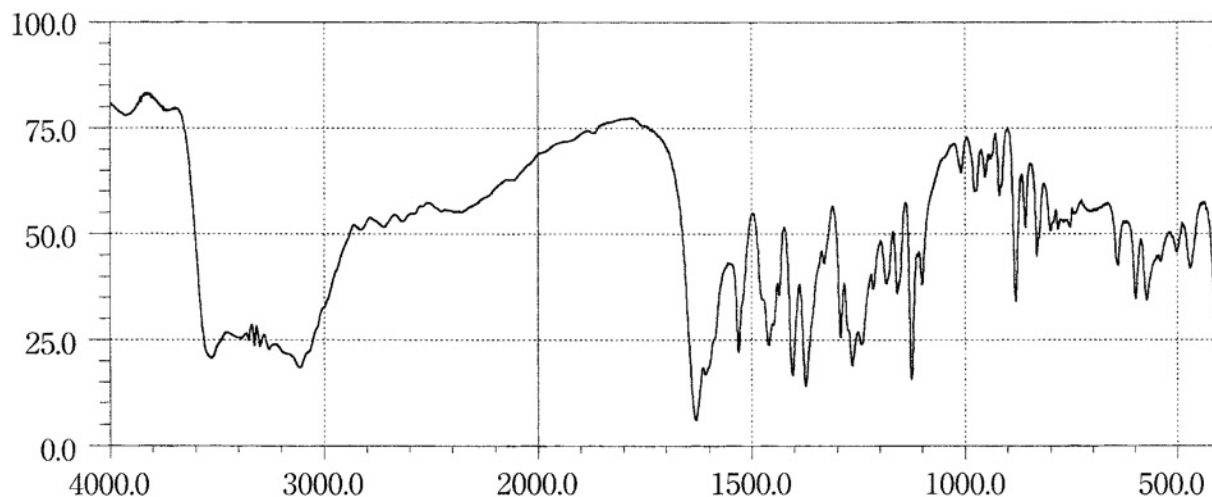
Captopril

Preparation of sample: Potassium bromide disk method

Carbazochrome Sodium Sulfonate Hydrate

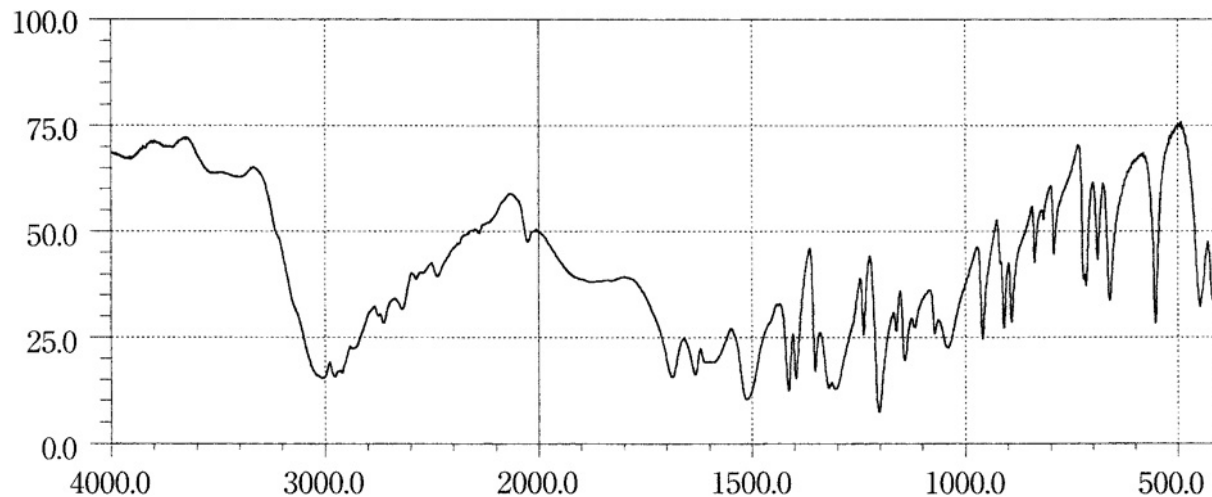
Preparation of sample: Potassium bromide disk method

Carbidopa Hydrate



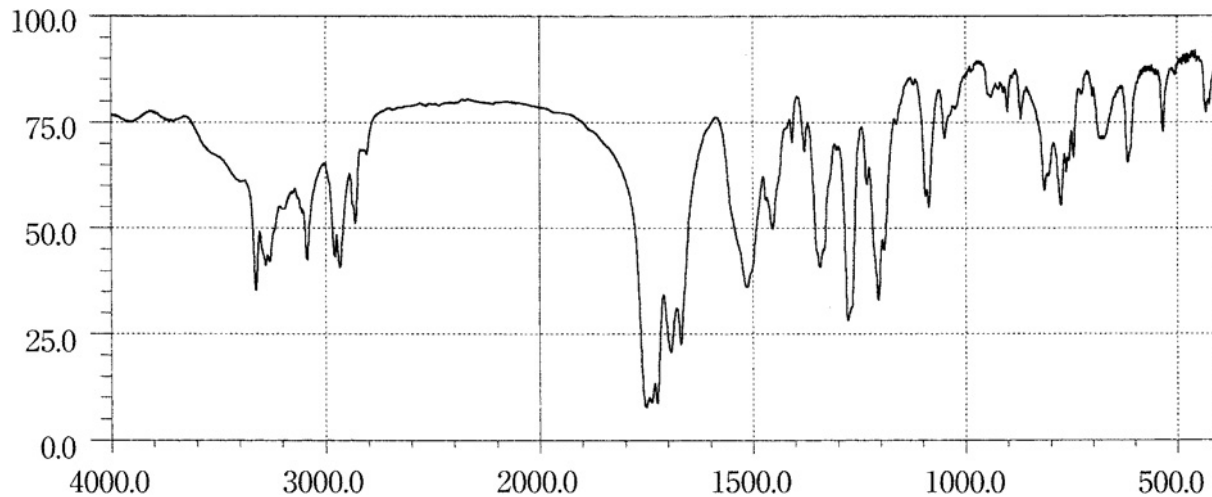
Preparation of sample: Potassium bromide disk method

L-Carbocisteine

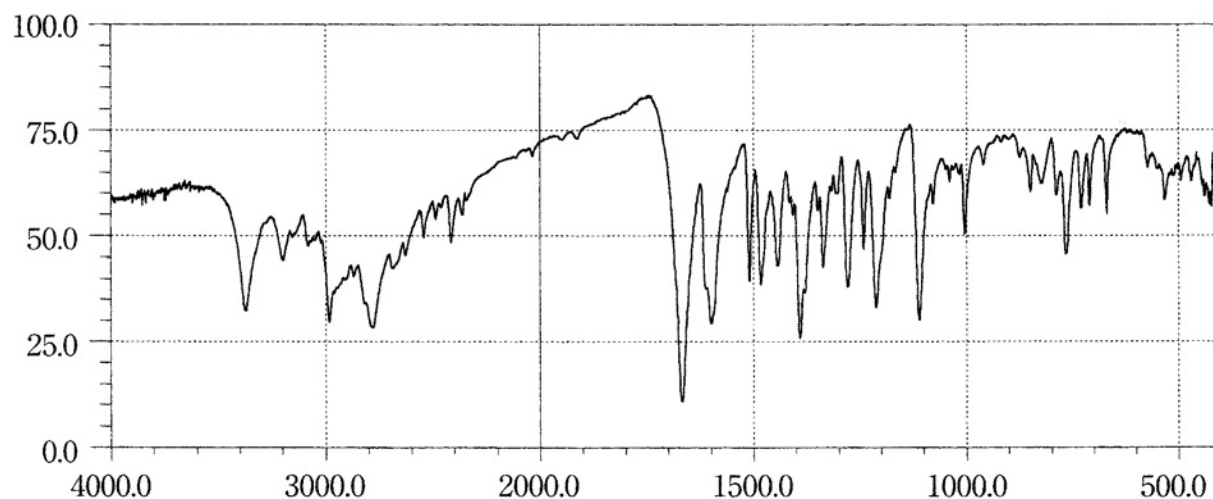


Preparation of sample: Potassium bromide disk method

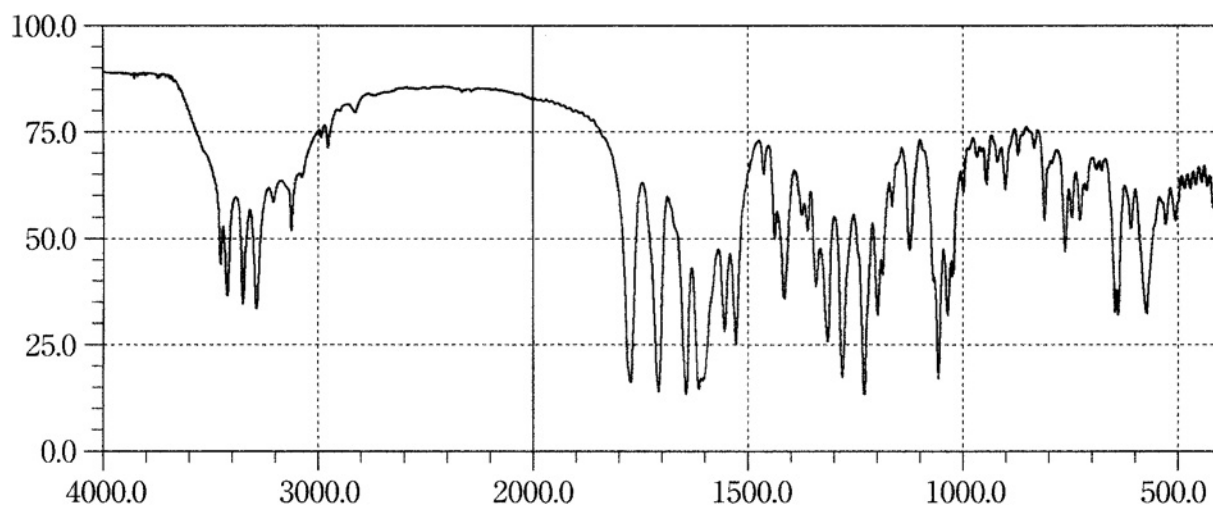
Carmofur



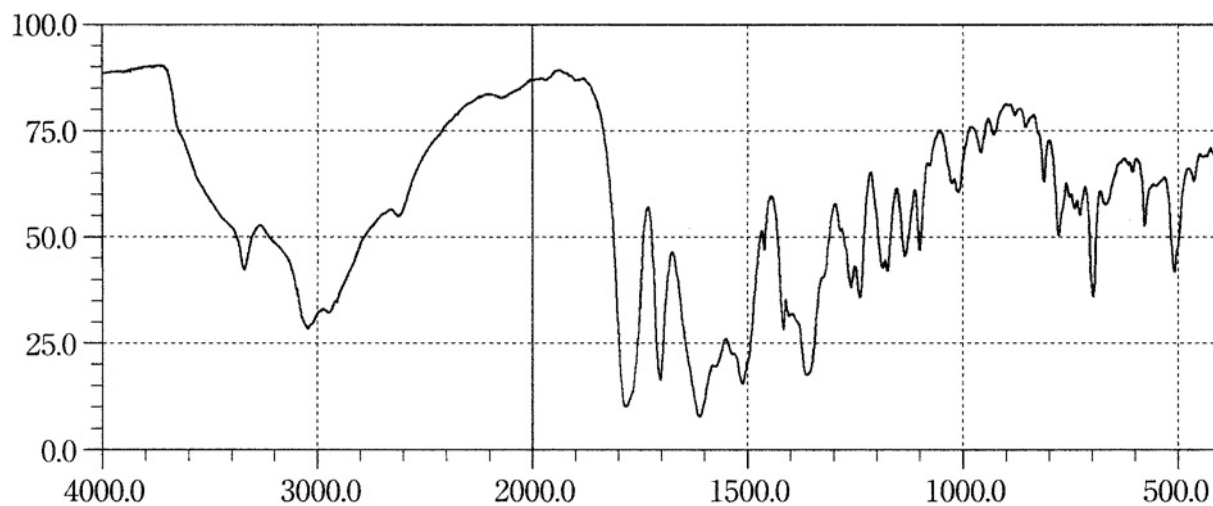
Preparation of sample: Potassium bromide disk method

Carteolol Hydrochloride

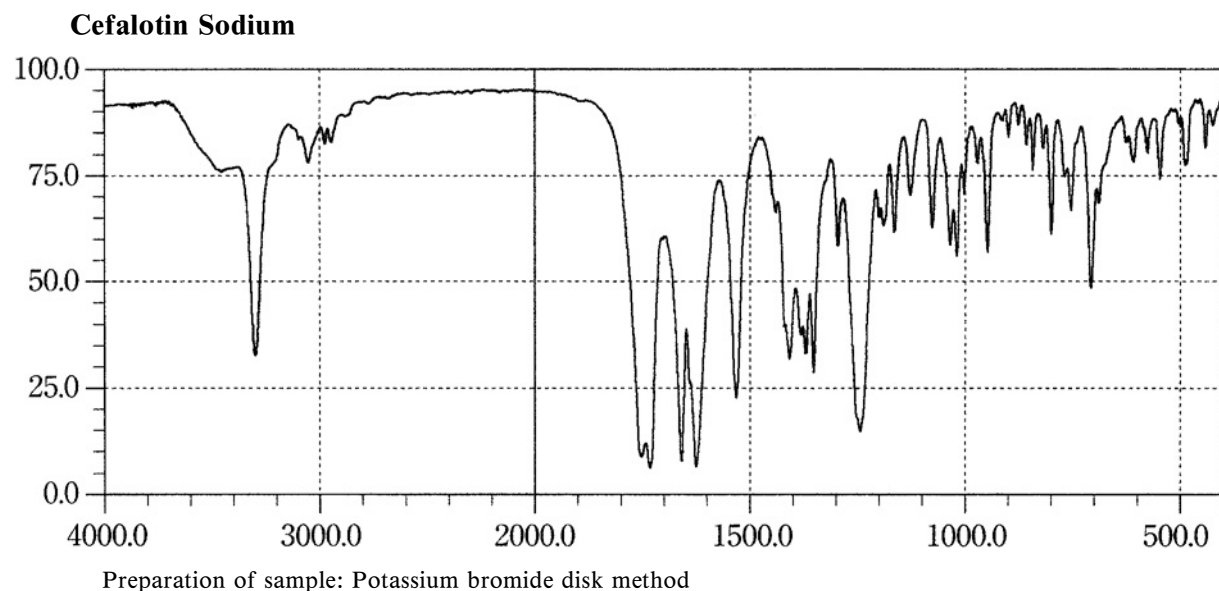
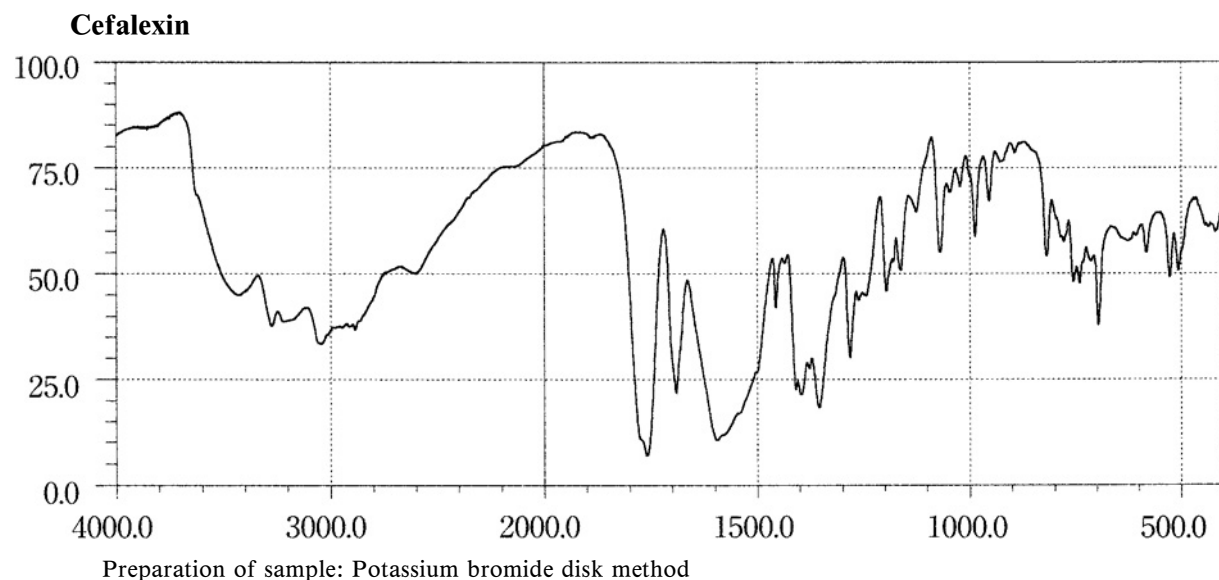
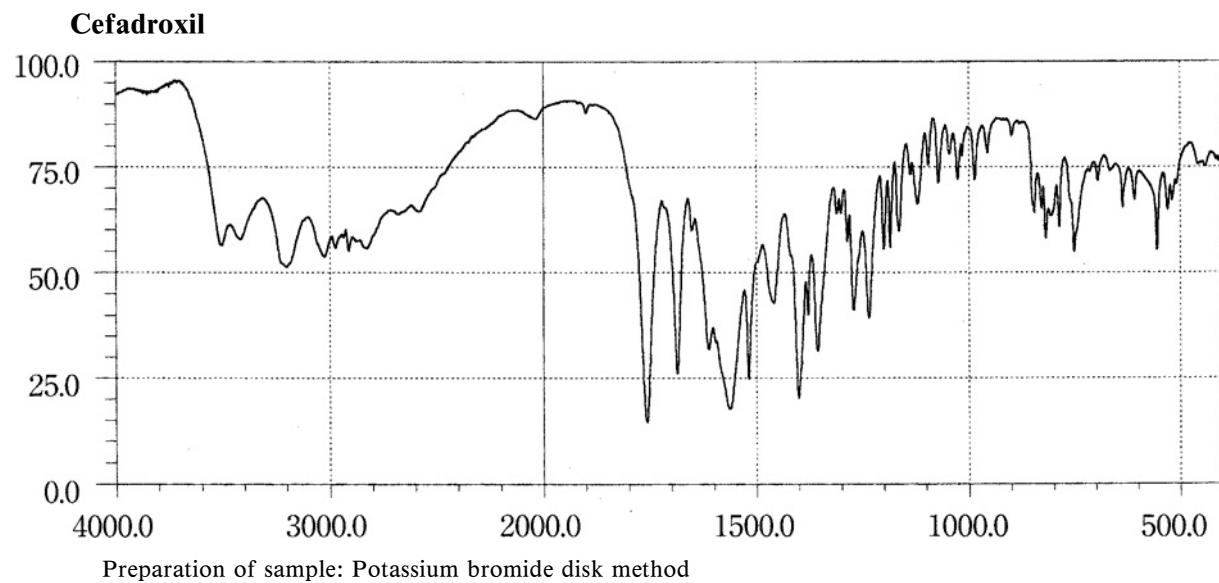
Preparation of sample: Potassium chloride disk method

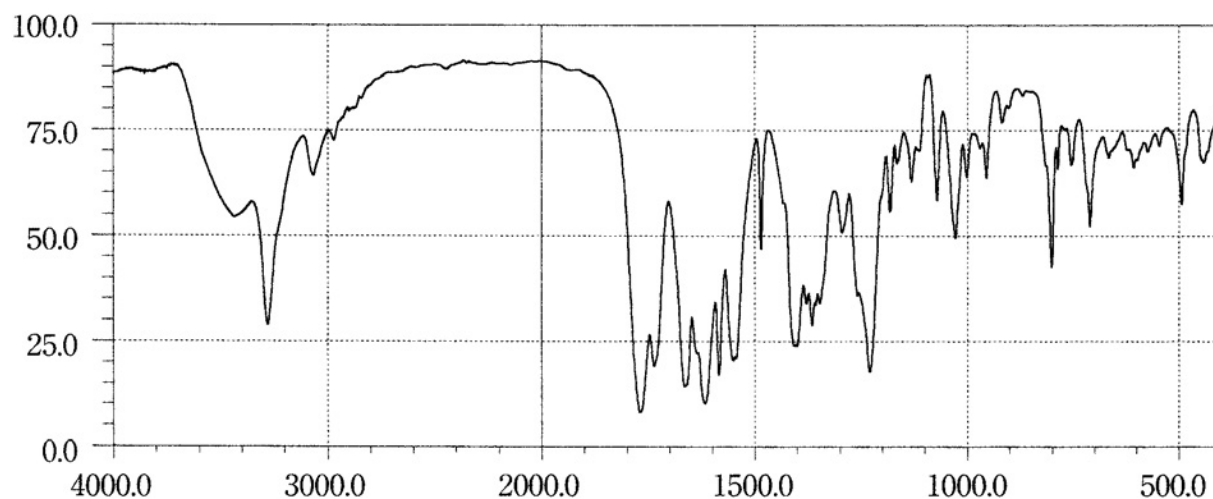
Carumonam Sodium

Preparation of sample: Potassium bromide disk method

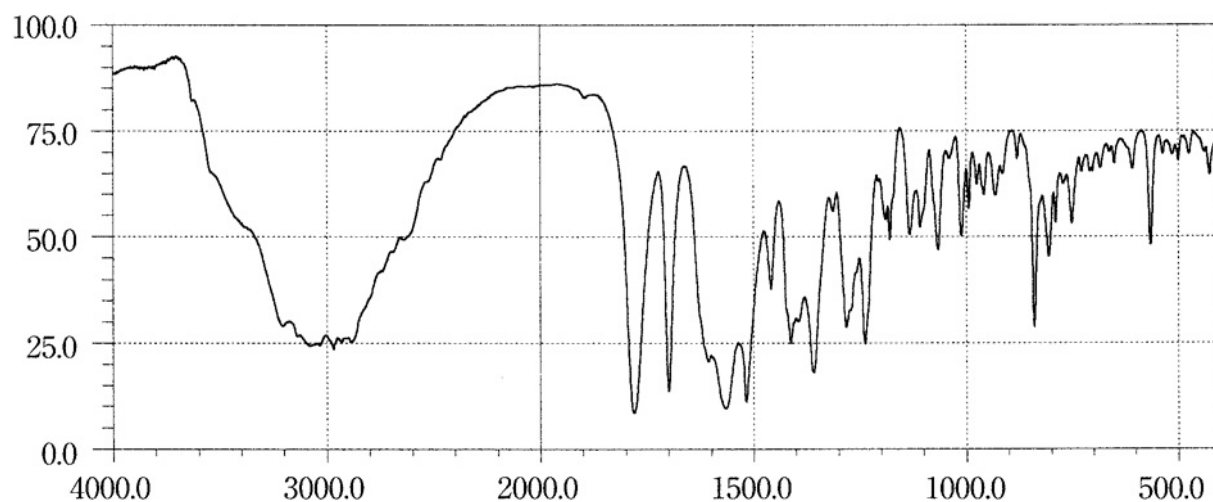
Cefactor

Preparation of sample: Potassium bromide disk method

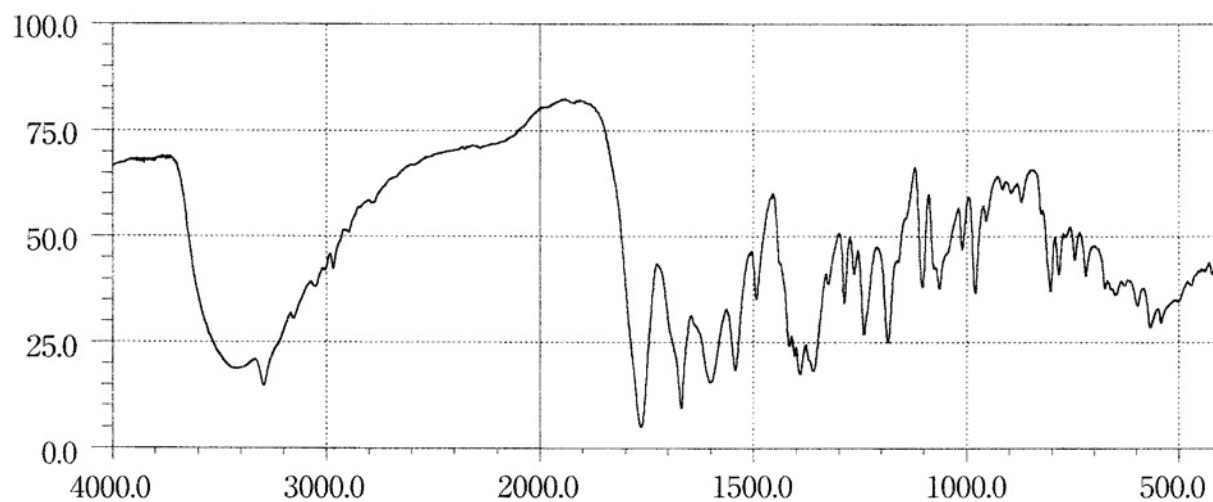


Cefapirin Sodium

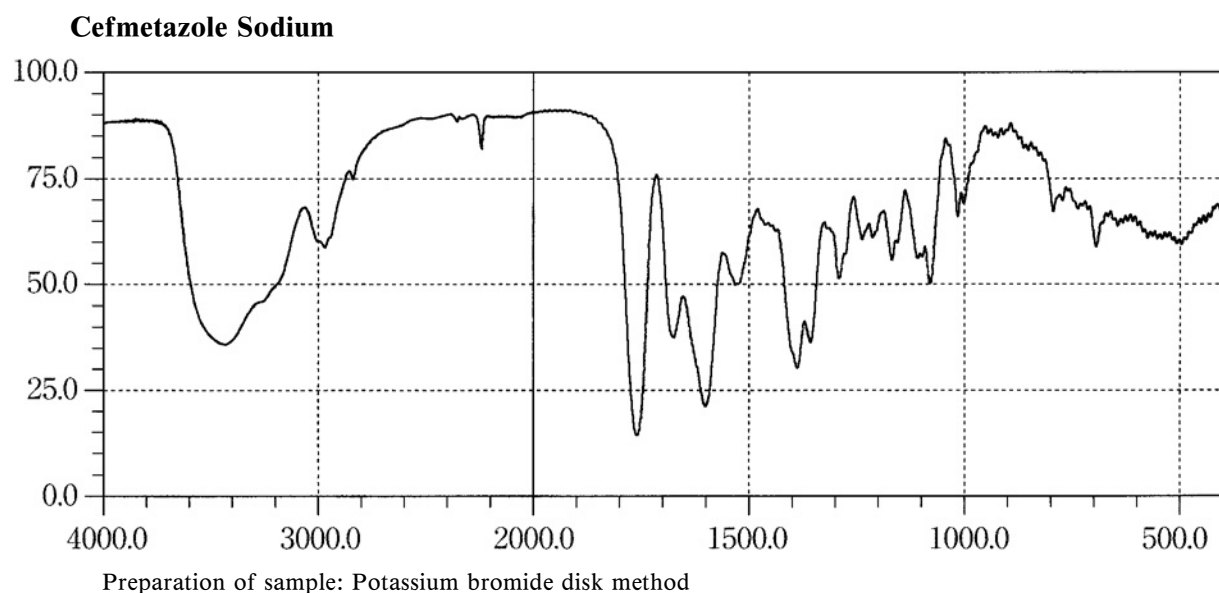
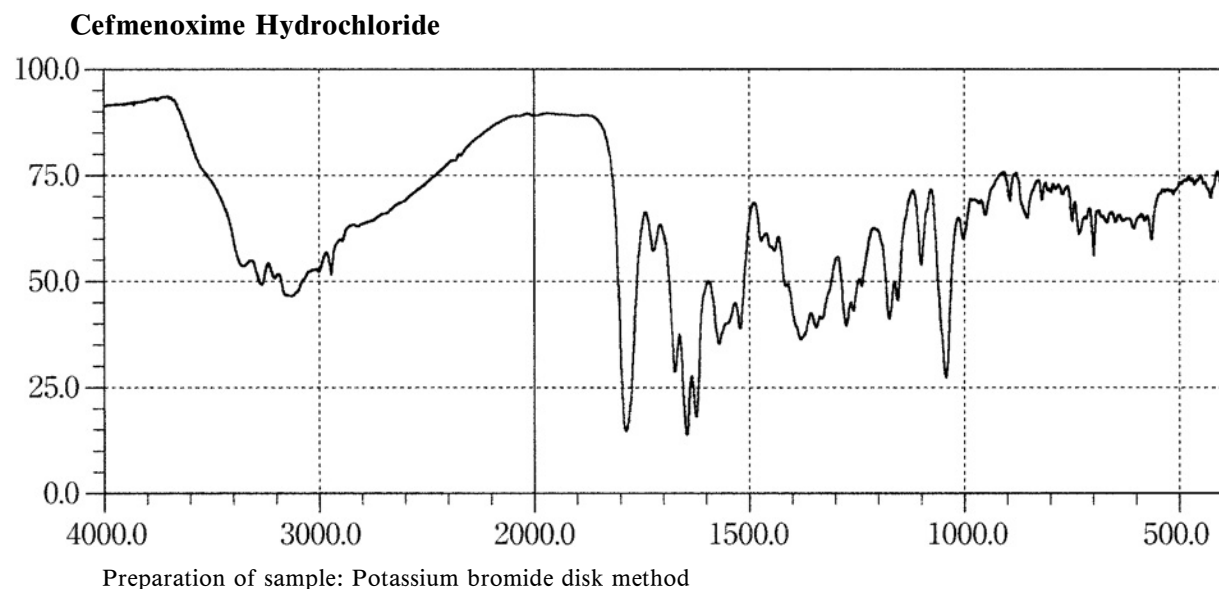
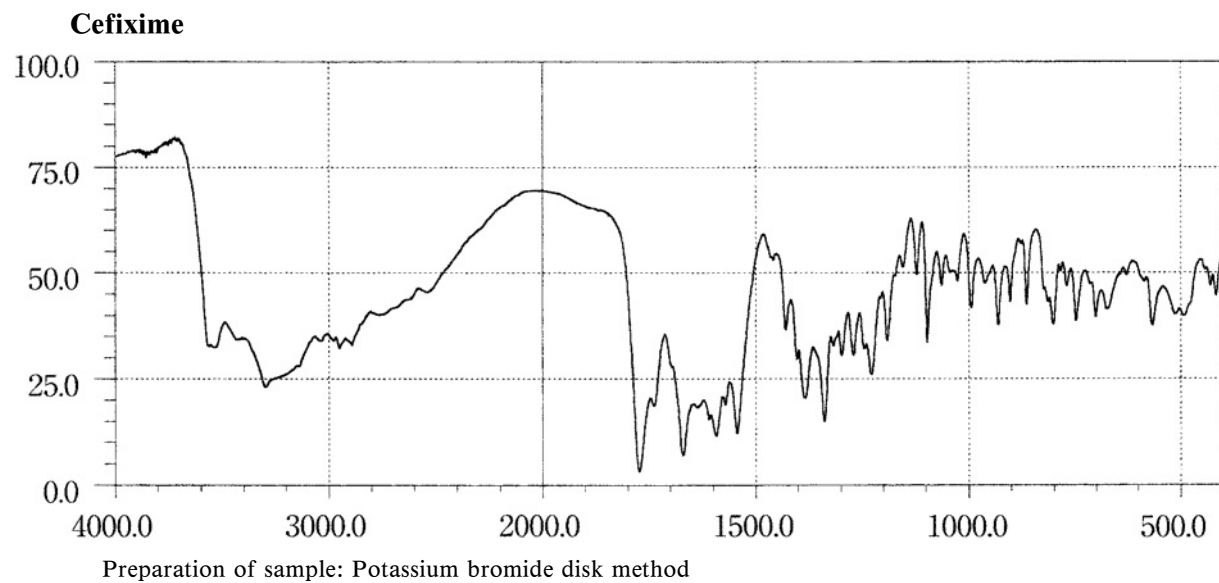
Preparation of sample: Potassium bromide disk method

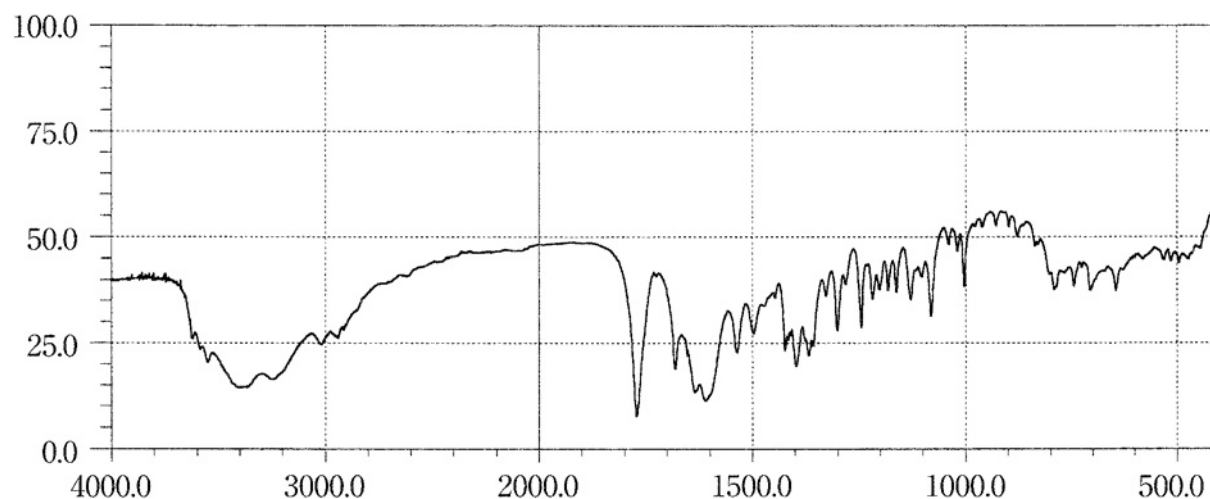
Cefatrizine Propylene Glycolate

Preparation of sample: Potassium bromide disk method

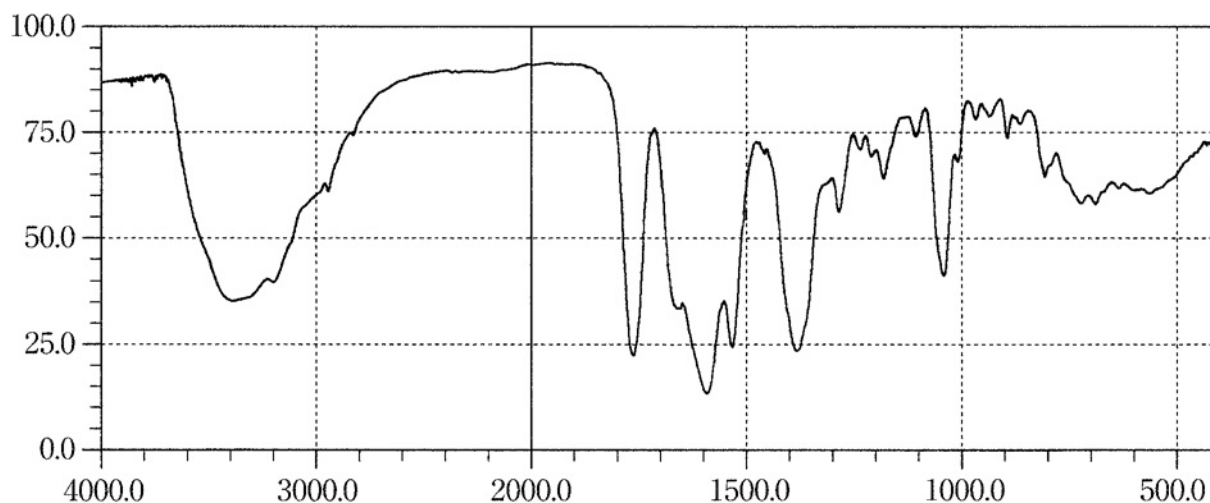
Cefazolin Sodium

Preparation of sample: Potassium bromide disk method

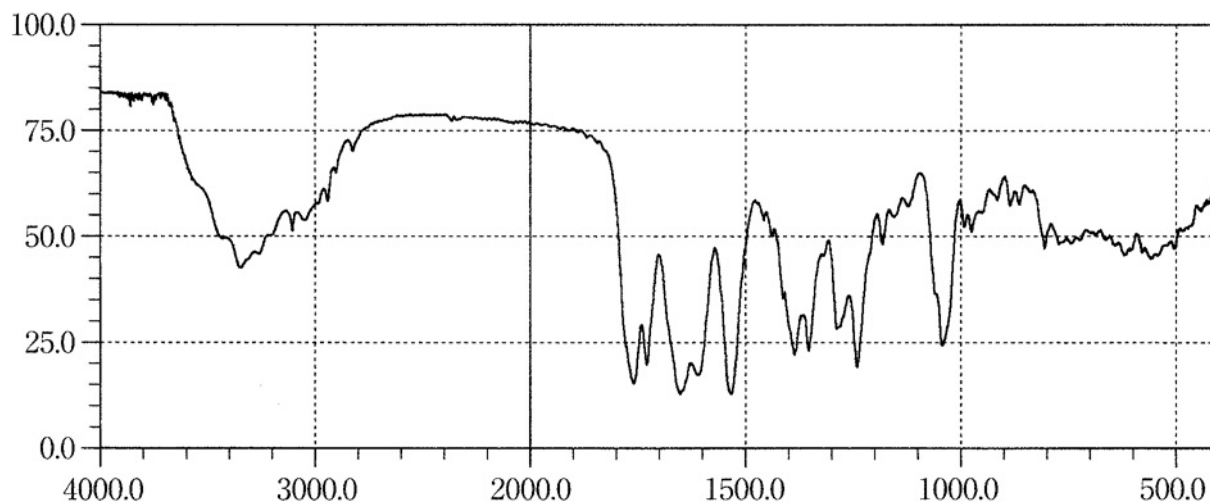


Cefminox Sodium Hydrate

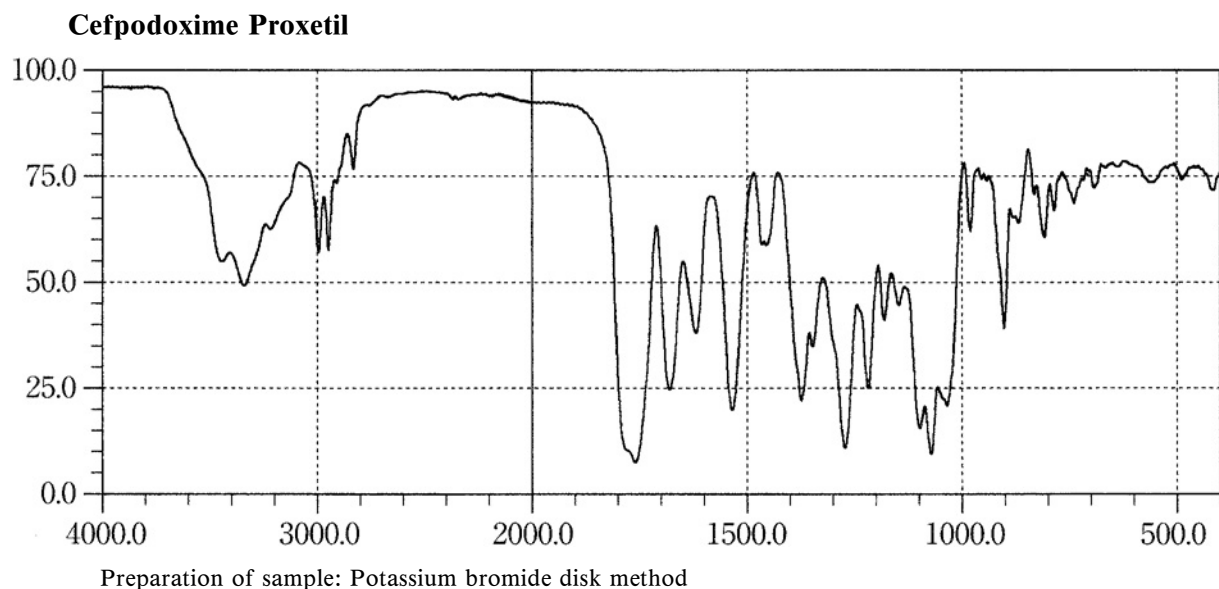
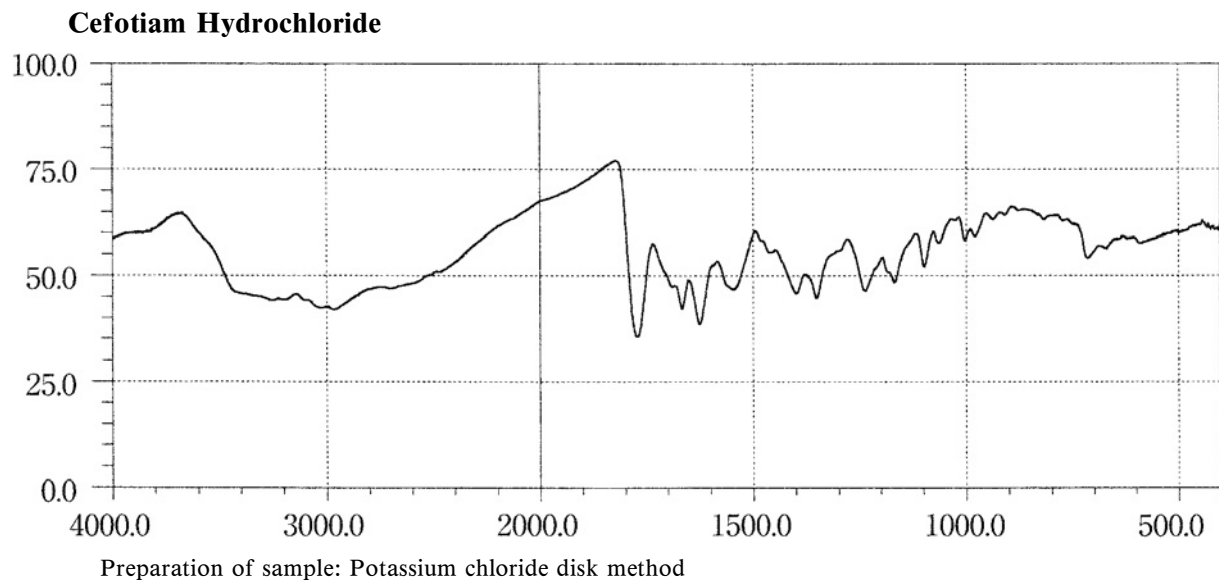
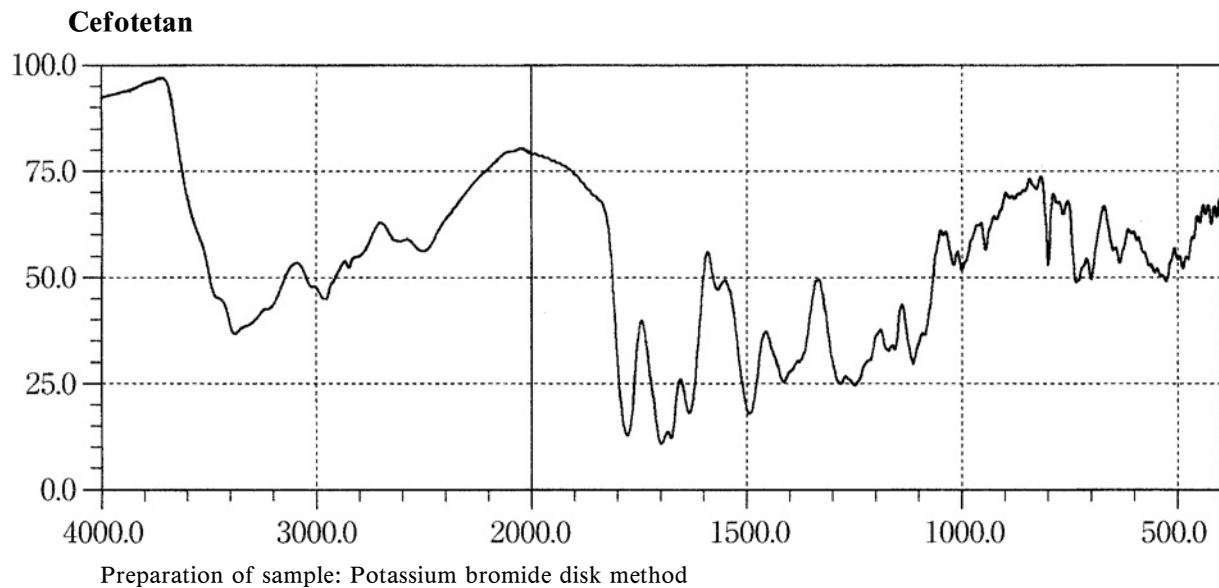
Preparation of sample: Potassium bromide disk method

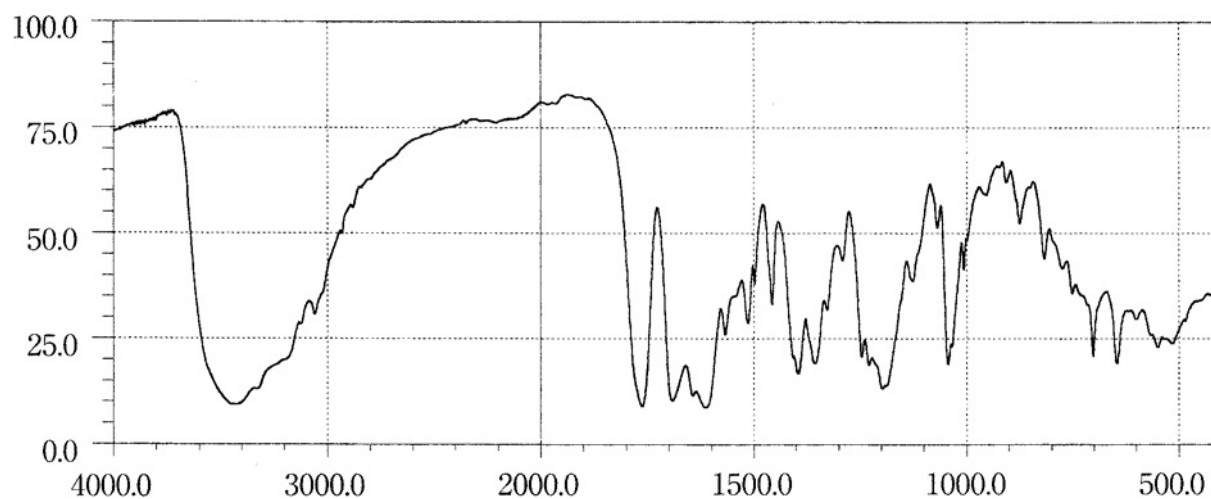
Cefodizime Sodium

Preparation of sample: Potassium bromide disk method

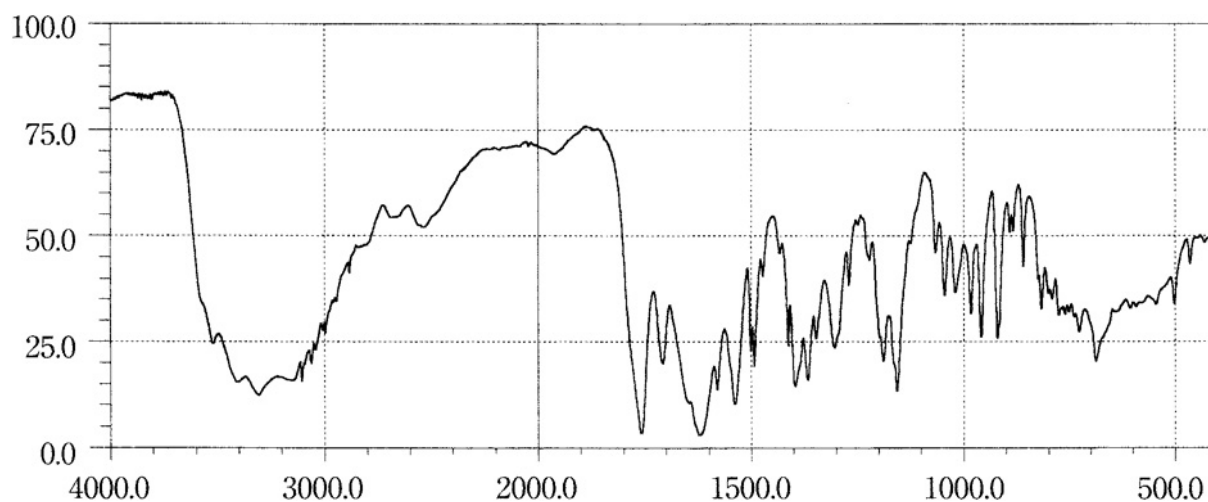
Cefotaxime Sodium

Preparation of sample: Potassium bromide disk method

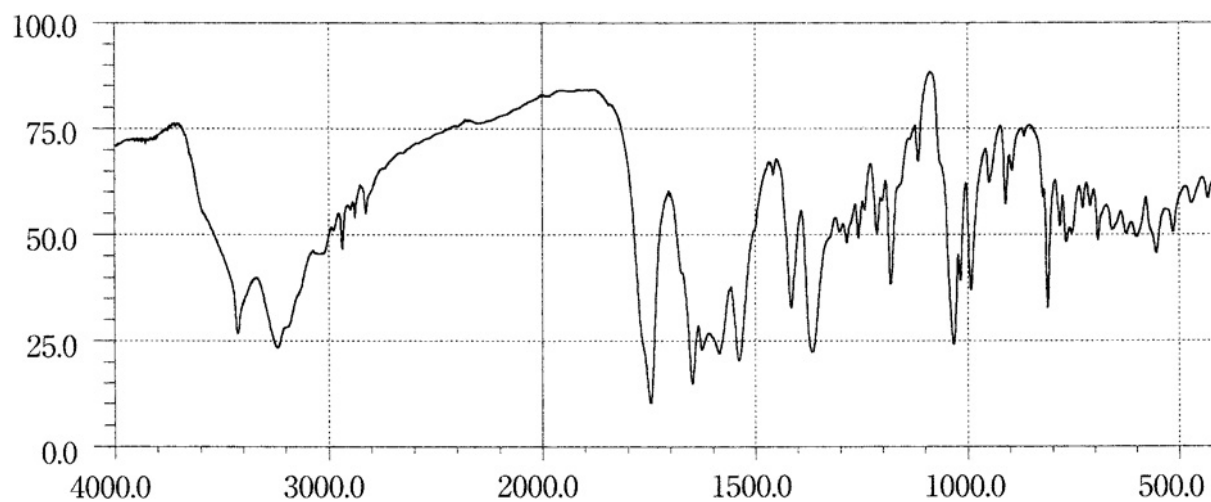


Cefsulodin Sodium

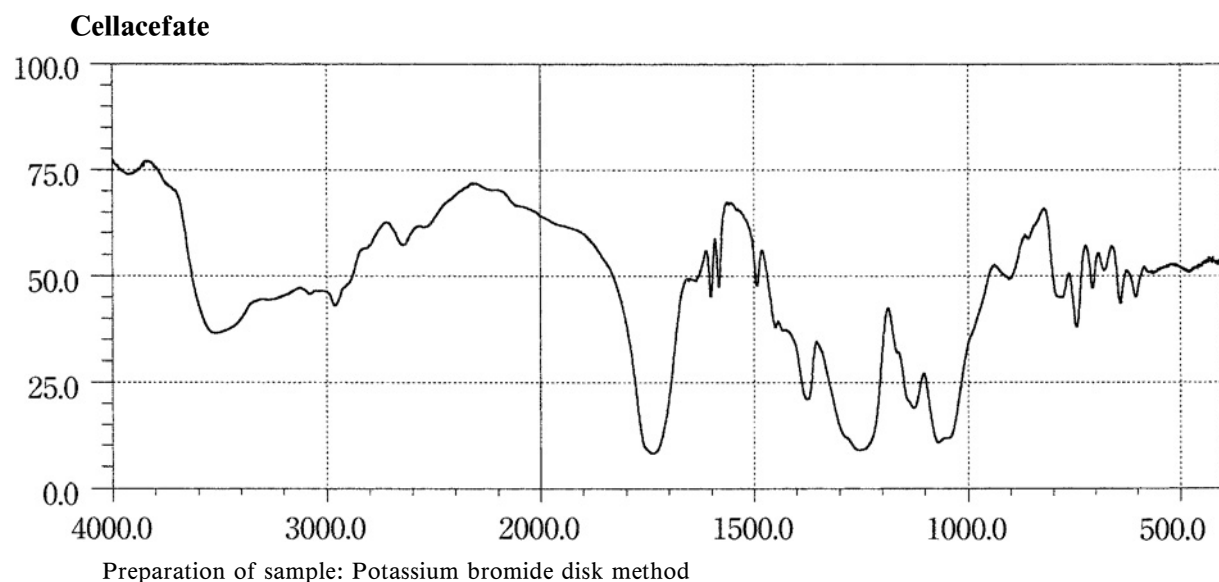
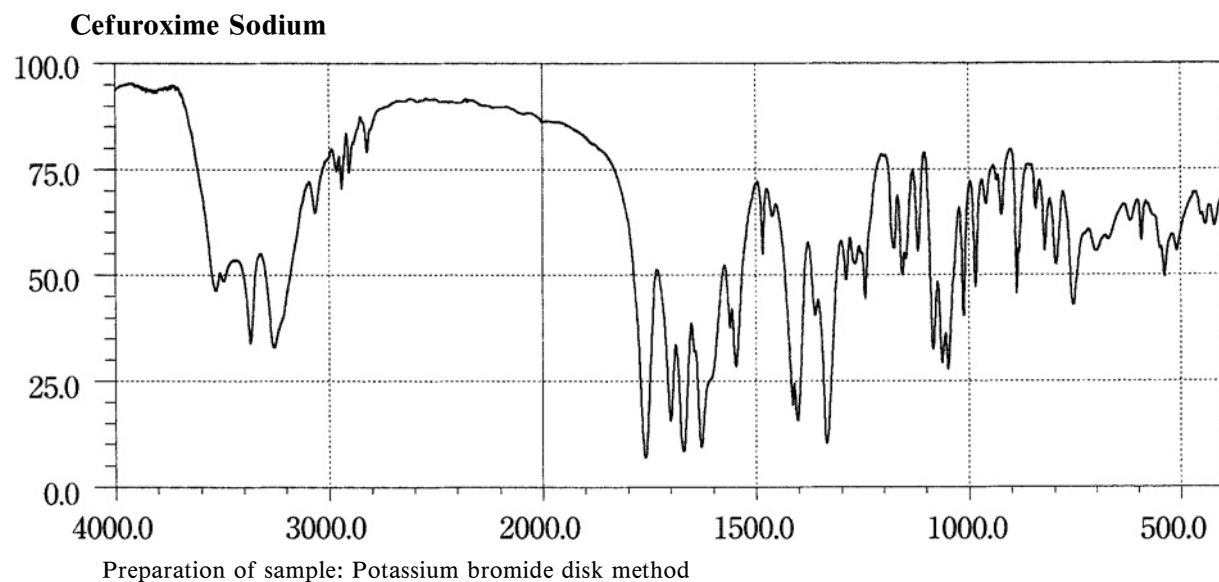
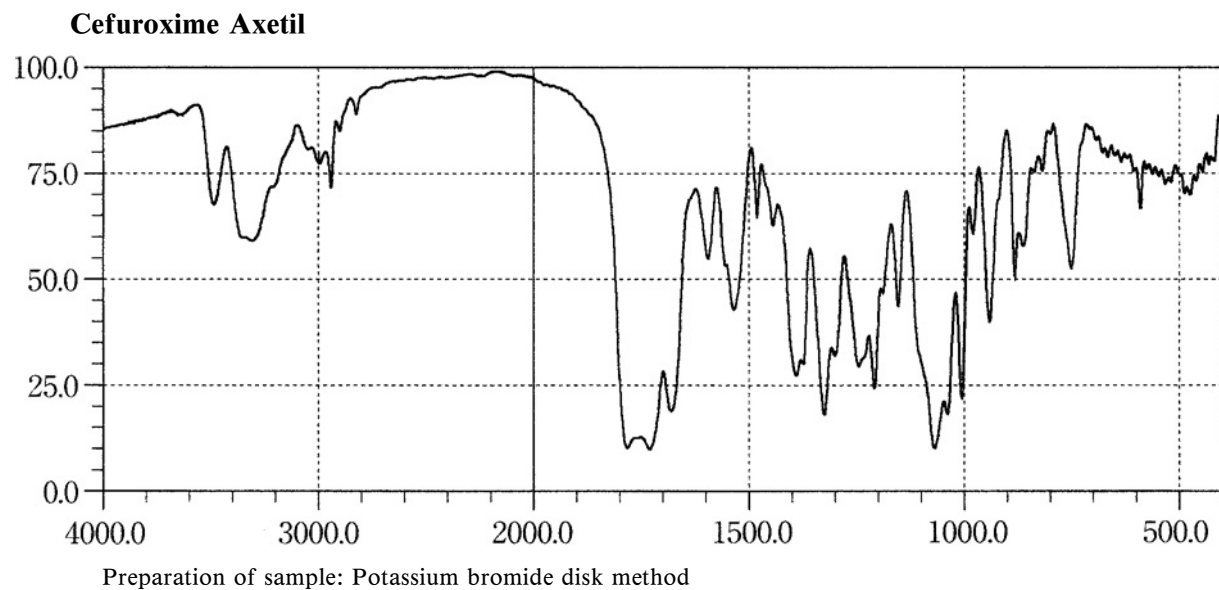
Preparation of sample: Potassium bromide disk method

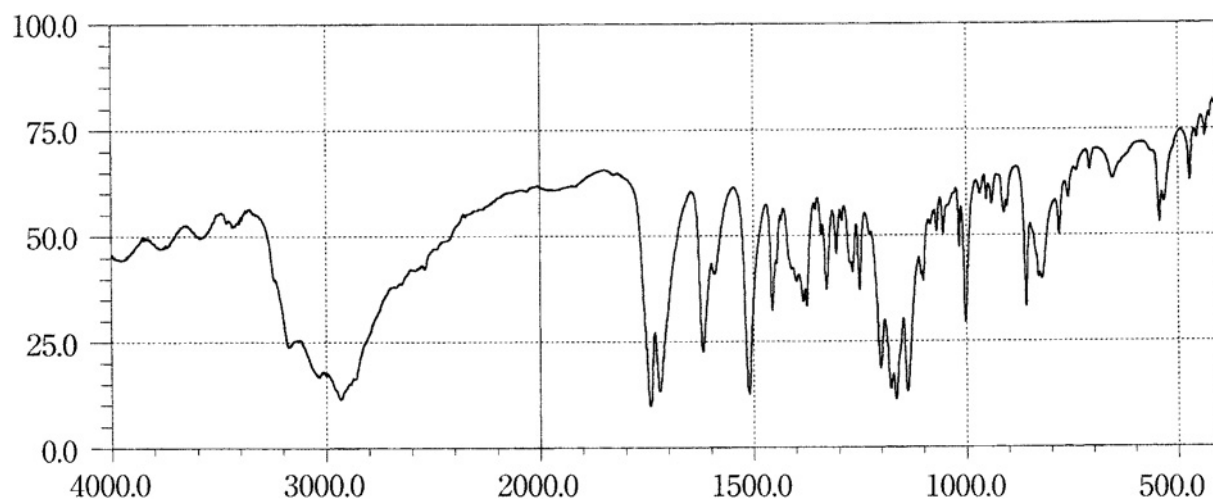
Ceftazidime Hydrate

Preparation of sample: Potassium bromide disk method

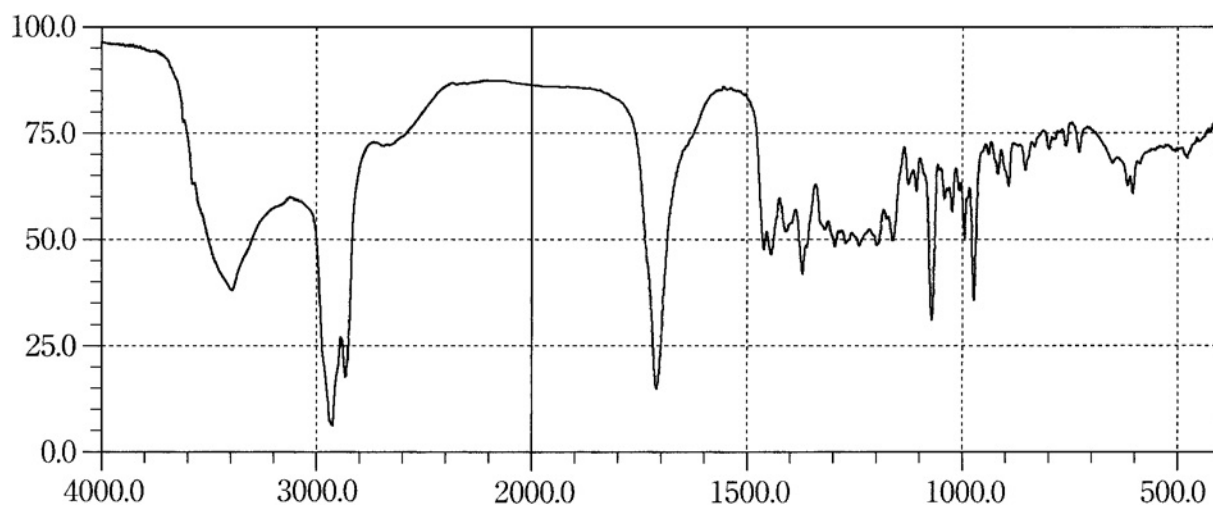
Ceftizoxime Sodium

Preparation of sample: Potassium bromide disk method

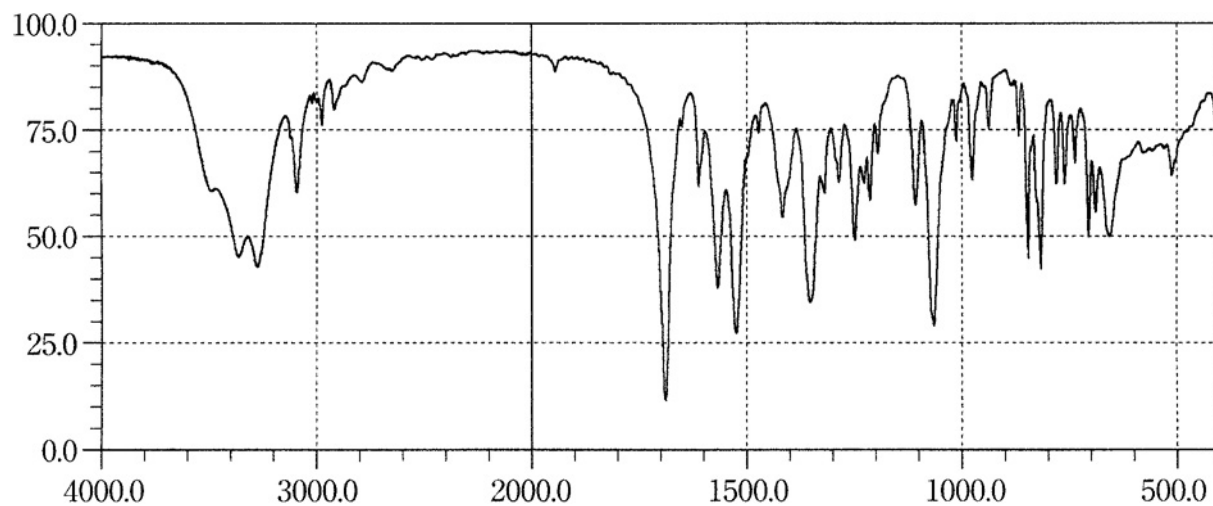


Cetraxate Hydrochloride

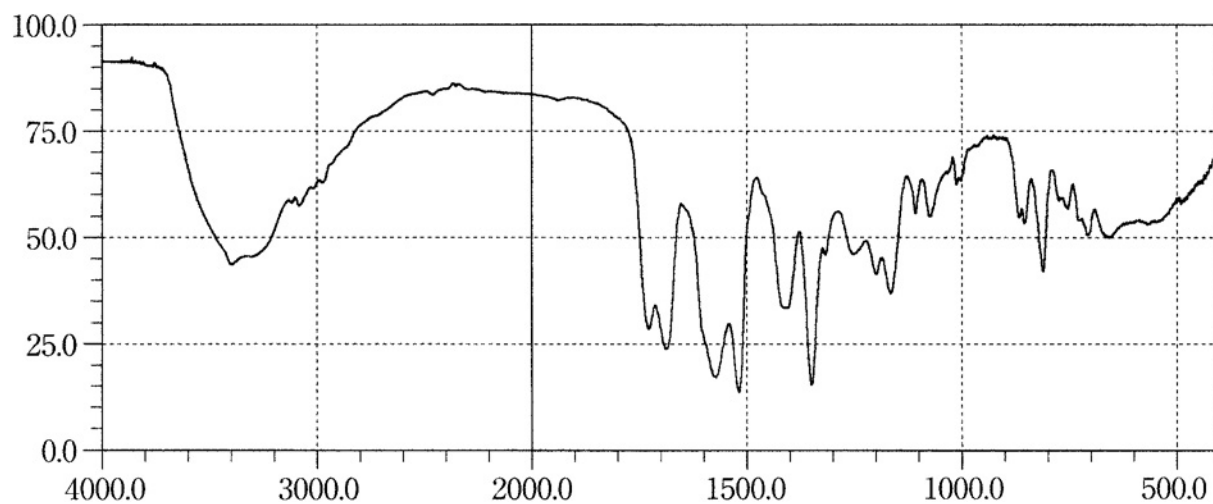
Preparation of sample: Potassium chloride disk method

Chenodeoxycholic Acid

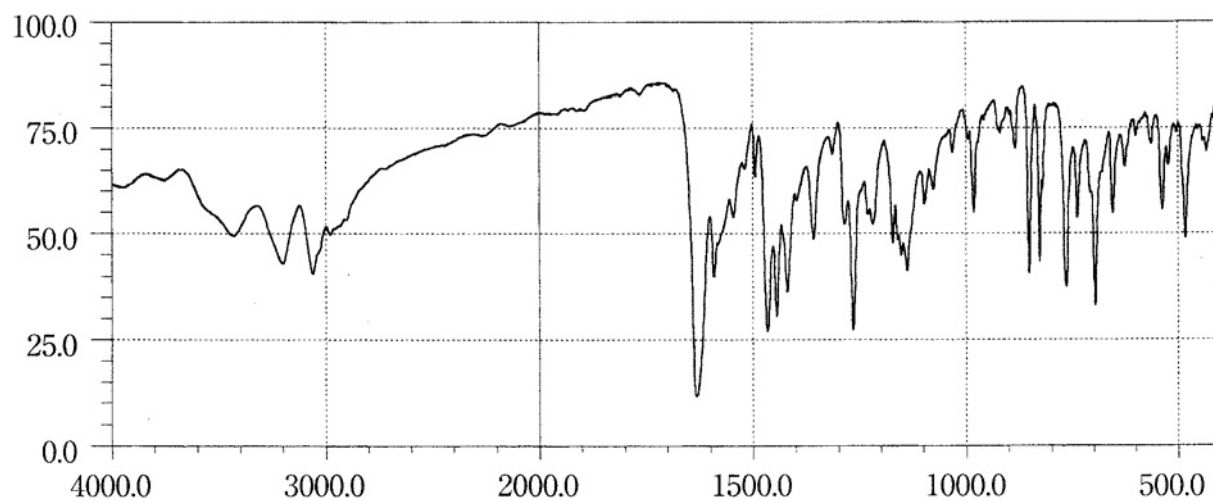
Preparation of sample: Potassium bromide disk method

Chloramphenicol

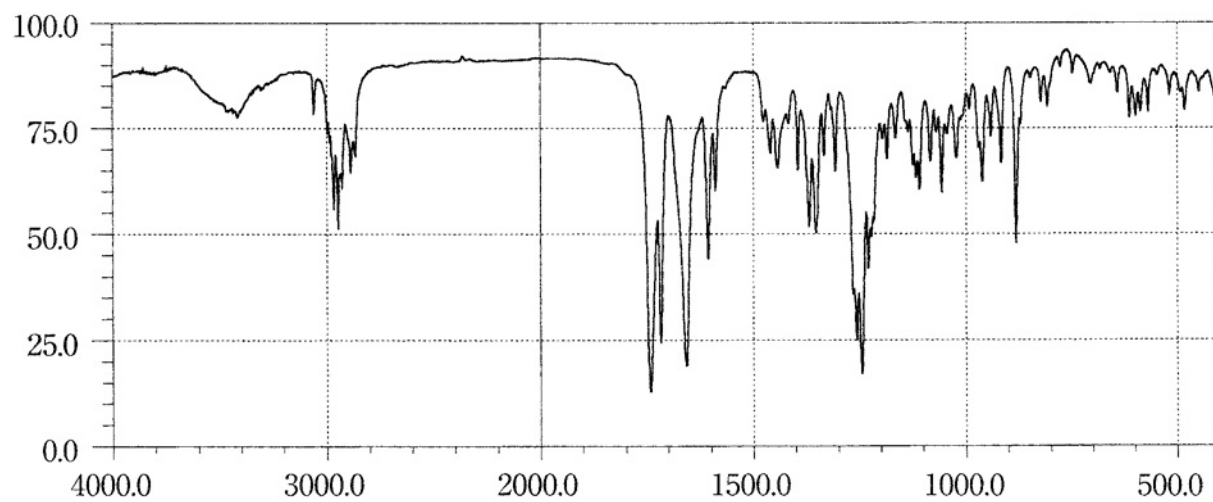
Preparation of sample: Potassium bromide disk method

Chloramphenicol Sodium Succinate

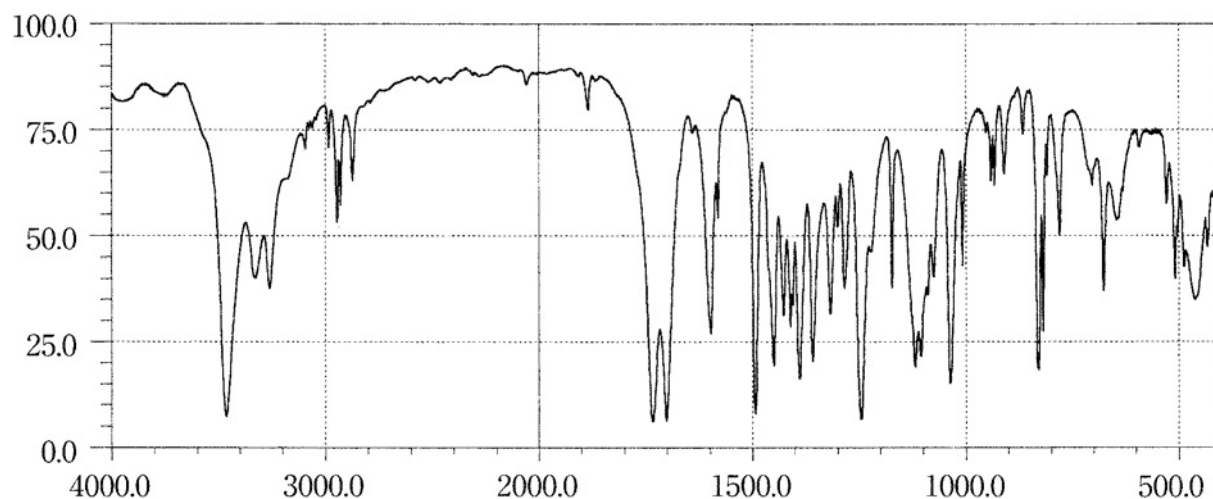
Preparation of sample: Potassium bromide disk method

Chlordiazepoxide

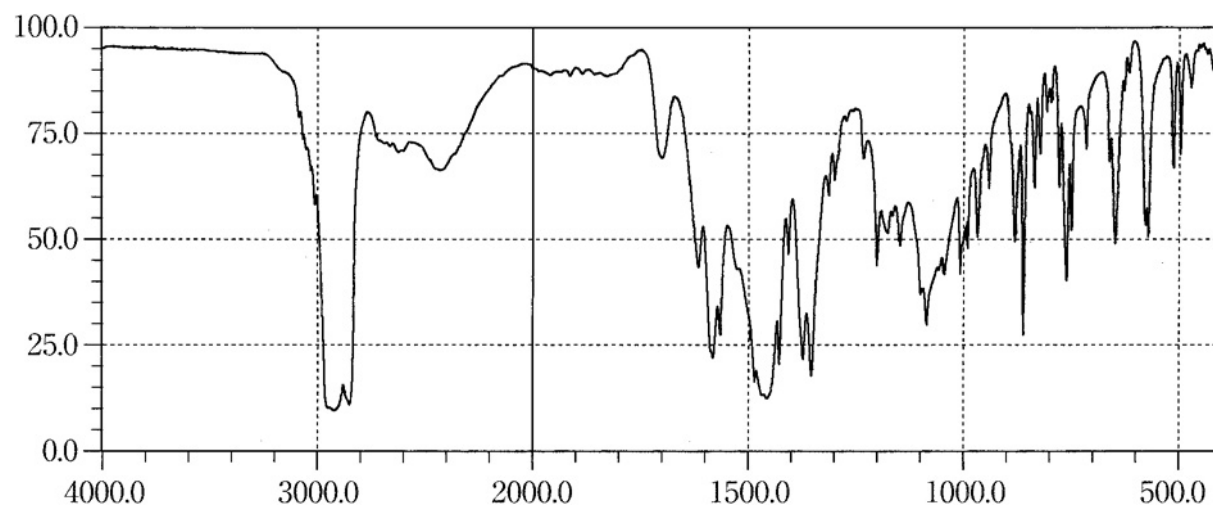
Preparation of sample: Potassium bromide disk method

Chlormadinone Acetate

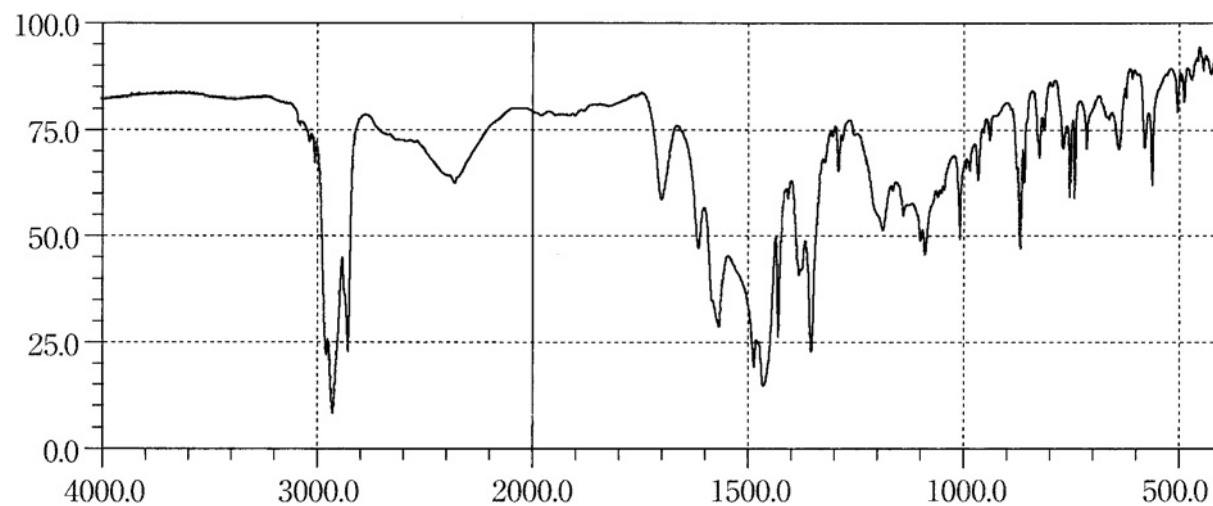
Preparation of sample: Potassium bromide disk method

Chlorphenesin Carbamate

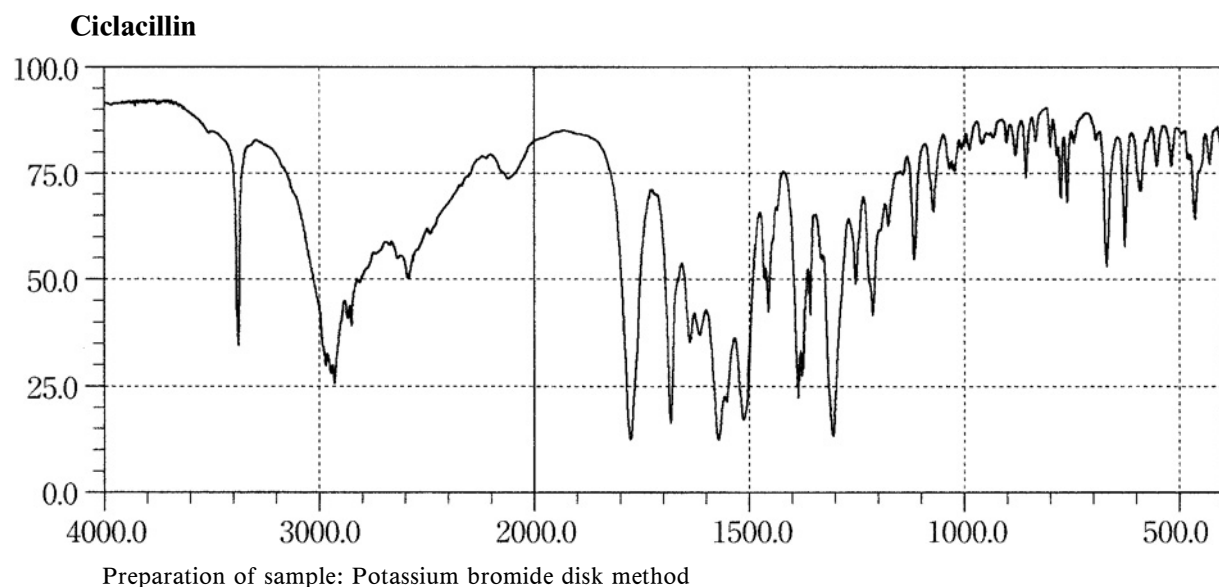
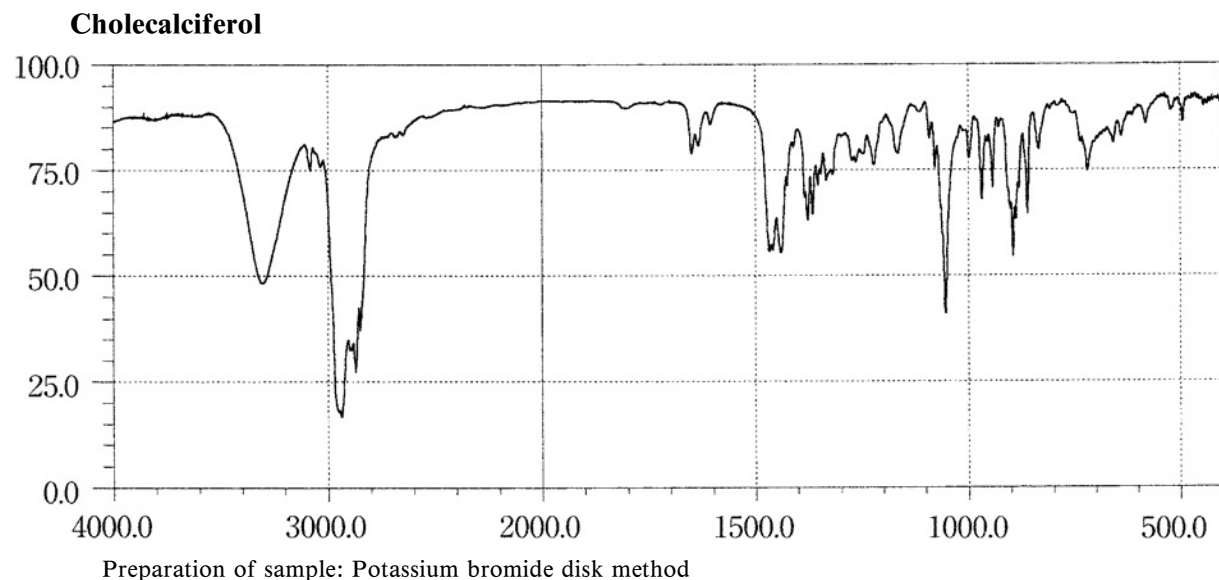
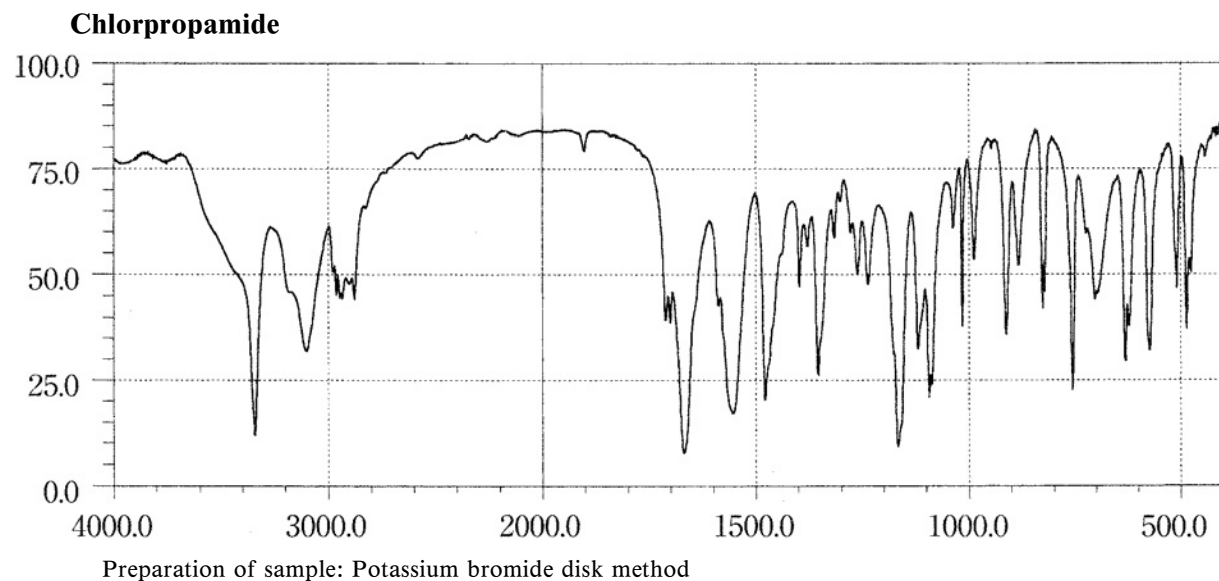
Preparation of sample: Potassium bromide disk method

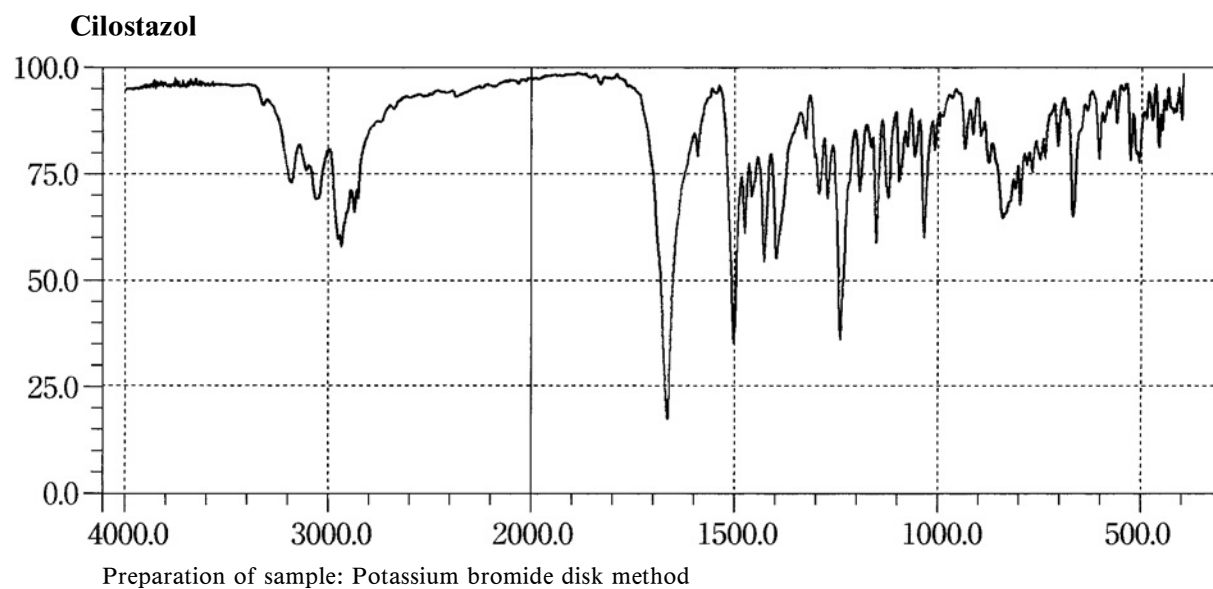
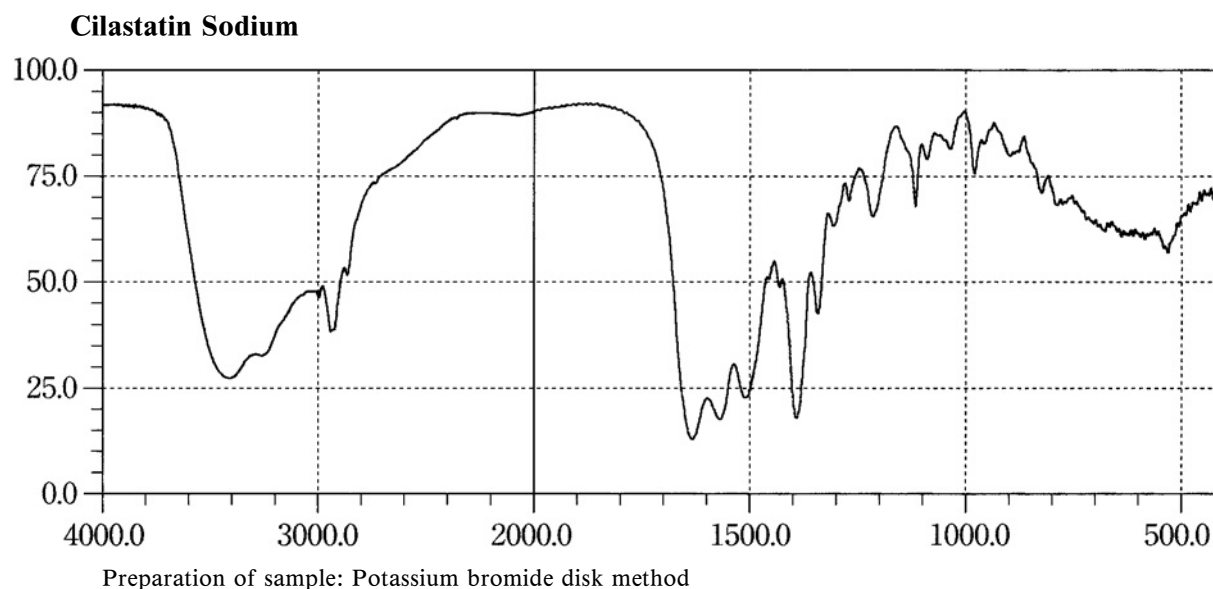
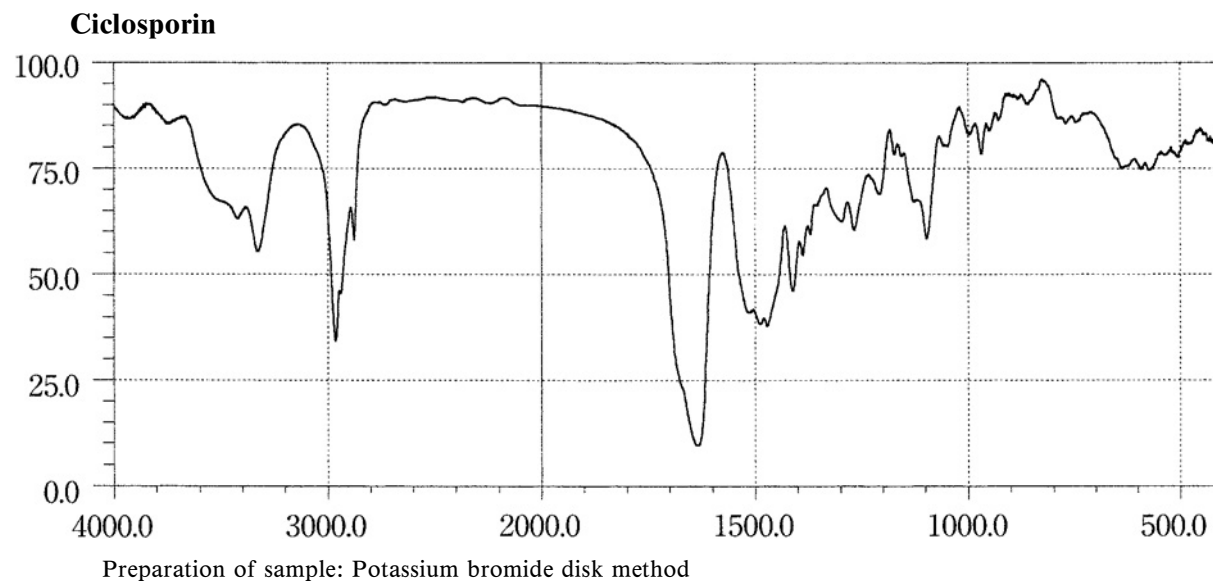
Chlorpheniramine Maleate

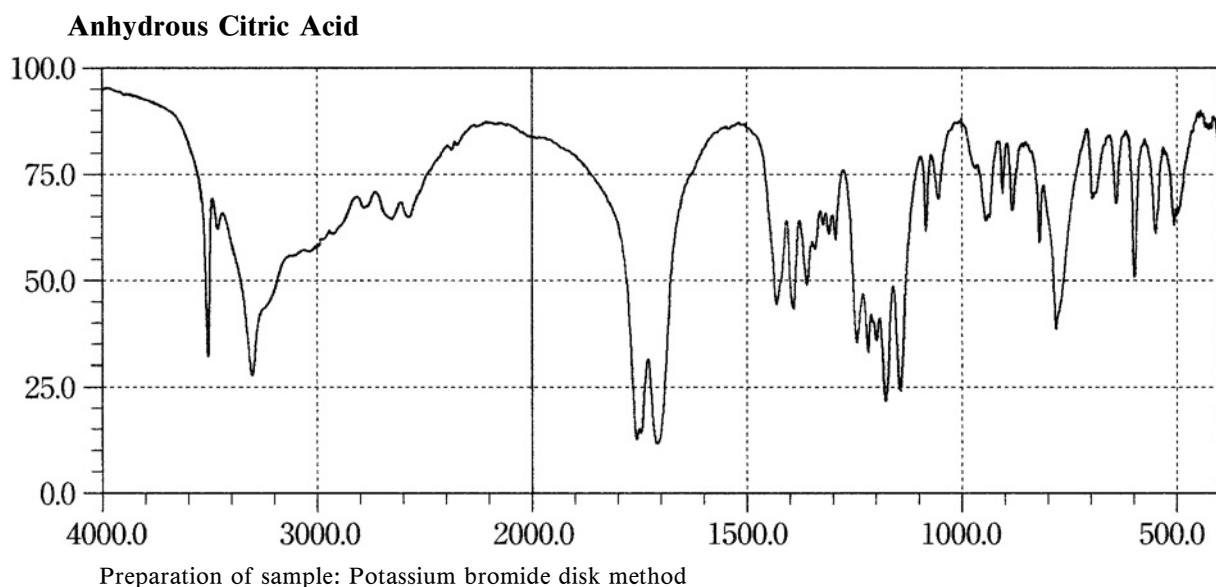
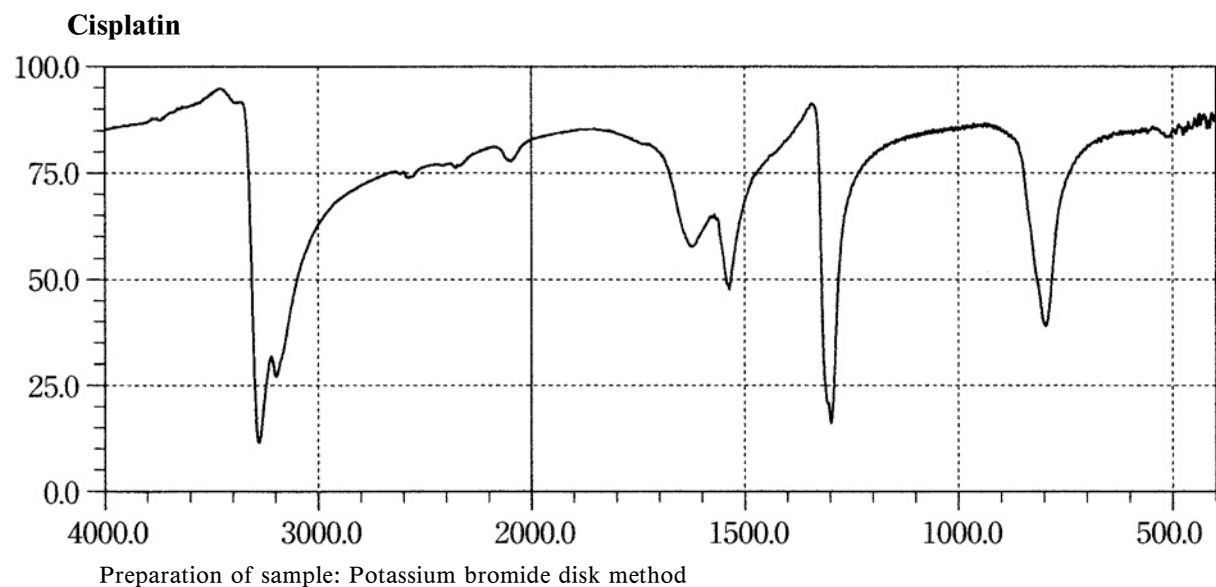
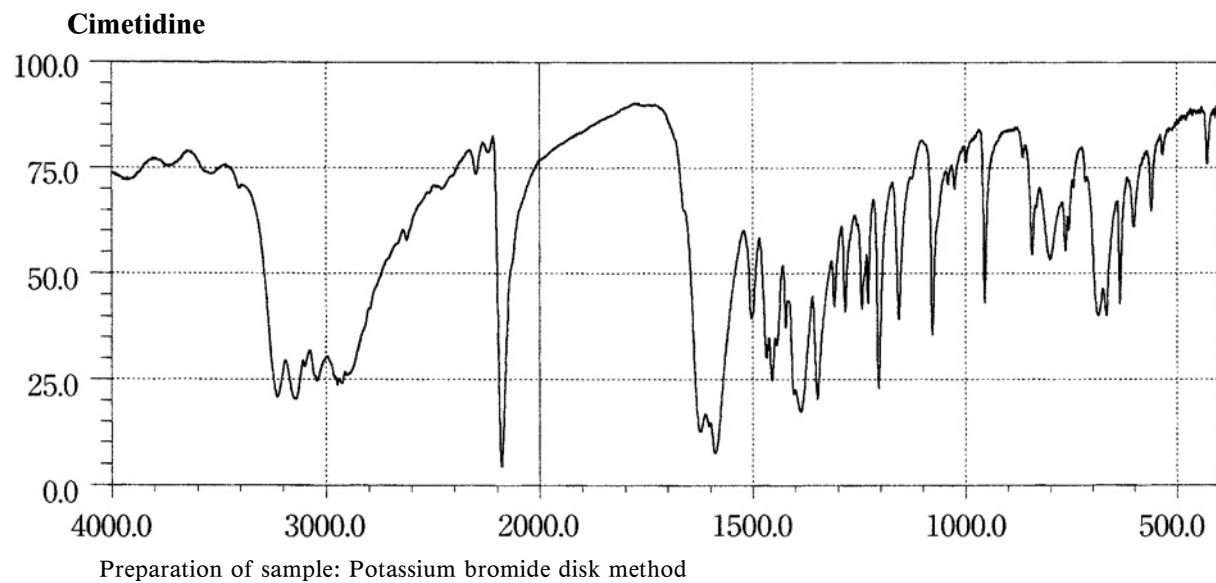
Preparation of sample: Paste method

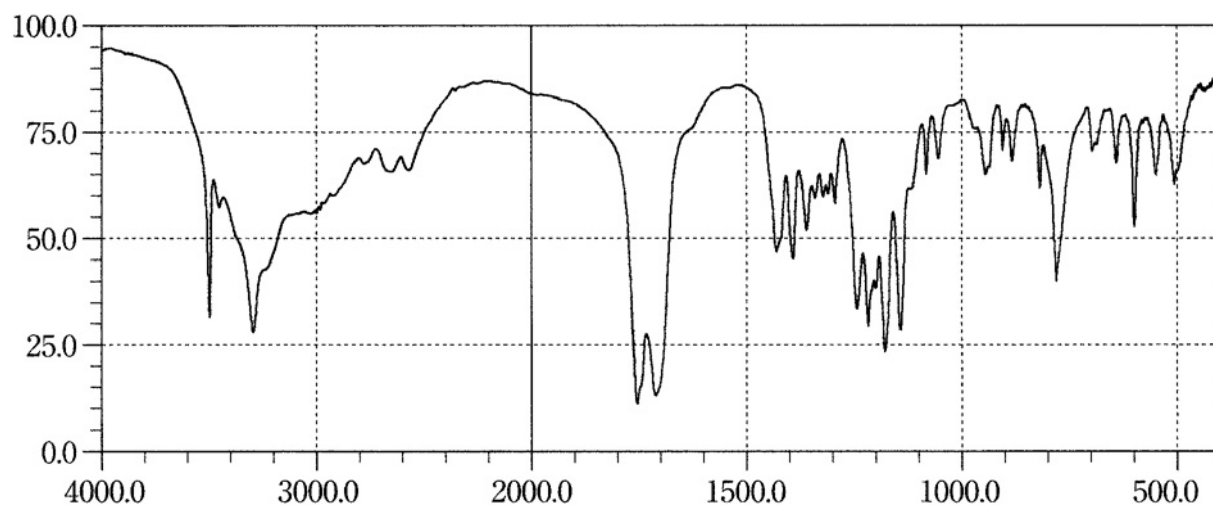
***d*-Chlorpheniramine Maleate**

Preparation of sample: Paste method

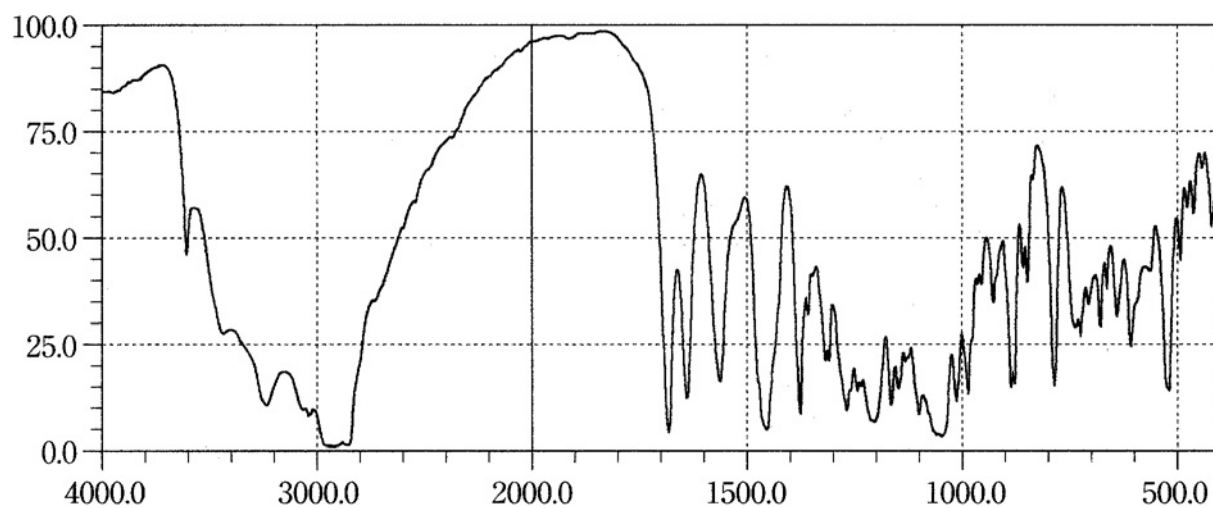




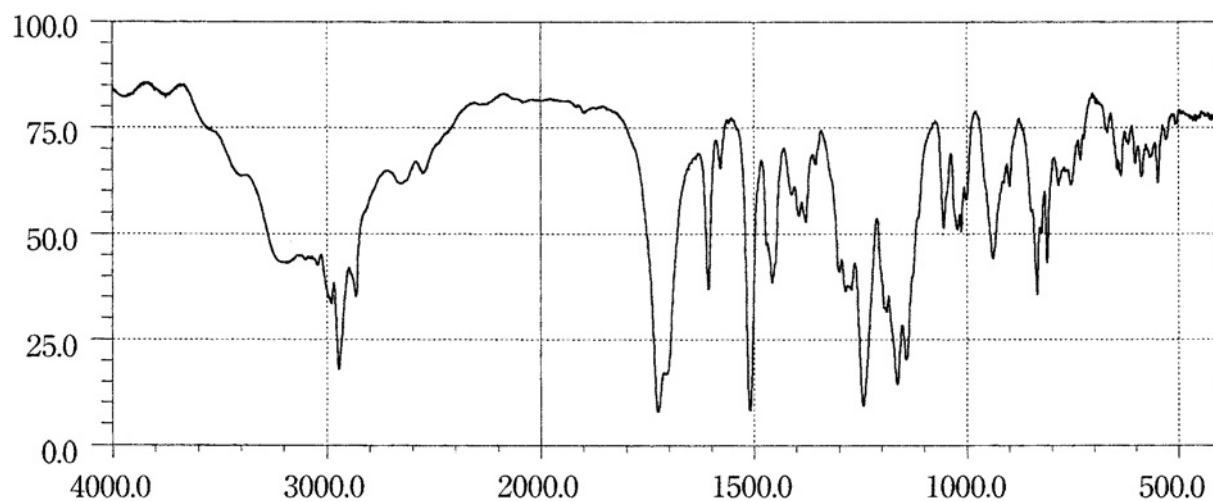


Citric Acid Hydrate

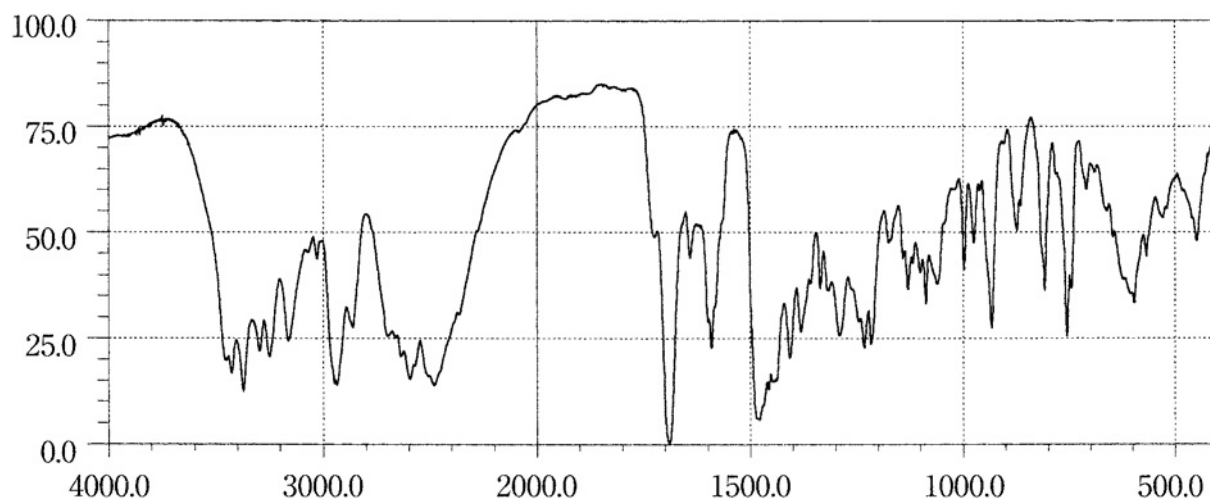
Preparation of sample: Potassium bromide disk method

Clindamycin Phosphate

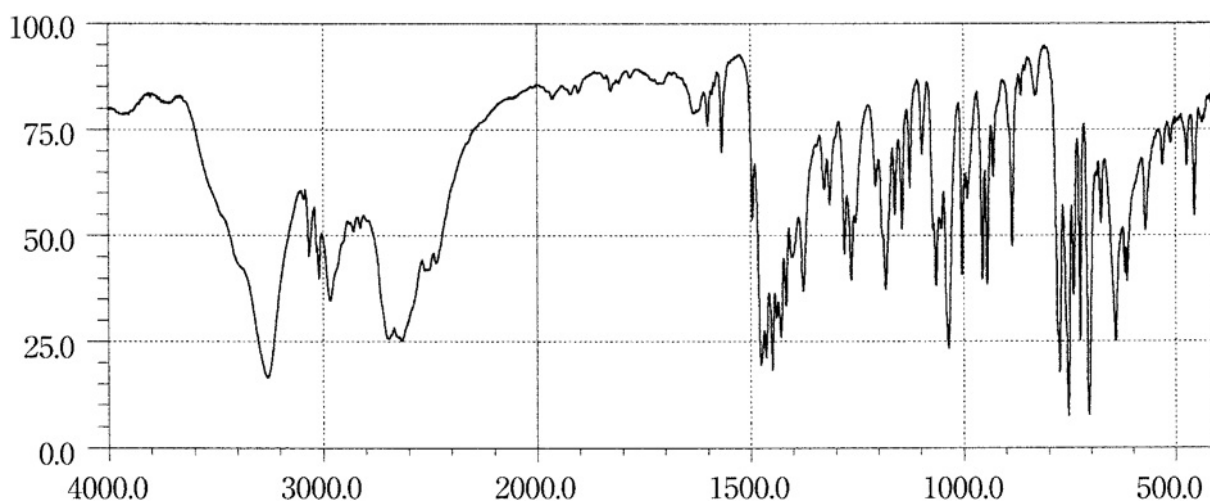
Preparation of sample: Paste method

Clinofibrate

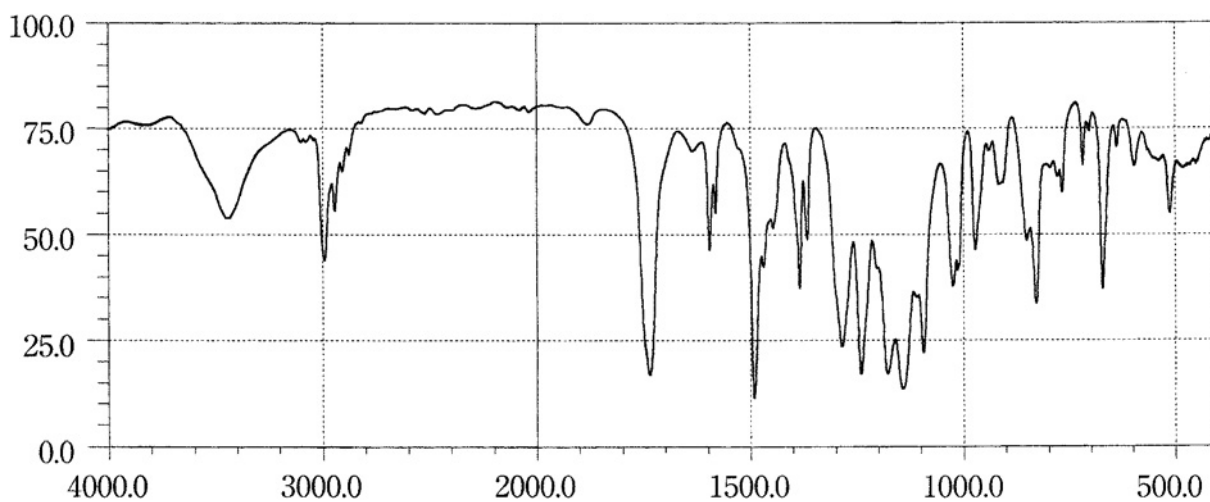
Preparation of sample: Potassium bromide disk method

Clocapramine Hydrochloride Hydrate

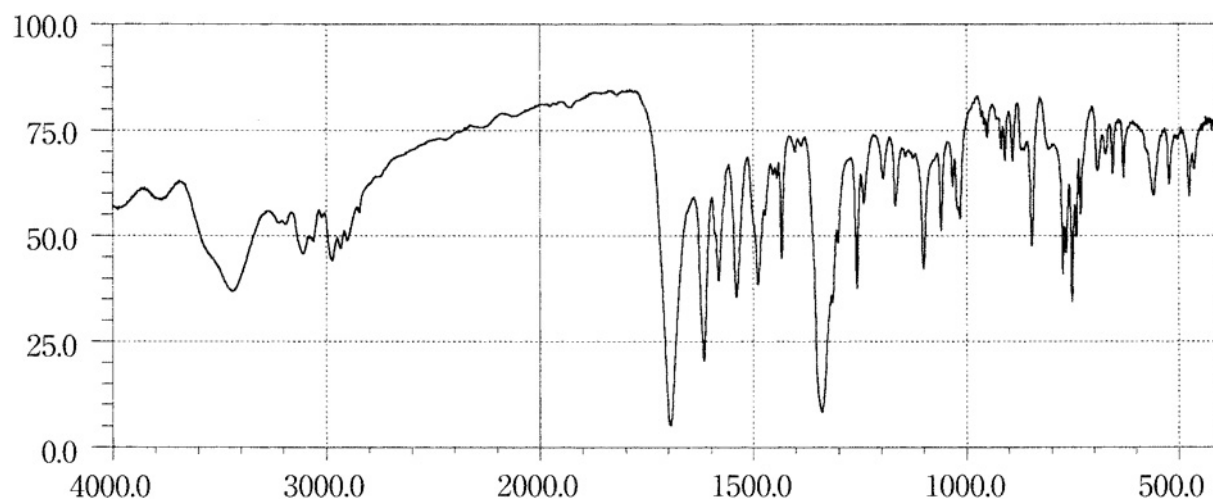
Preparation of sample: Potassium bromide disk method

Clofedanol Hydrochloride

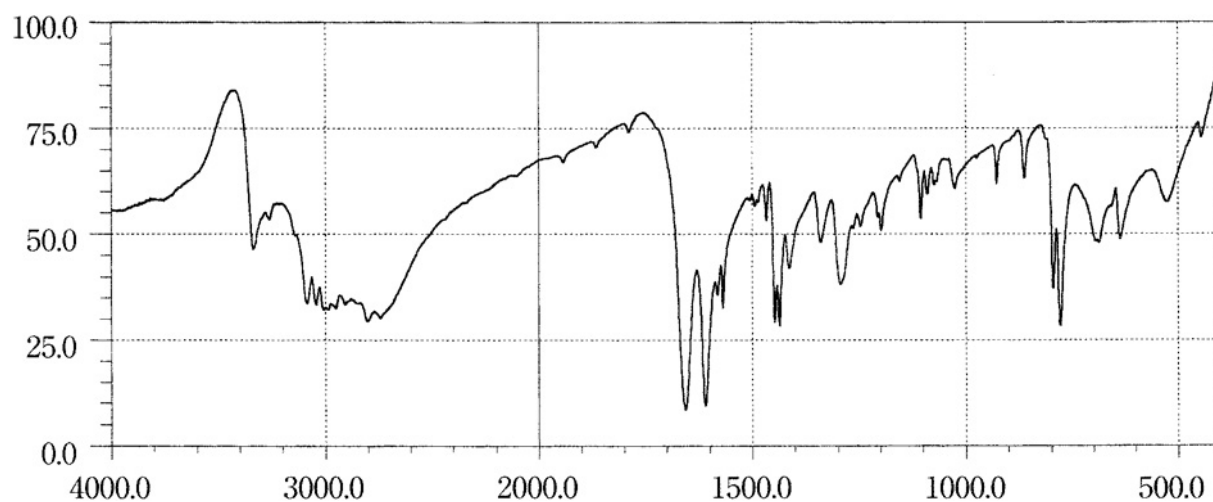
Preparation of sample: Potassium bromide disk method

Clofibrate

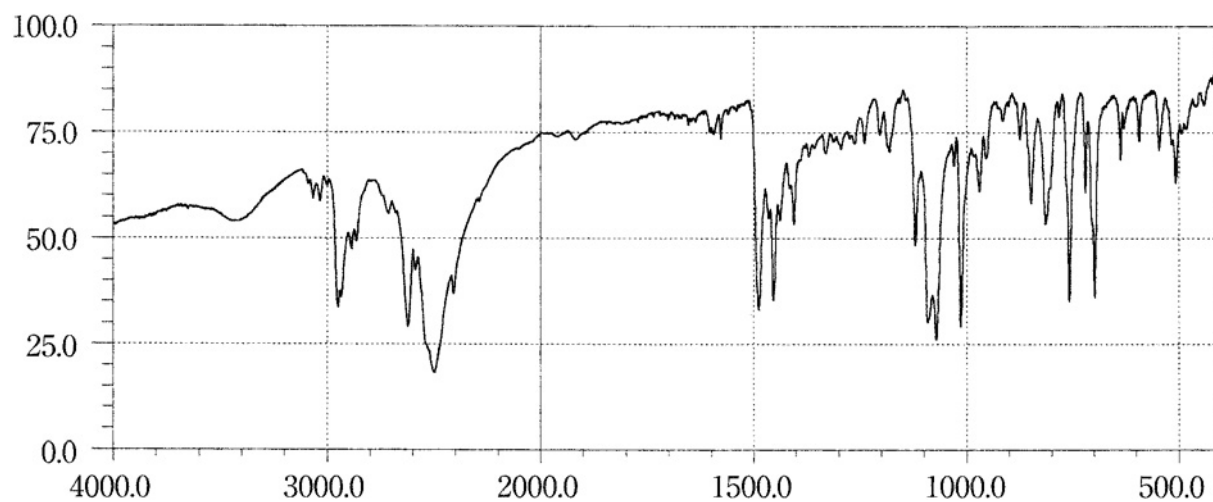
Preparation of sample: Liquid film method

Clonazepam

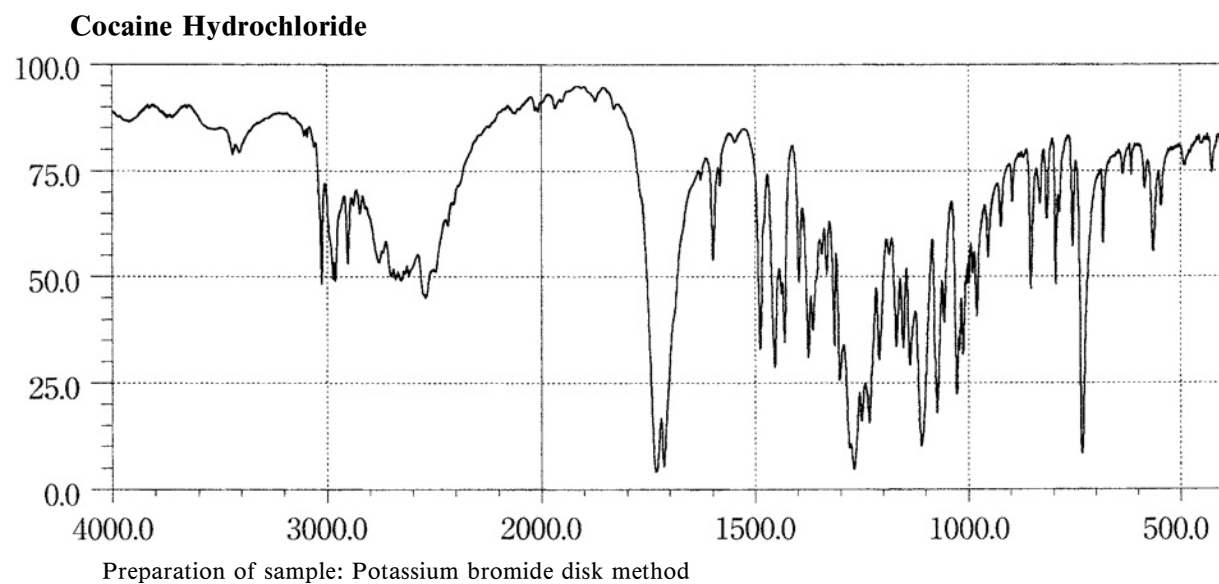
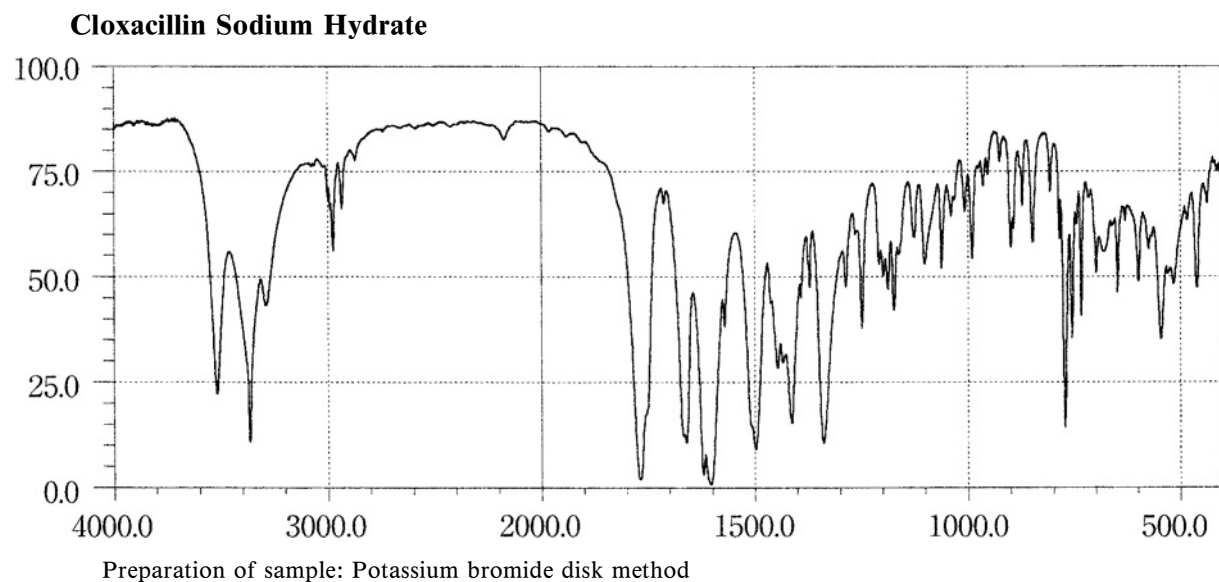
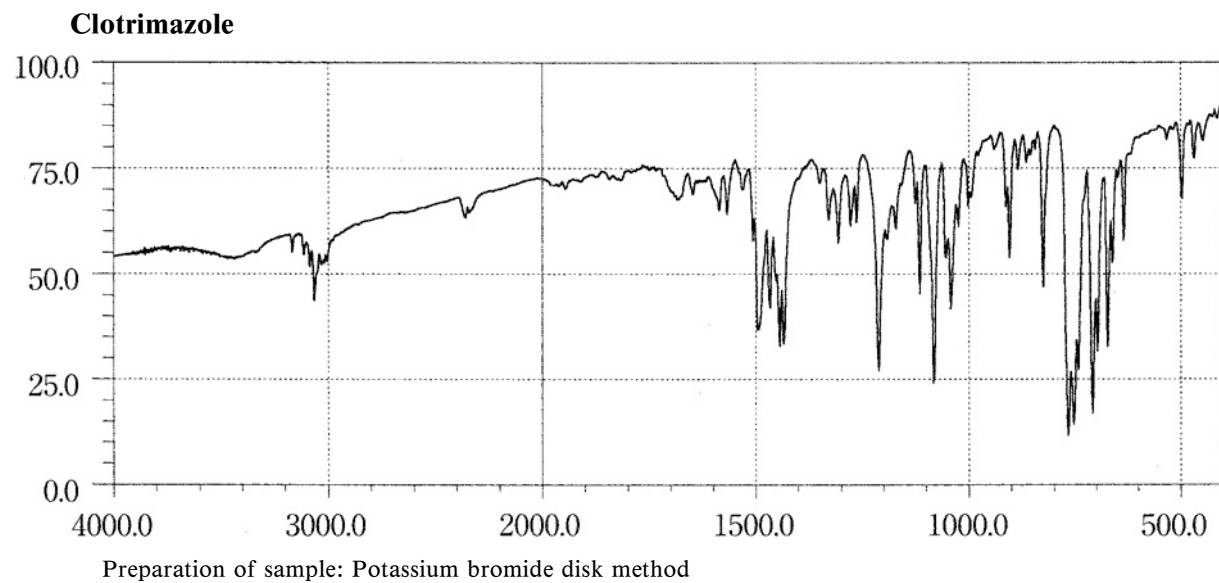
Preparation of sample: Potassium bromide disk method

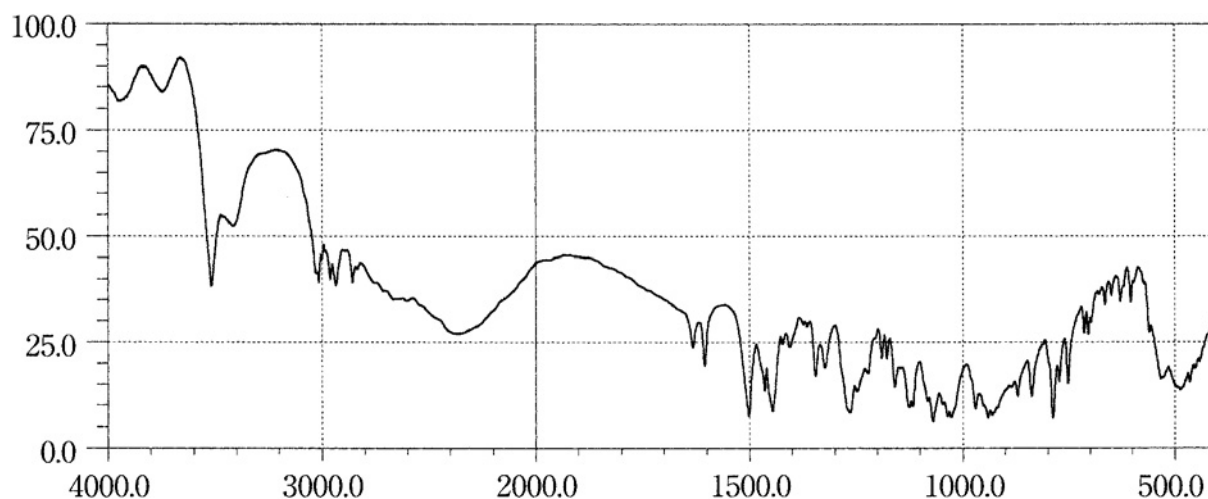
Clonidine Hydrochloride

Preparation of sample: Potassium chloride disk method

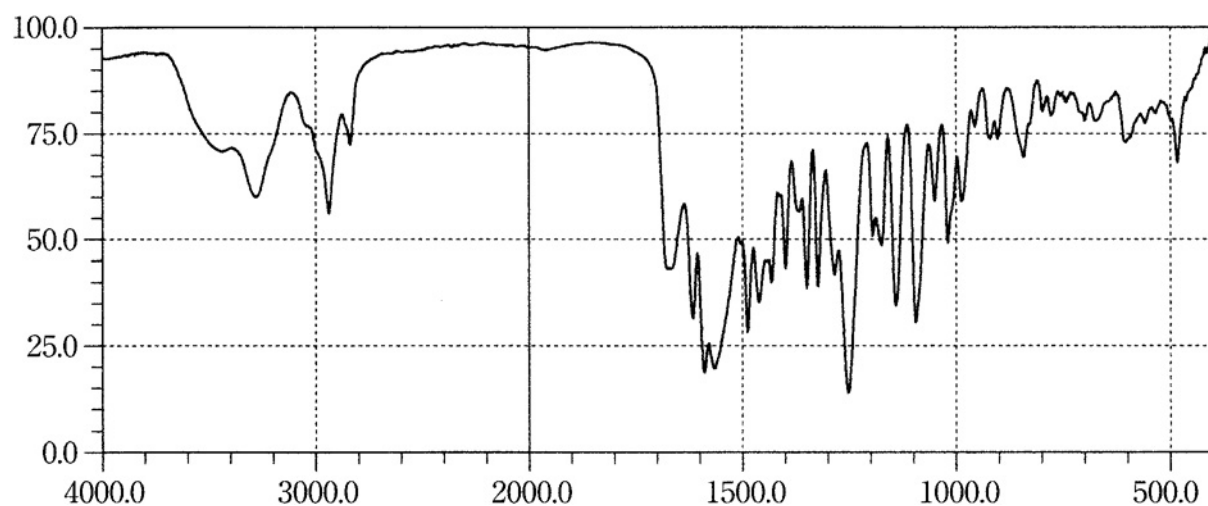
Cloperastine Hydrochloride

Preparation of sample: Potassium chloride disk method

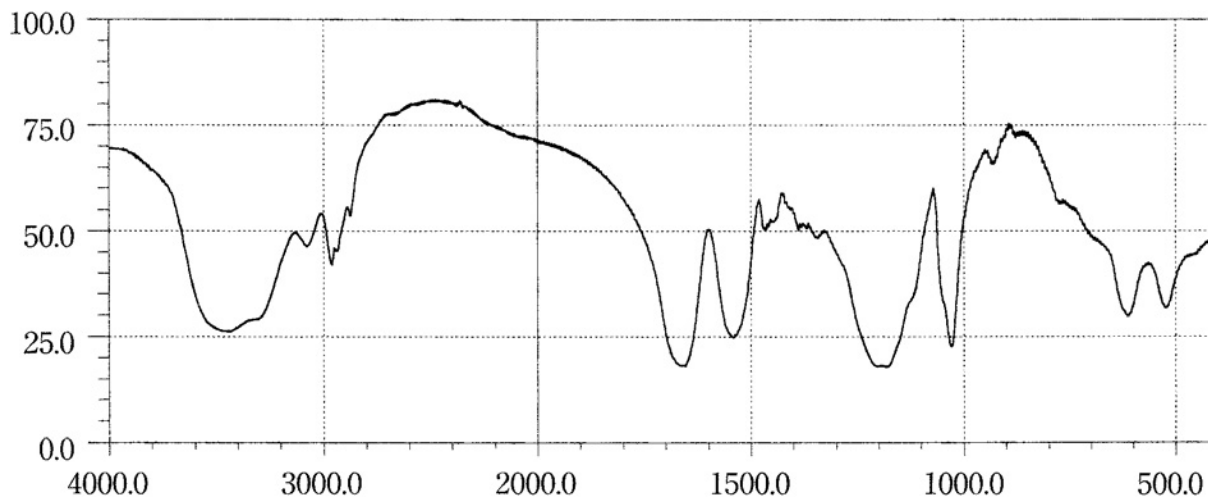


Codeine Phosphate Hydrate

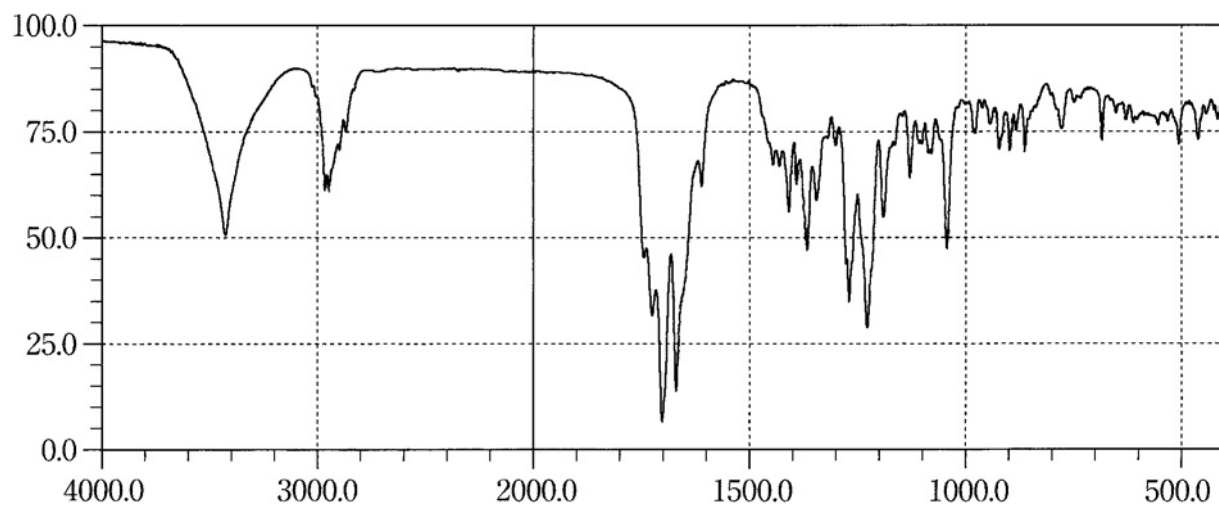
Preparation of sample: Potassium bromide disk method

Colchicine

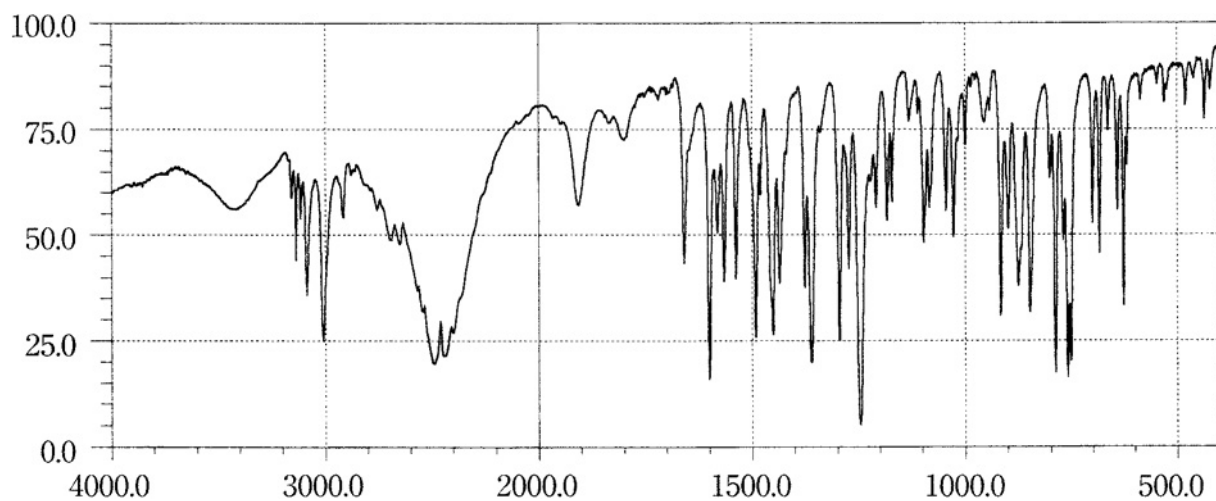
Preparation of sample: Potassium bromide disk method [To 0.5 mL of a solution of Colchicine in methanol (1 in 50) add 1 g of potassium bromide, and dry under reduced pressure (80°C, 1 hour).]

Colistin Sodium Methanesulfonate

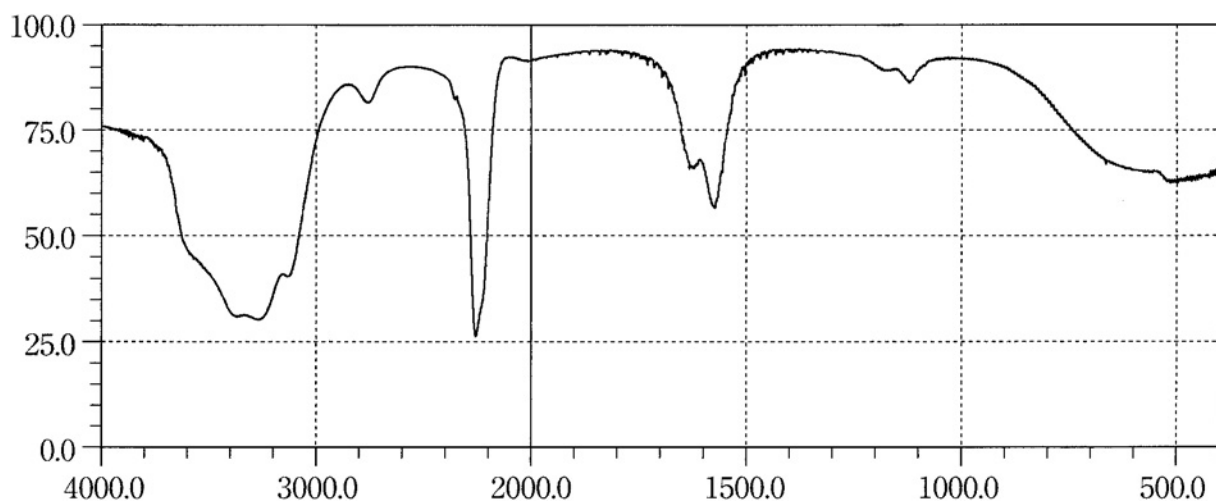
Preparation of sample: Potassium bromide disk method

Cortisone Acetate

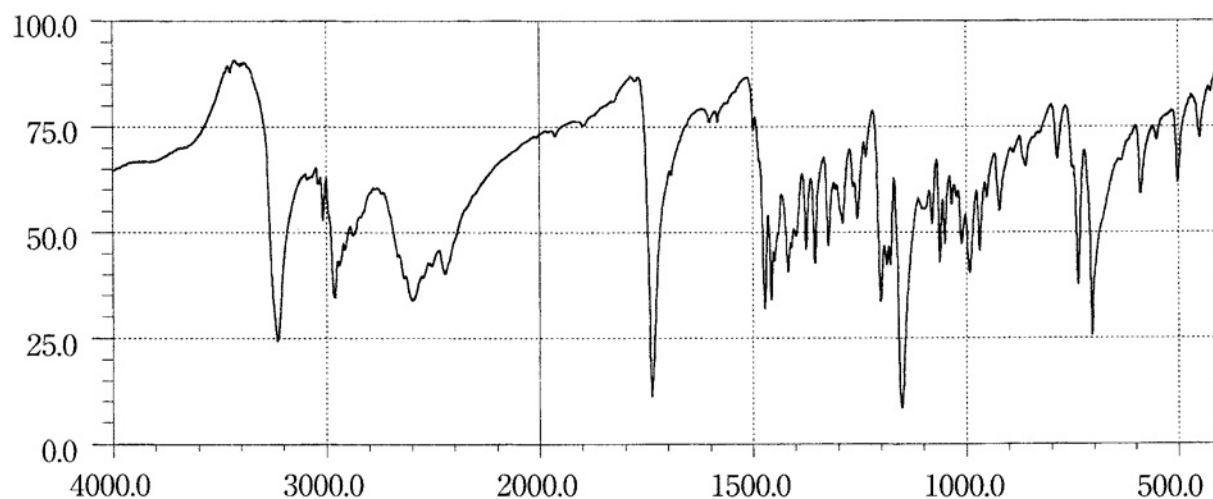
Preparation of sample: Potassium bromide disk method

Croconazole Hydrochloride

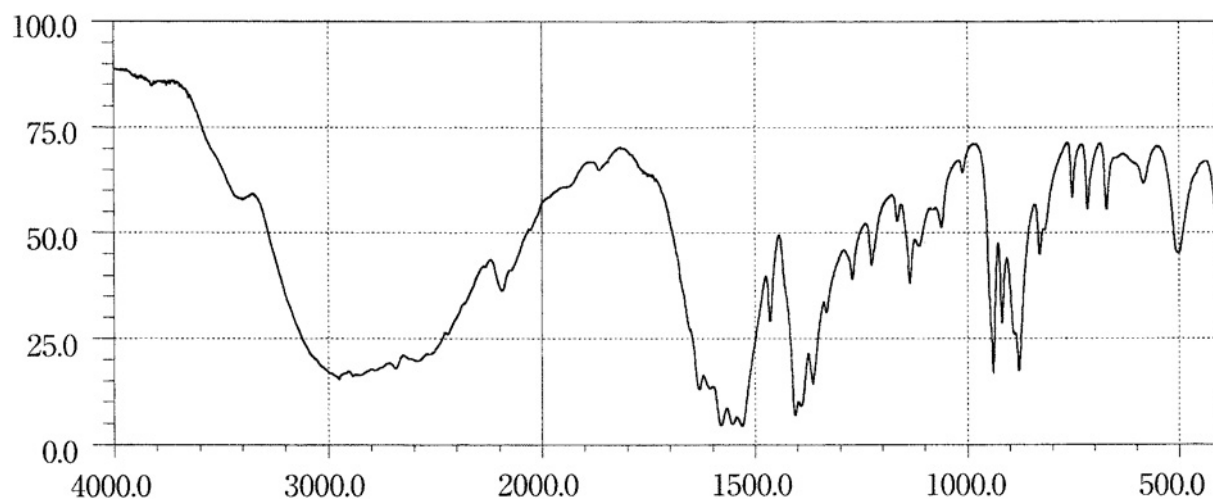
Preparation of sample: Potassium chloride disk method

Cyanamid

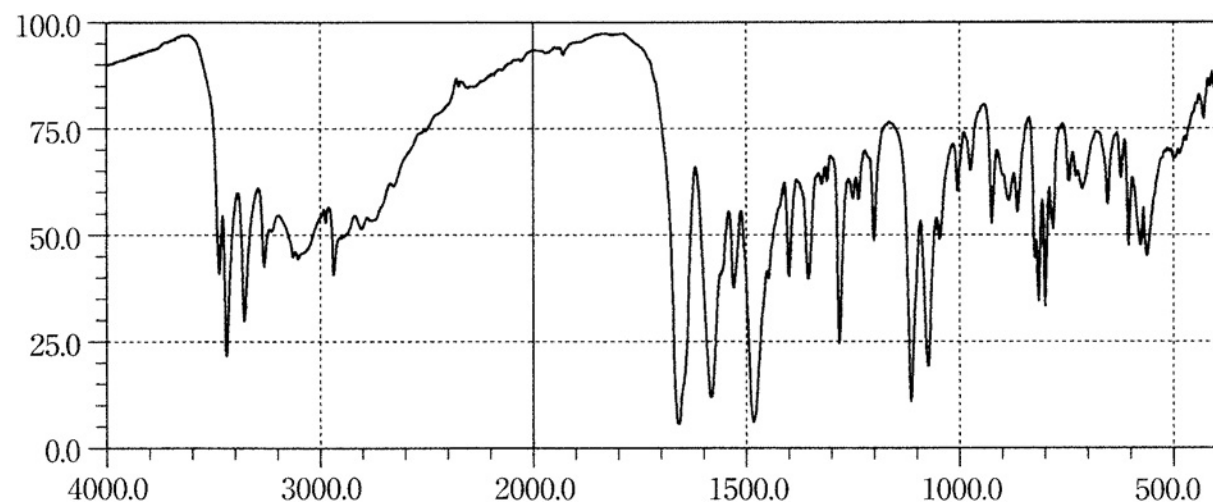
Preparation of sample: Film method (Drop one or two drops of a solution in acetone (1 in 100) onto a potassium bromide disk, and dry.)

Cyclopentolate Hydrochloride

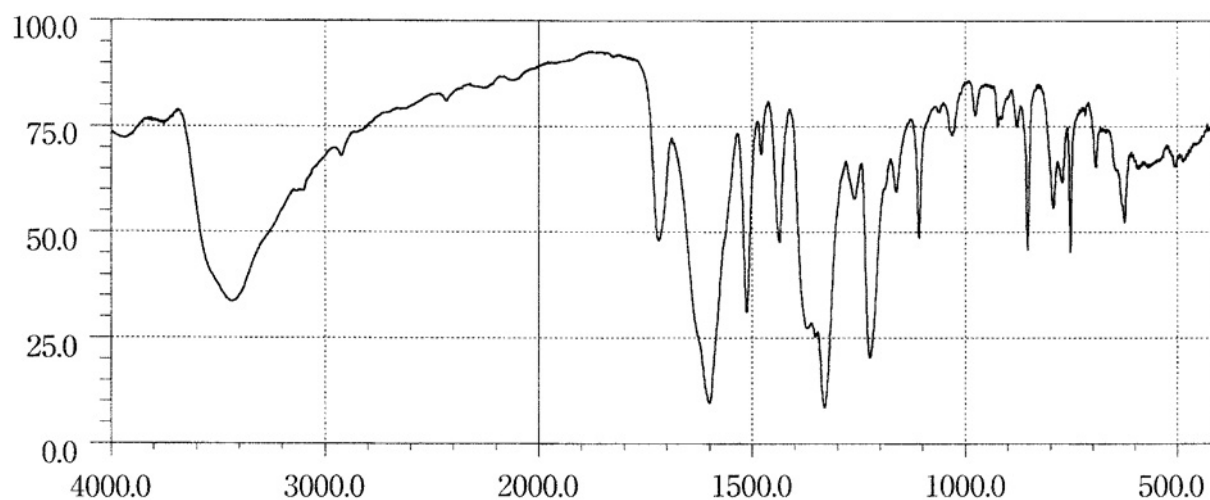
Preparation of sample: Potassium chloride disk method

Cycloserine

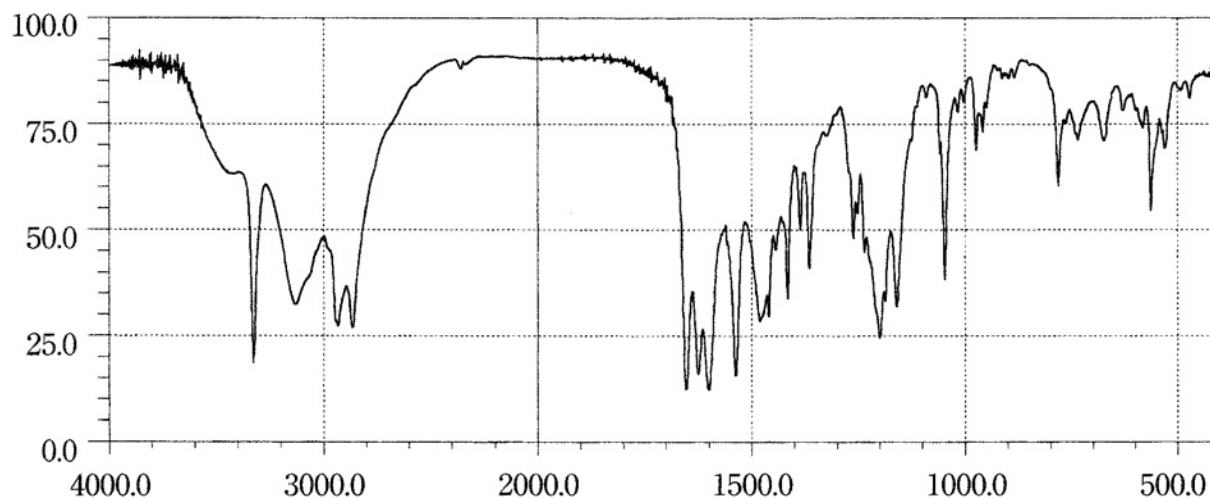
Preparation of sample: Potassium bromide disk method

Cytarabine

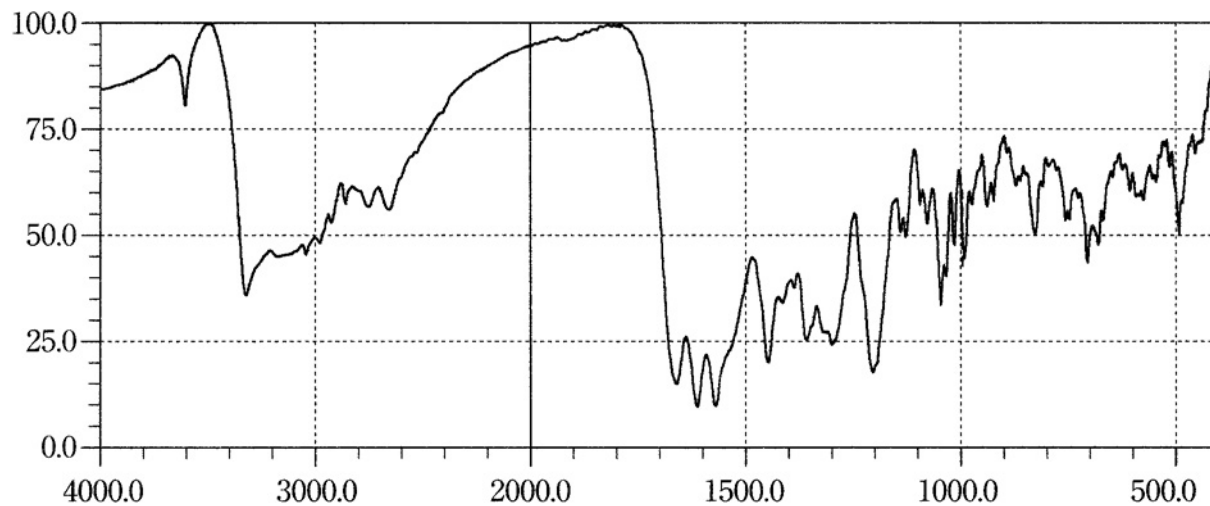
Preparation of sample: Potassium bromide disk method

Dantrolene Sodium Hydrate

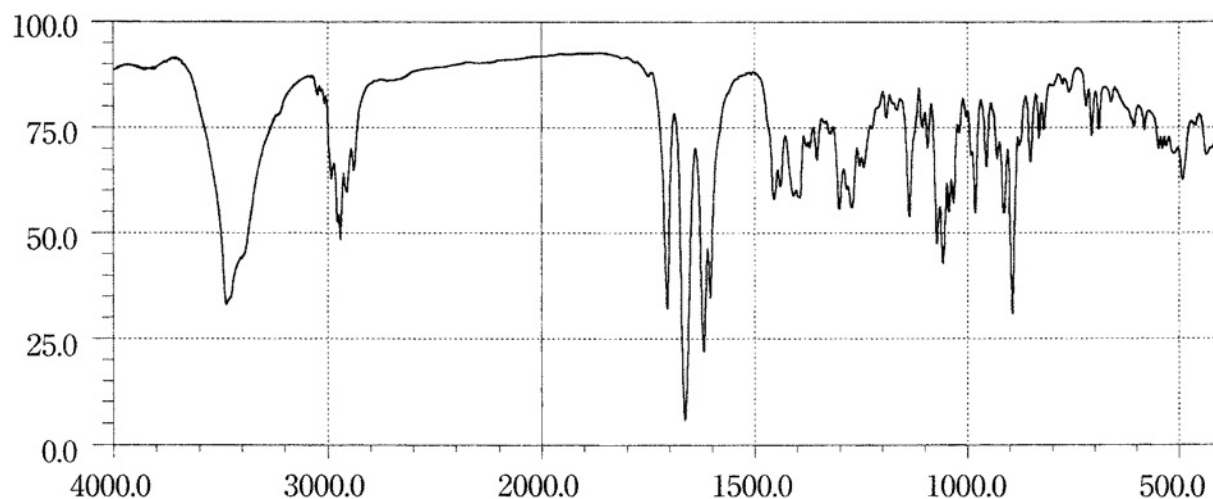
Preparation of sample: Potassium bromide disk method

Deferoxamine Mesilate

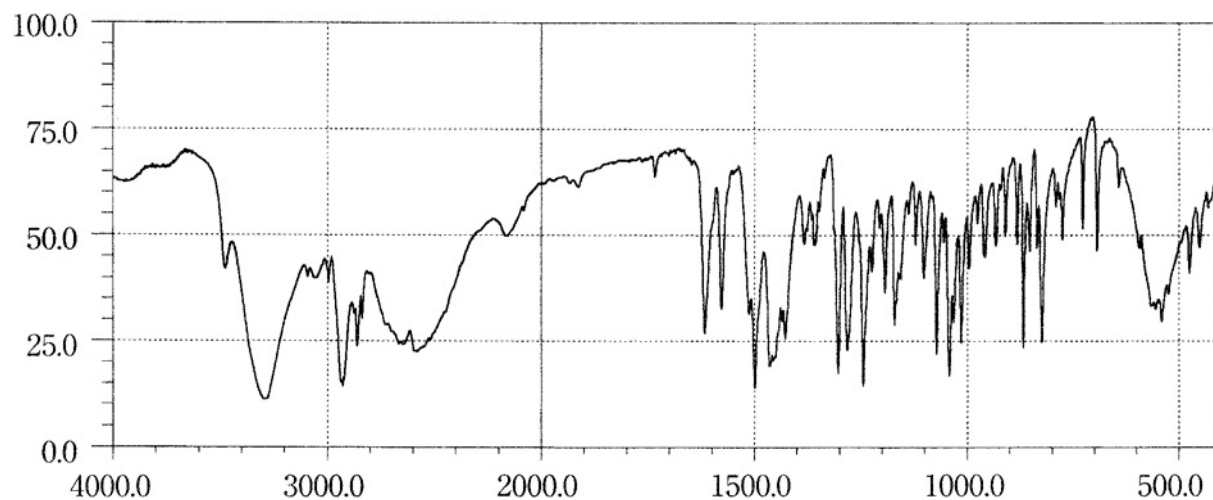
Preparation of sample: Potassium bromide disk method

Demethylchlortetracycline Hydrochloride

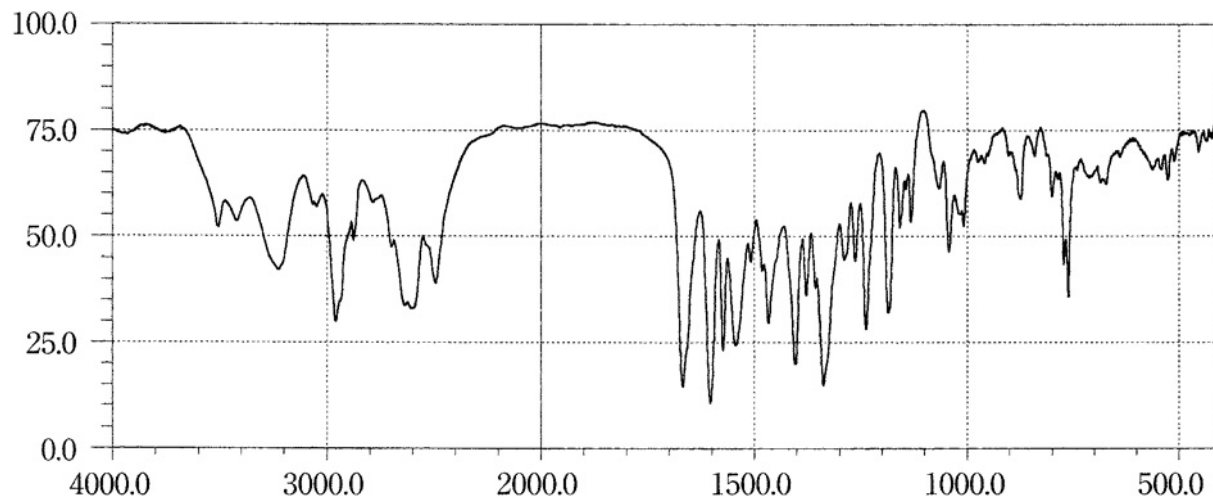
Preparation of sample: Potassium chloride disk method

Dexamethasone

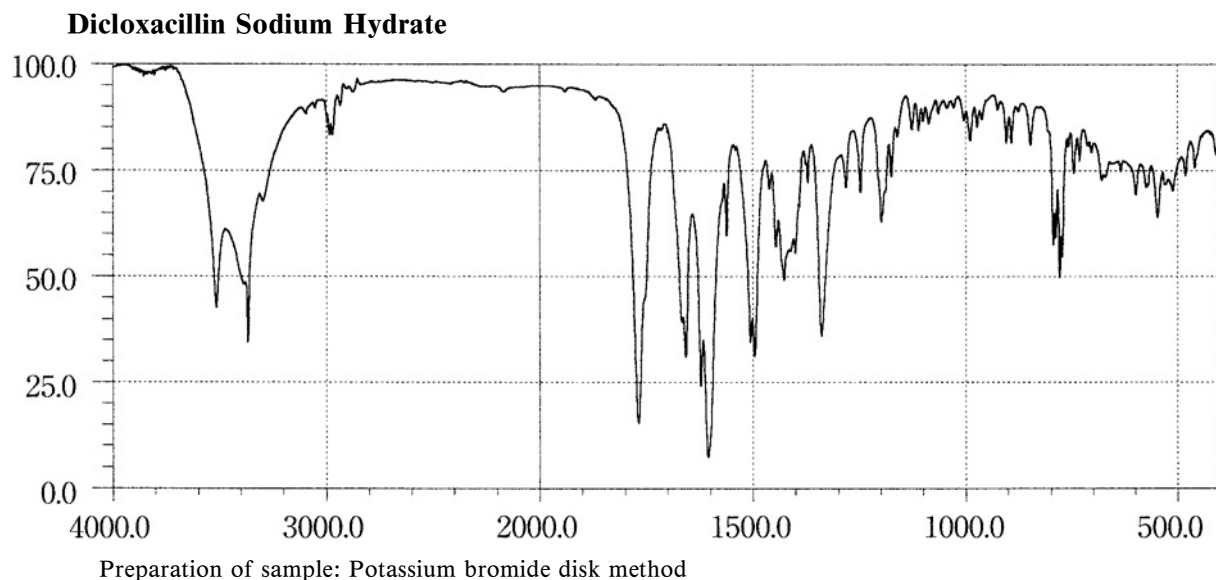
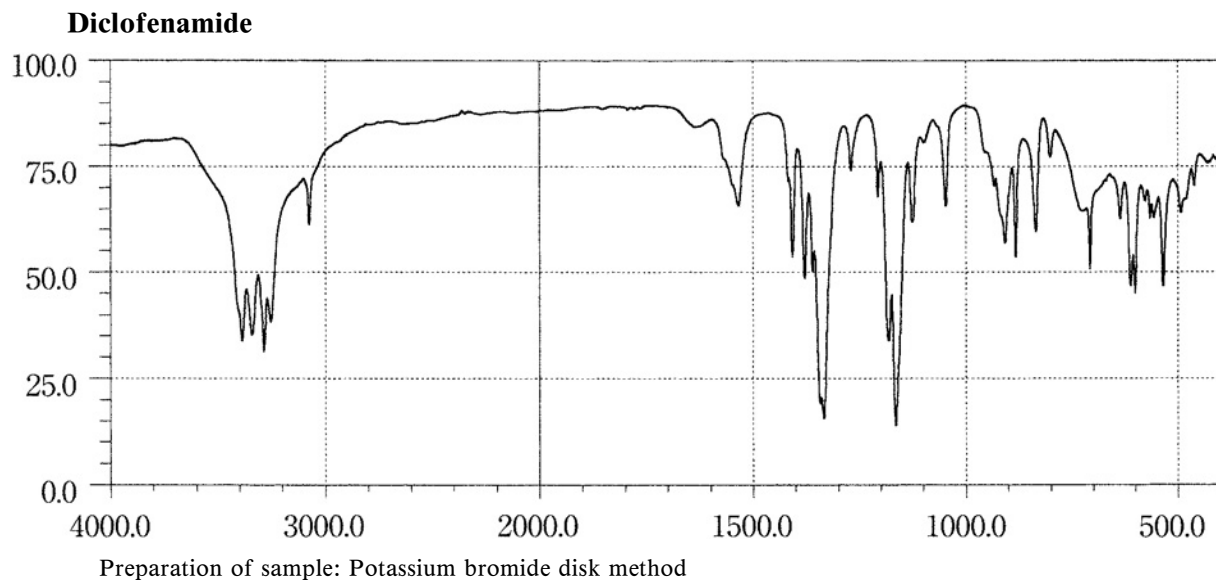
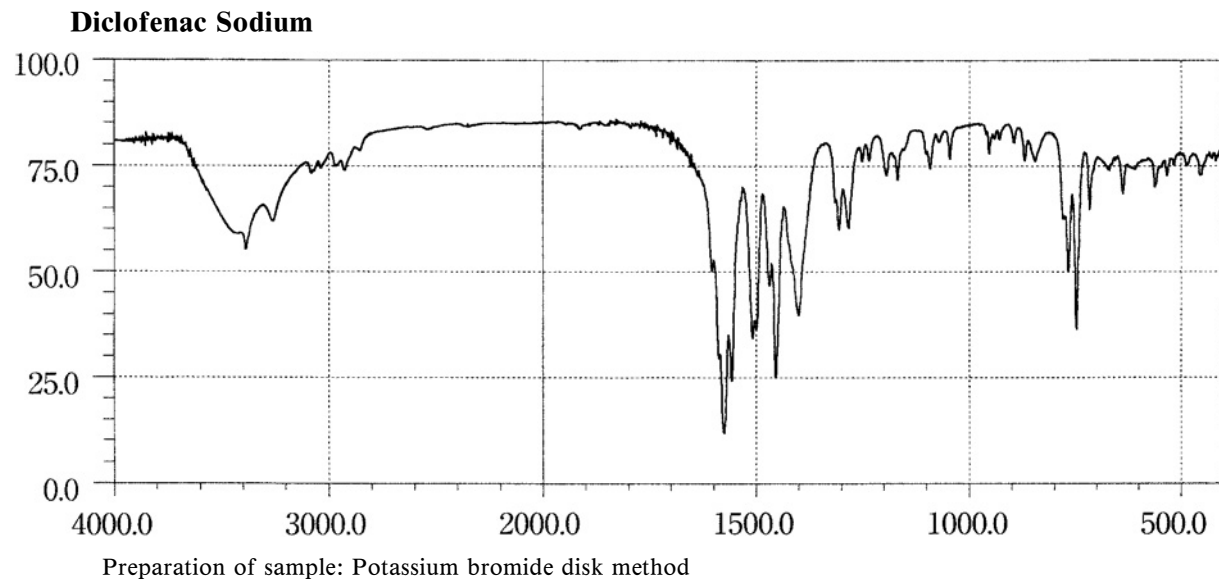
Preparation of sample: Potassium bromide disk method

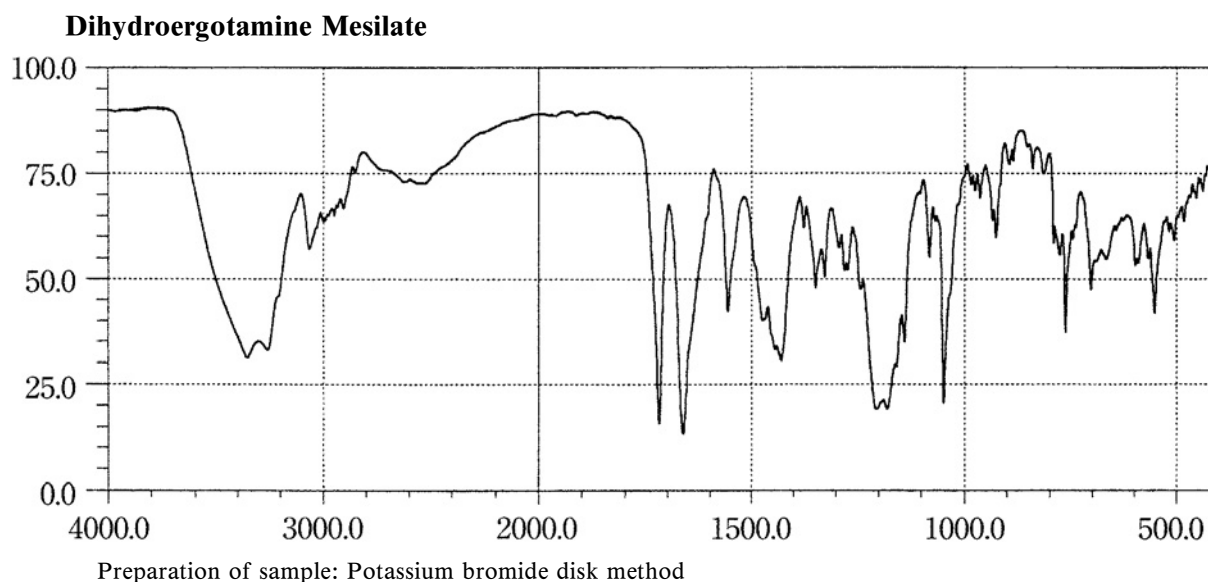
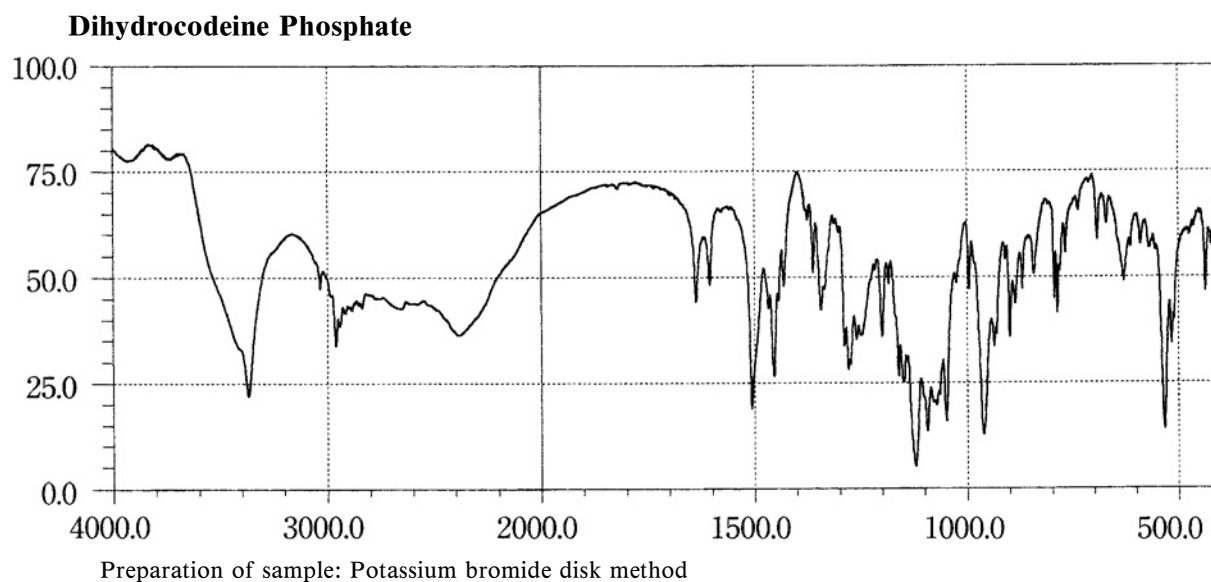
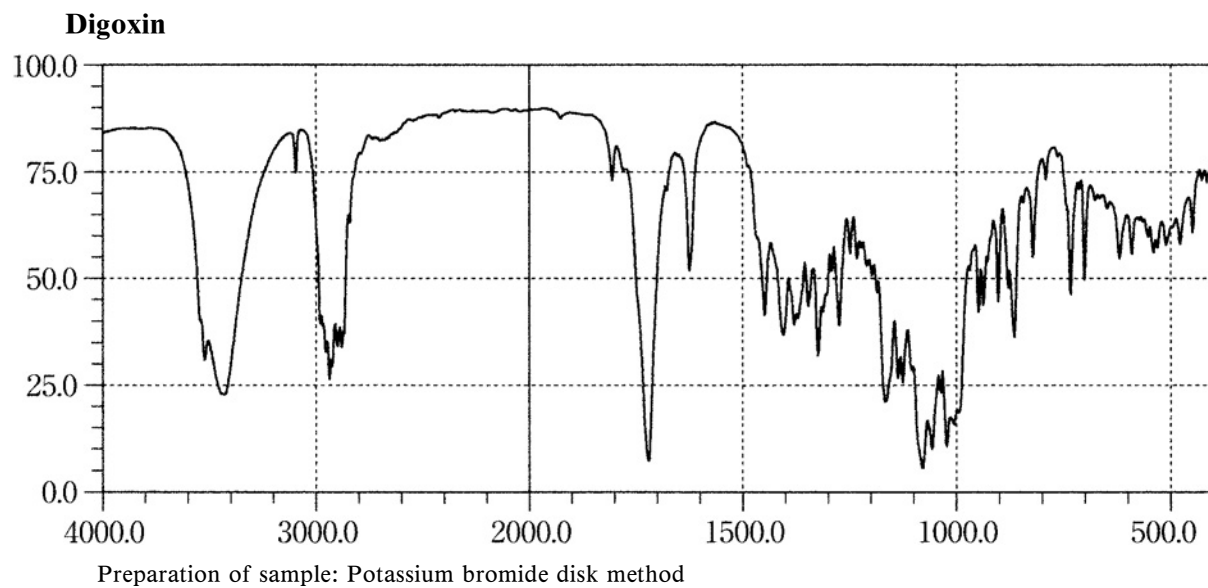
Dextromethorphan Hydrobromide Hydrate

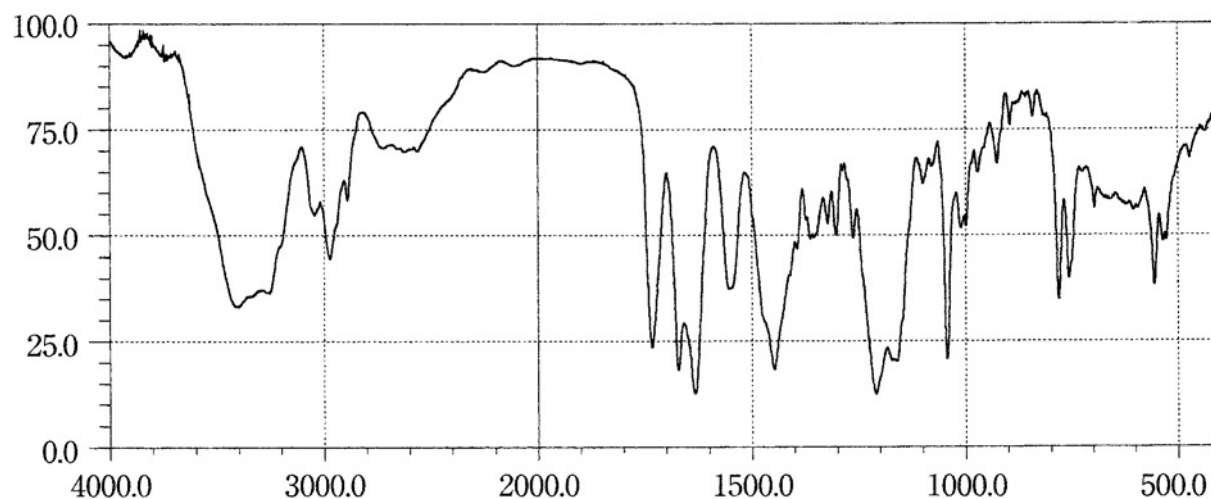
Preparation of sample: Potassium bromide disk method

Dibucaine Hydrochloride

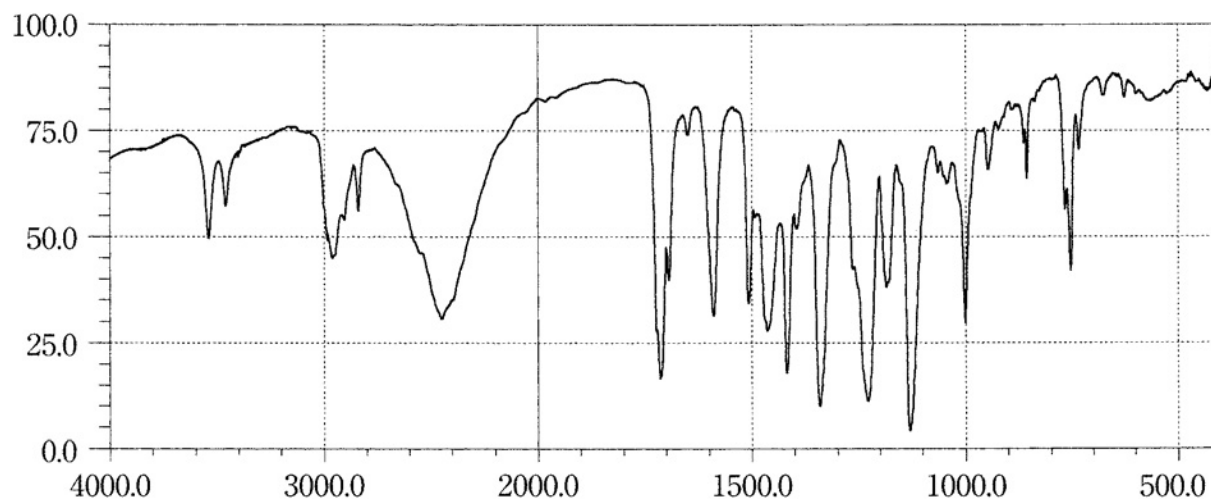
Preparation of sample: Potassium bromide disk method



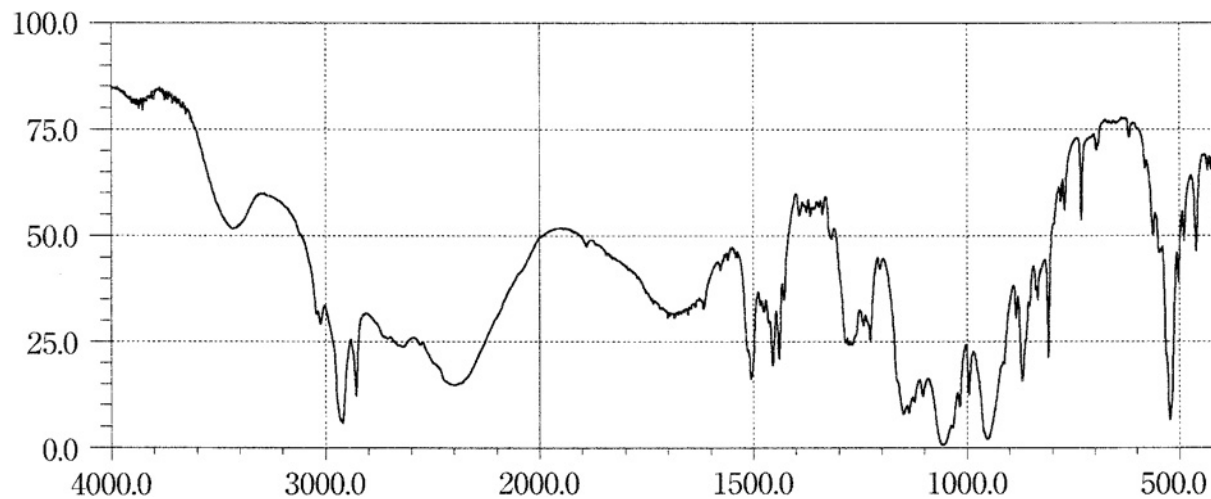


Dihydroergotoxine Mesilate

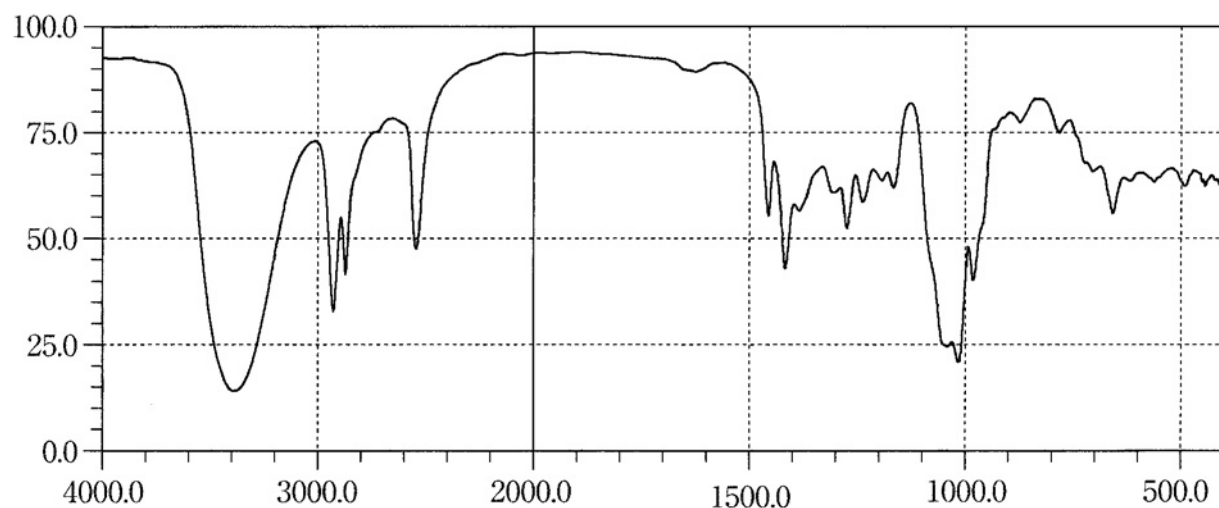
Preparation of sample: Potassium bromide disk method

Dilazep Hydrochloride Hydrate

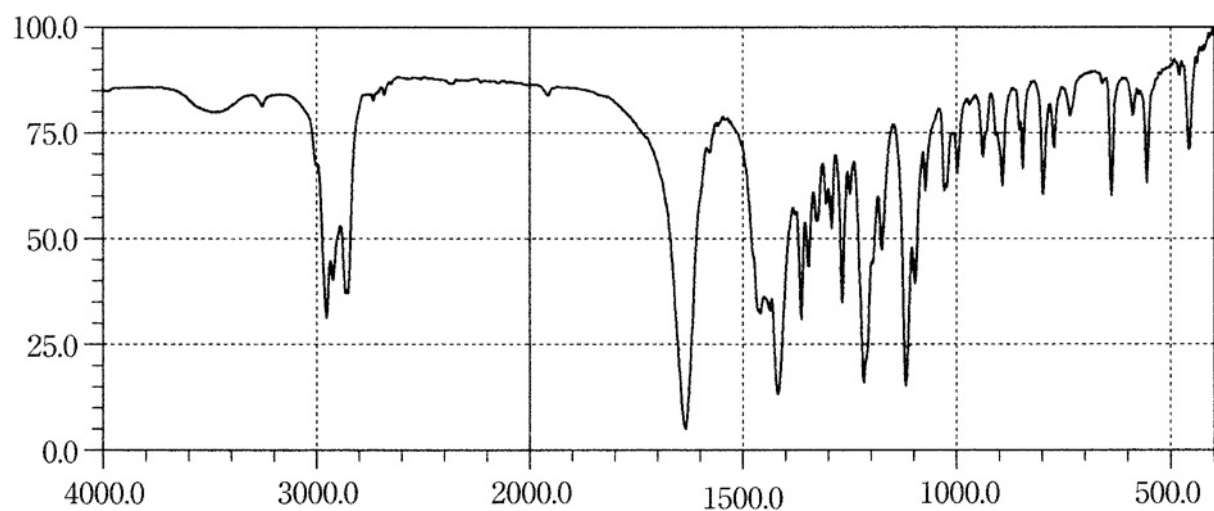
Preparation of sample: Potassium chloride disk method

Dimemorfan Phosphate

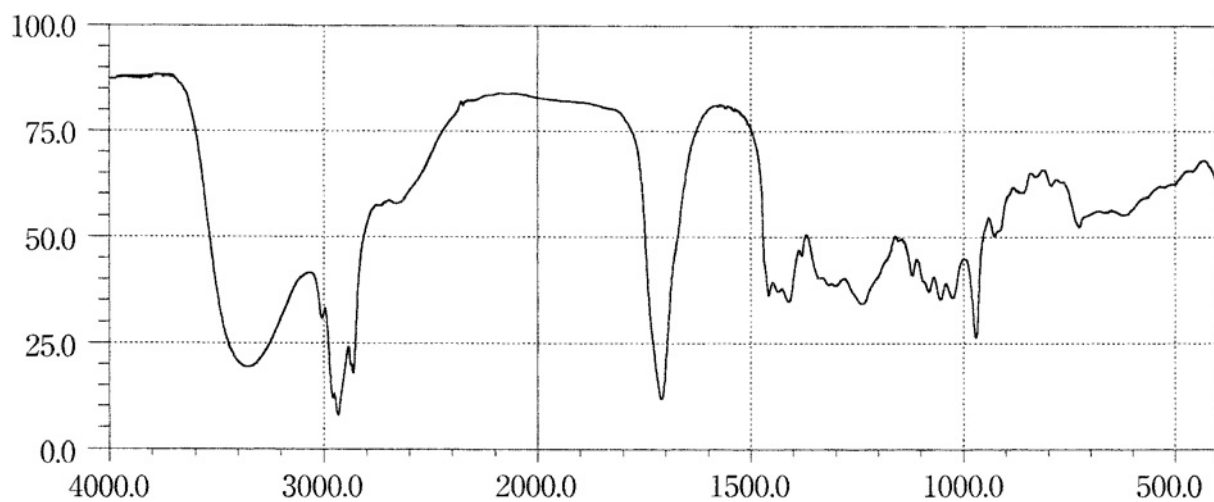
Preparation of sample: Potassium bromide disk method

Dimercaprol

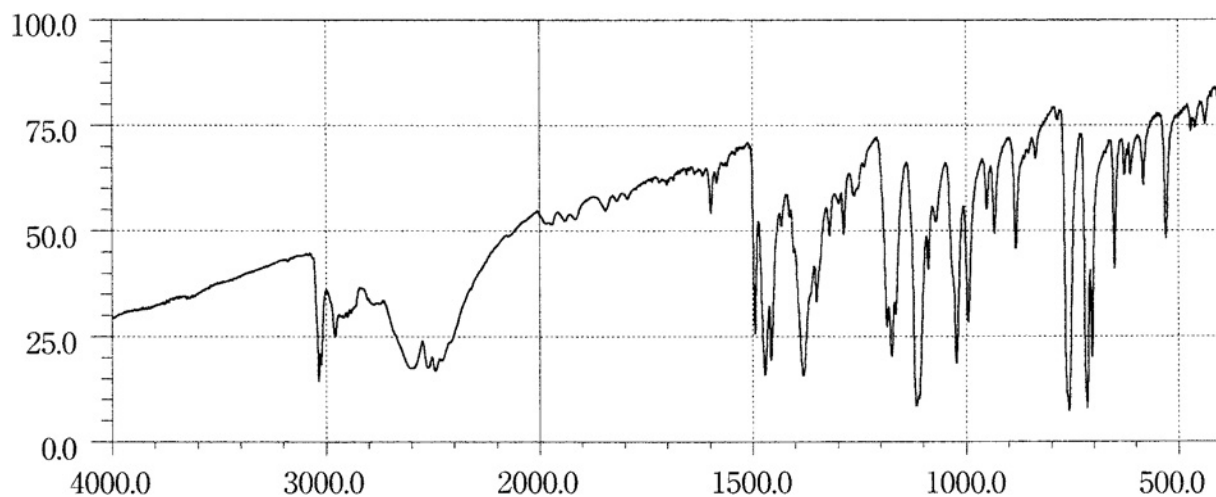
Preparation of sample: Liquid film method

Dimorpholamine

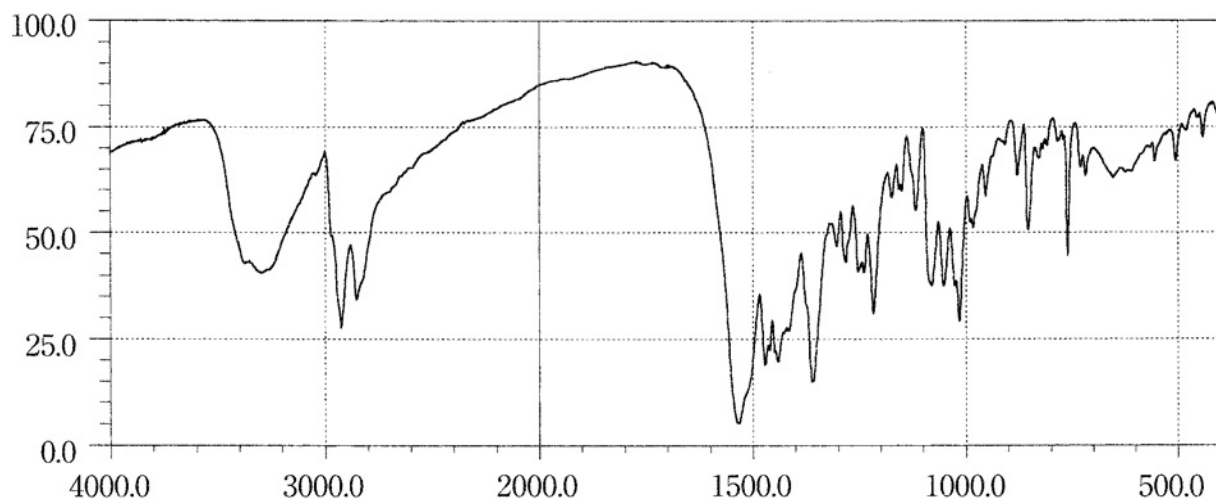
Preparation of sample: Potassium bromide disk method

Dinoprost

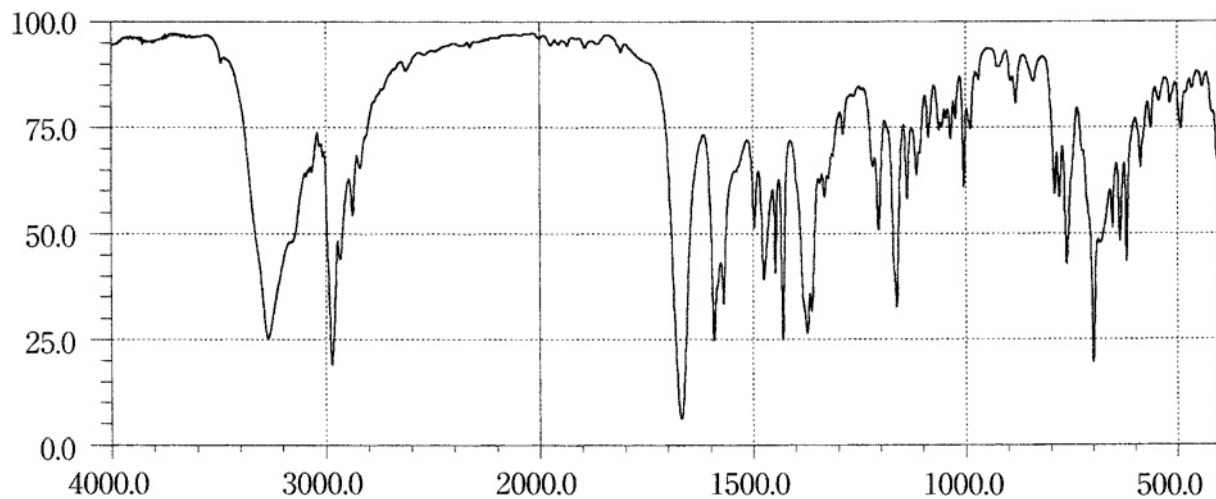
Preparation of sample: Liquid film method

Diphenhydramine Hydrochloride

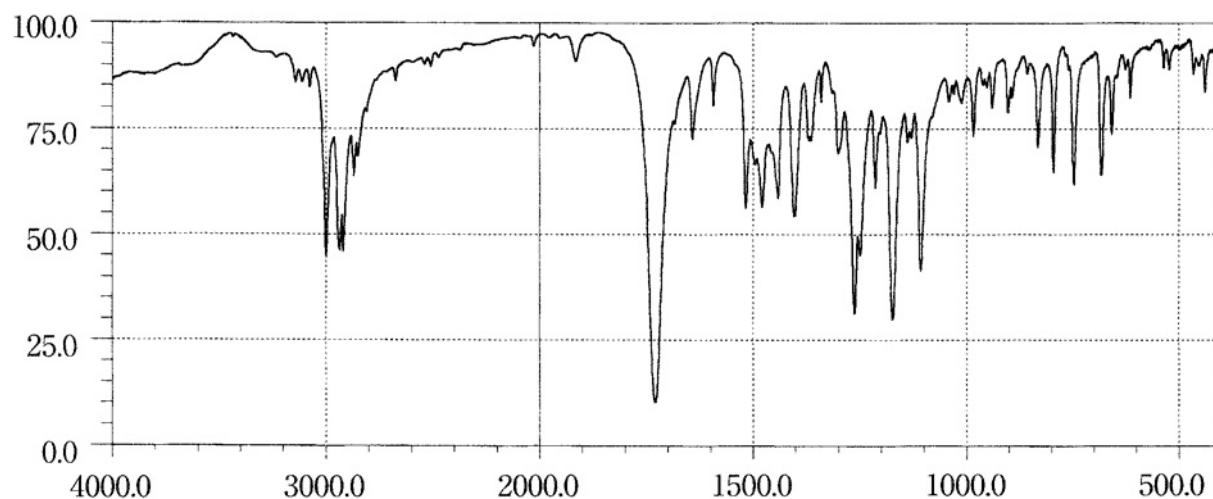
Preparation of sample: Potassium chloride disk method

Dipyridamole

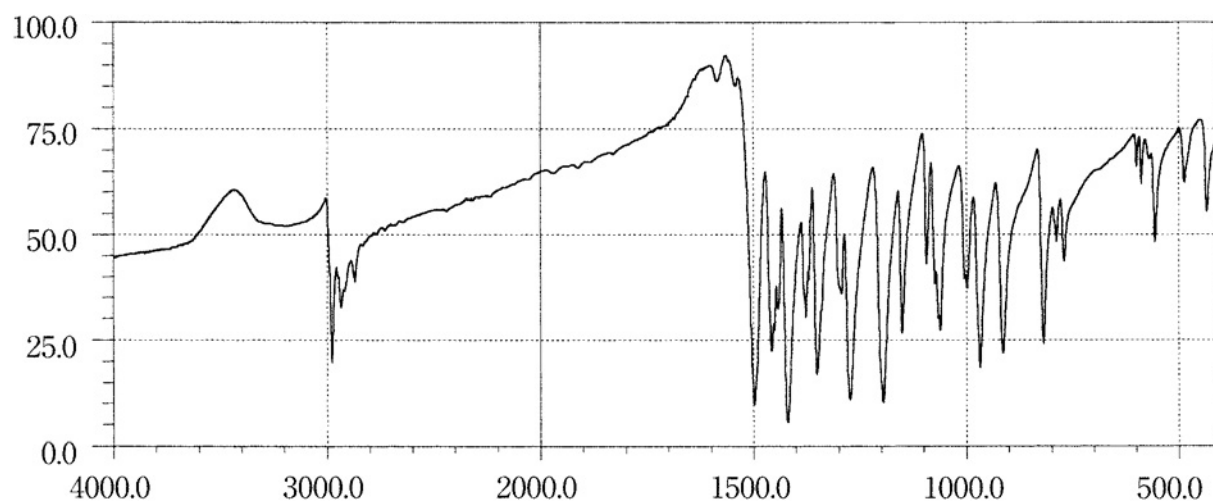
Preparation of sample: Potassium bromide disk method

Disopyramide

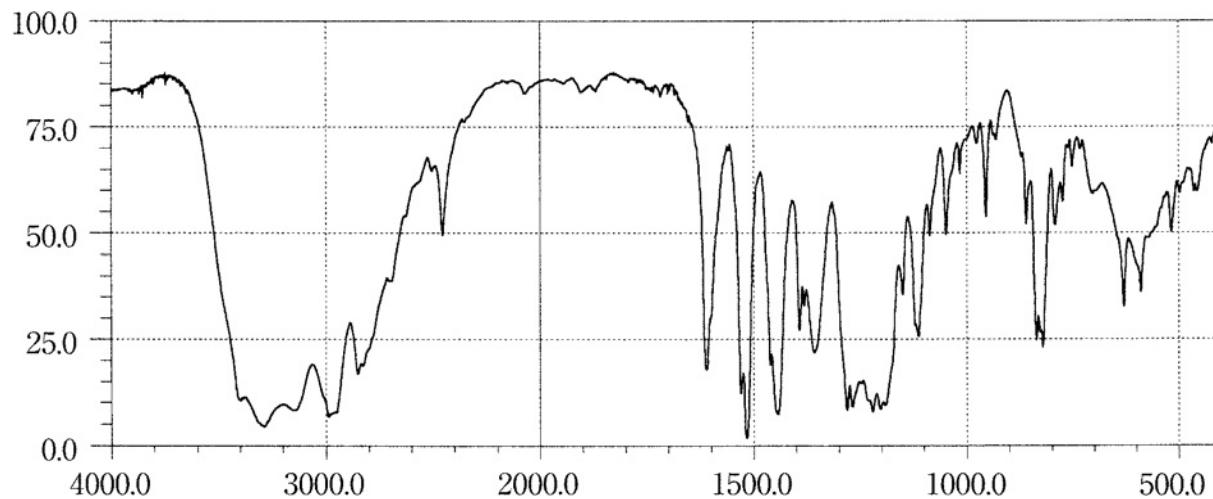
Preparation of sample: Potassium bromide disk method

Distigmine Bromide

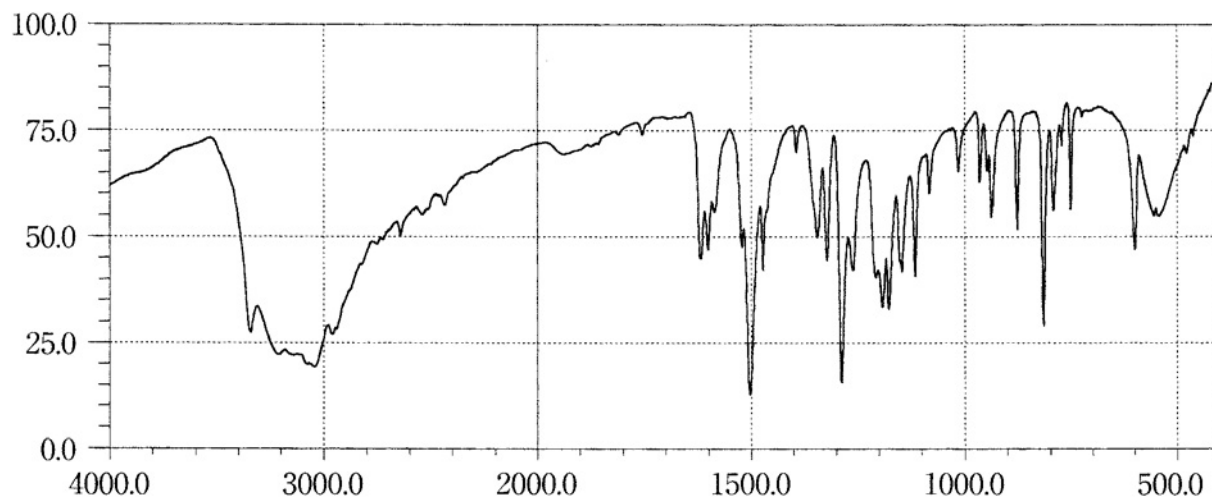
Preparation of sample: Potassium bromide disk method

Disulfiram

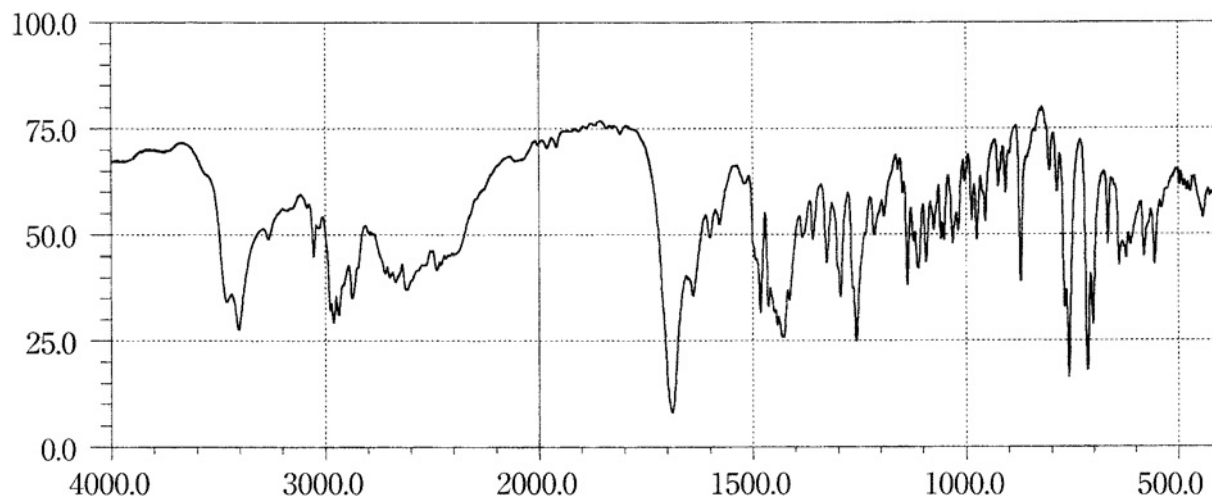
Preparation of sample: Potassium bromide disk method

Dobutamine Hydrochloride

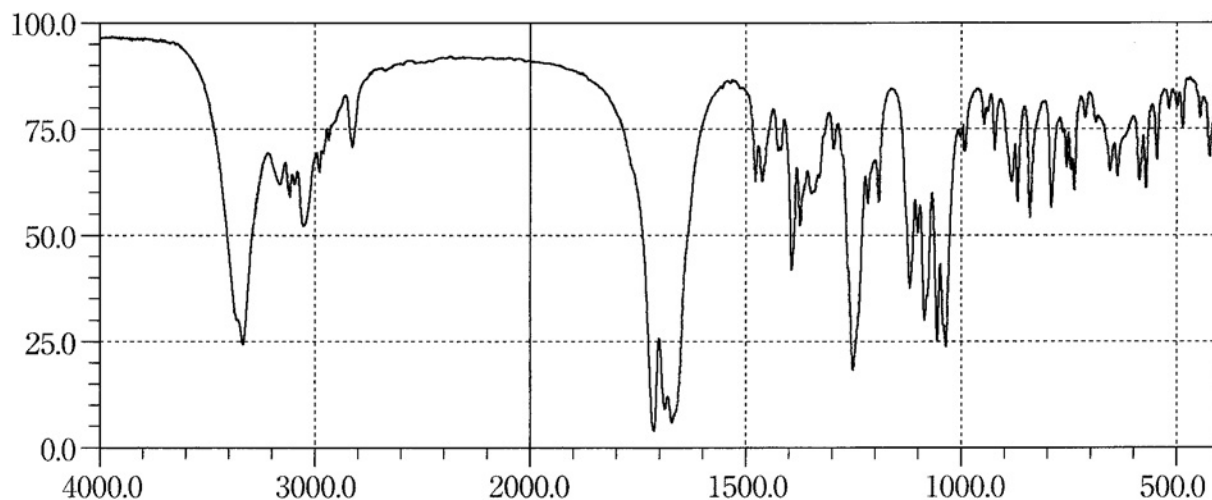
Preparation of sample: Potassium bromide disk method

Dopamine Hydrochloride

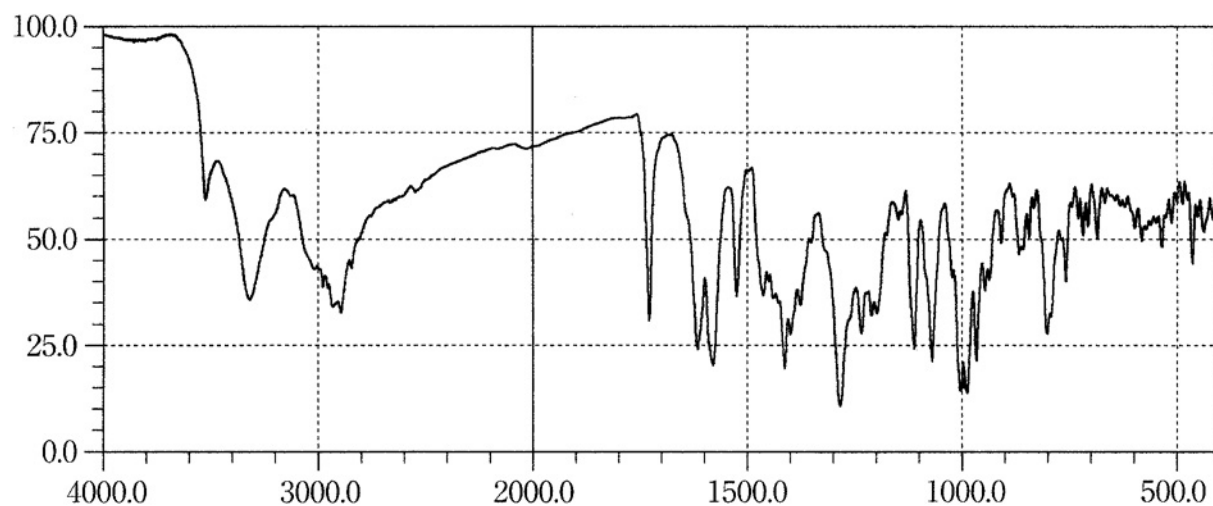
Preparation of sample: Potassium chloride disk method

Doxapram Hydrochloride Hydrate

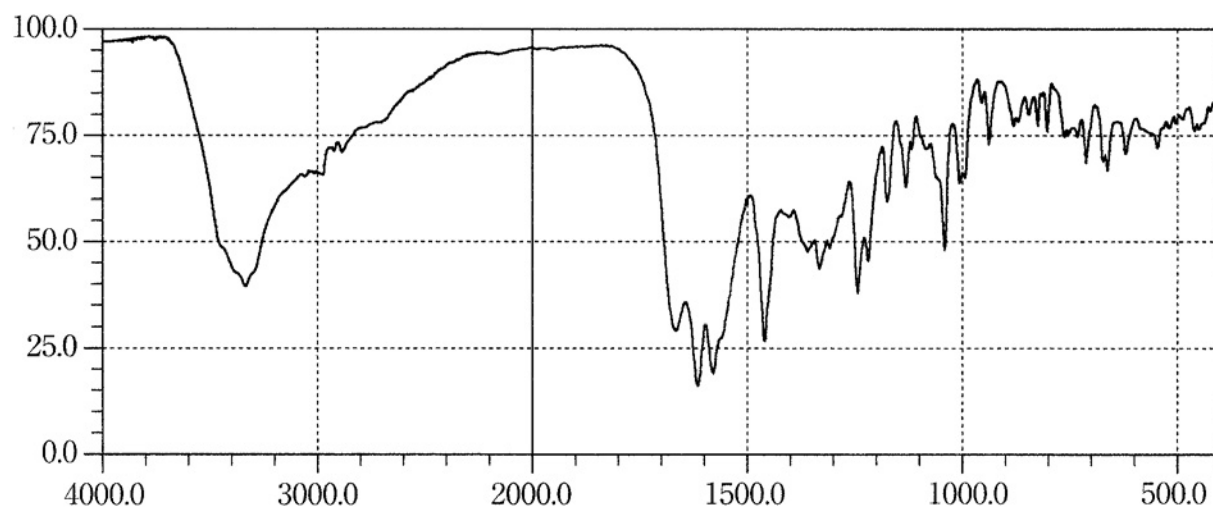
Preparation of sample: Potassium bromide disk method

Doxifluridine

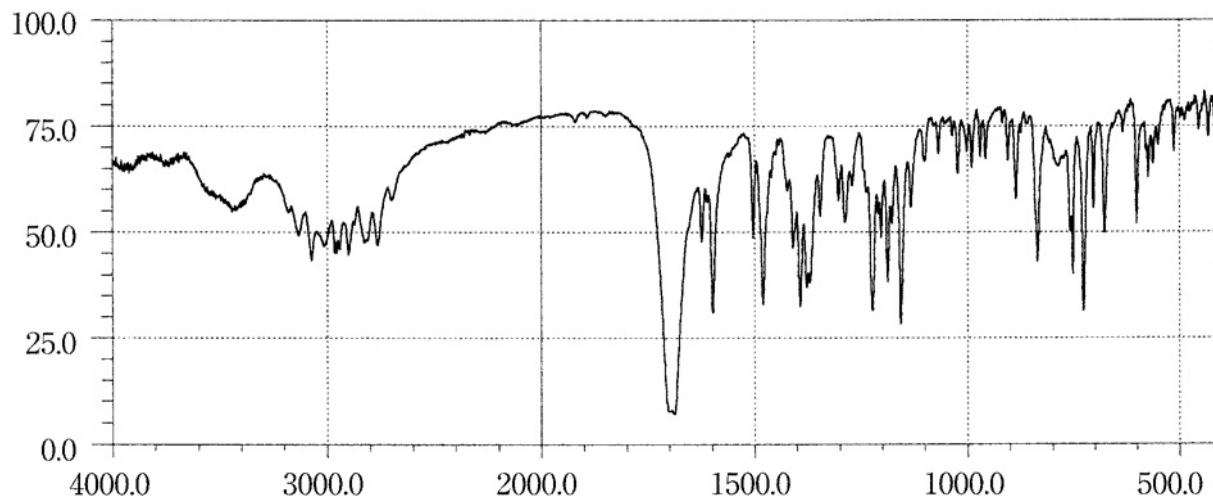
Preparation of sample: Potassium bromide disk method

Doxorubicin Hydrochloride

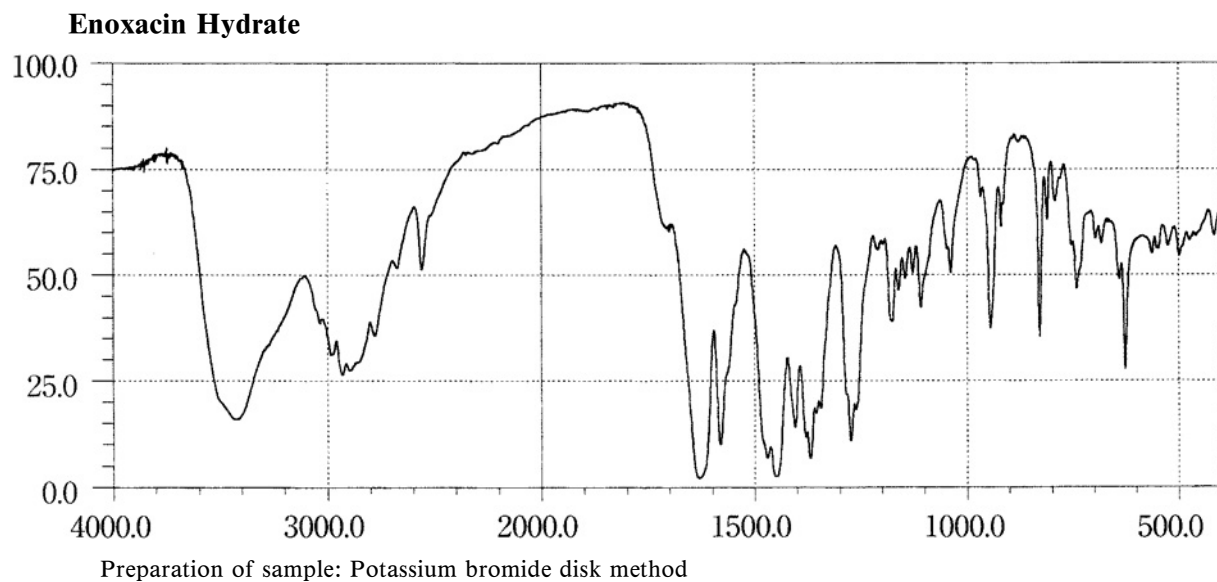
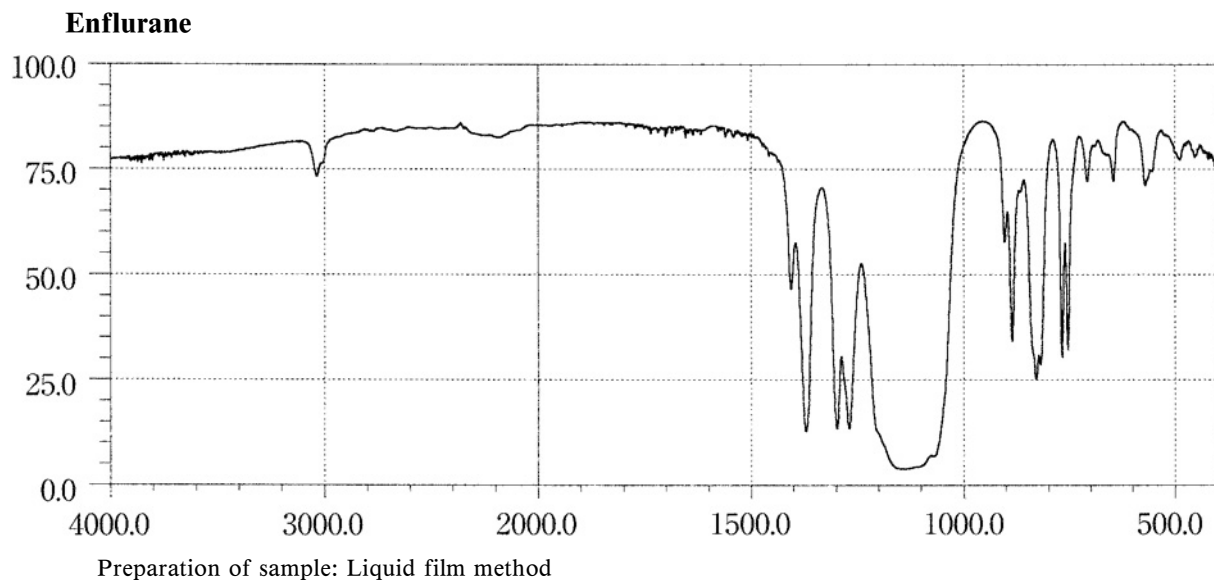
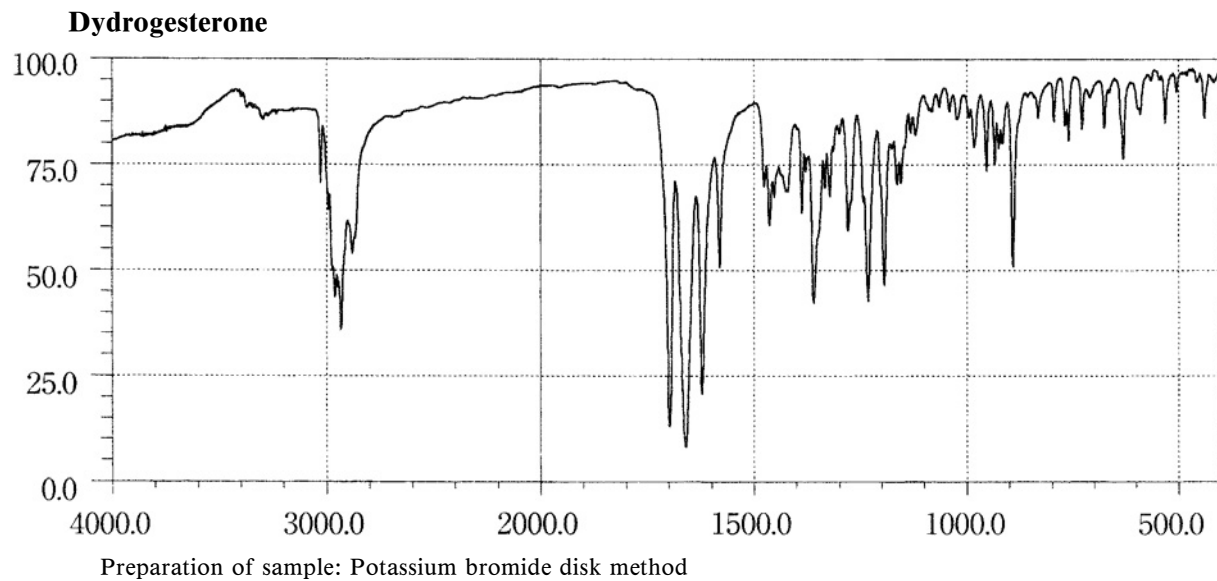
Preparation of sample: Potassium chloride disk method

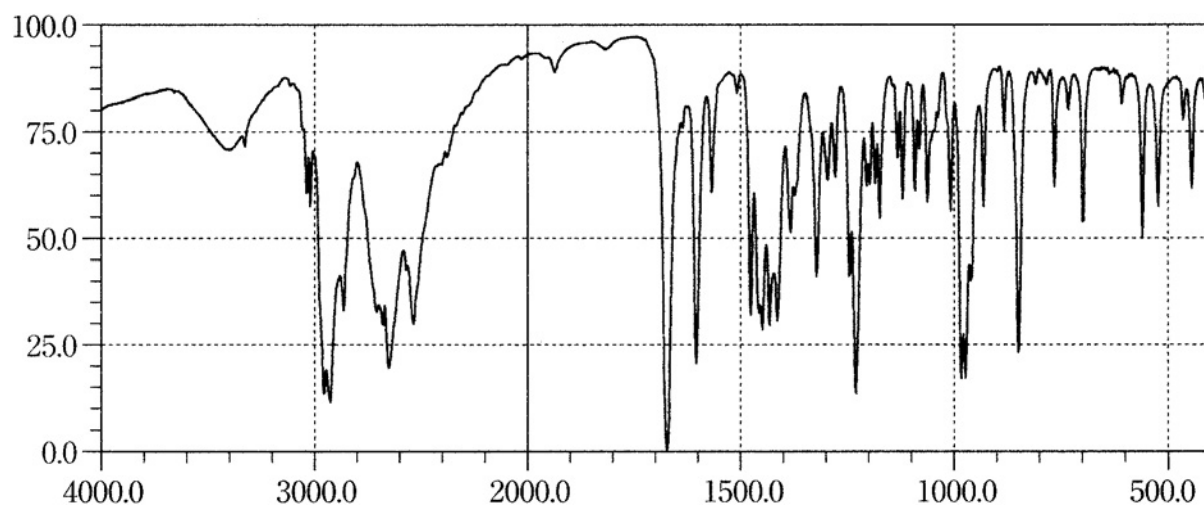
Doxycycline Hydrochloride Hydrate

Preparation of sample: Potassium bromide disk method

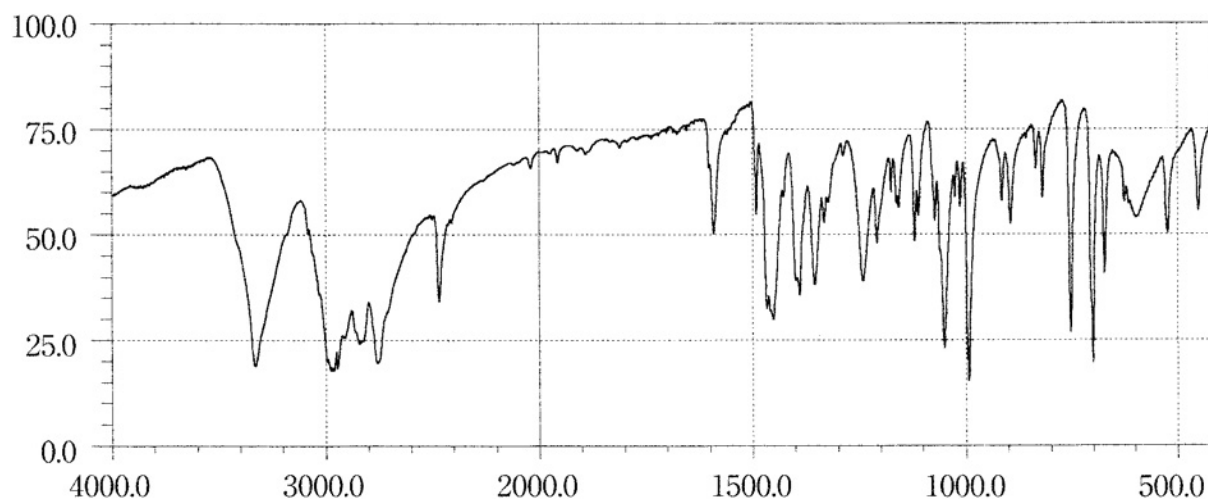
Droperidol

Preparation of sample: Potassium bromide disk method

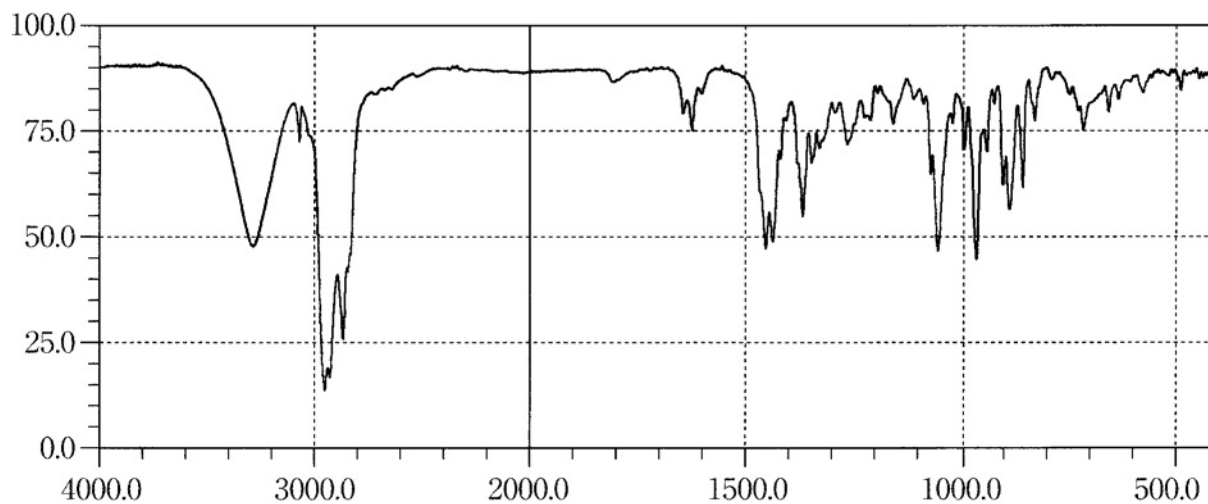


Eperisone Hydrochloride

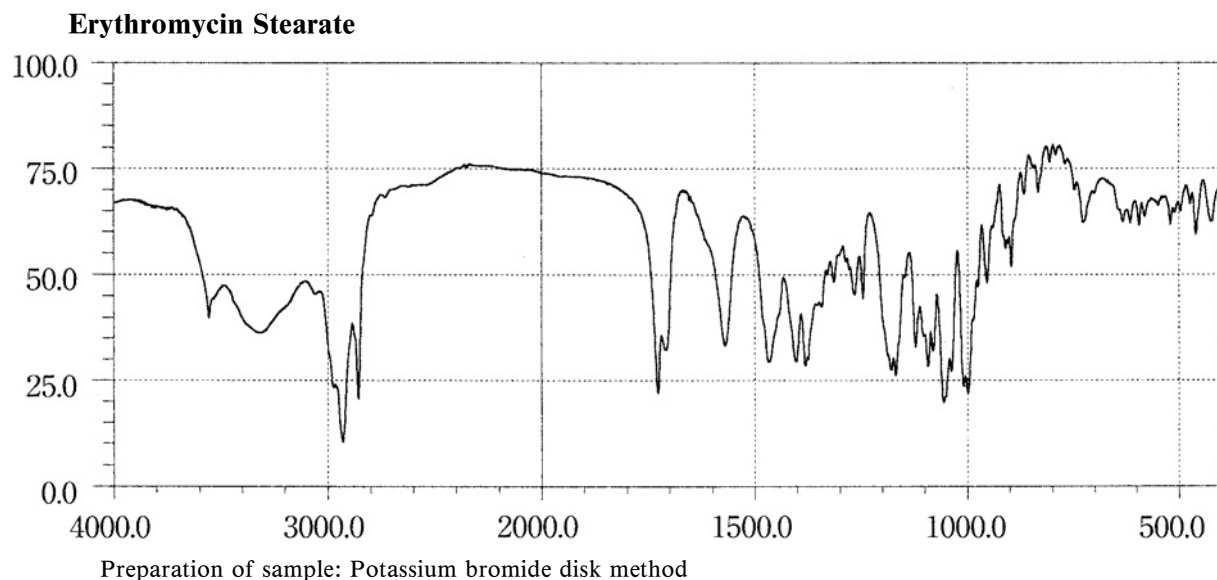
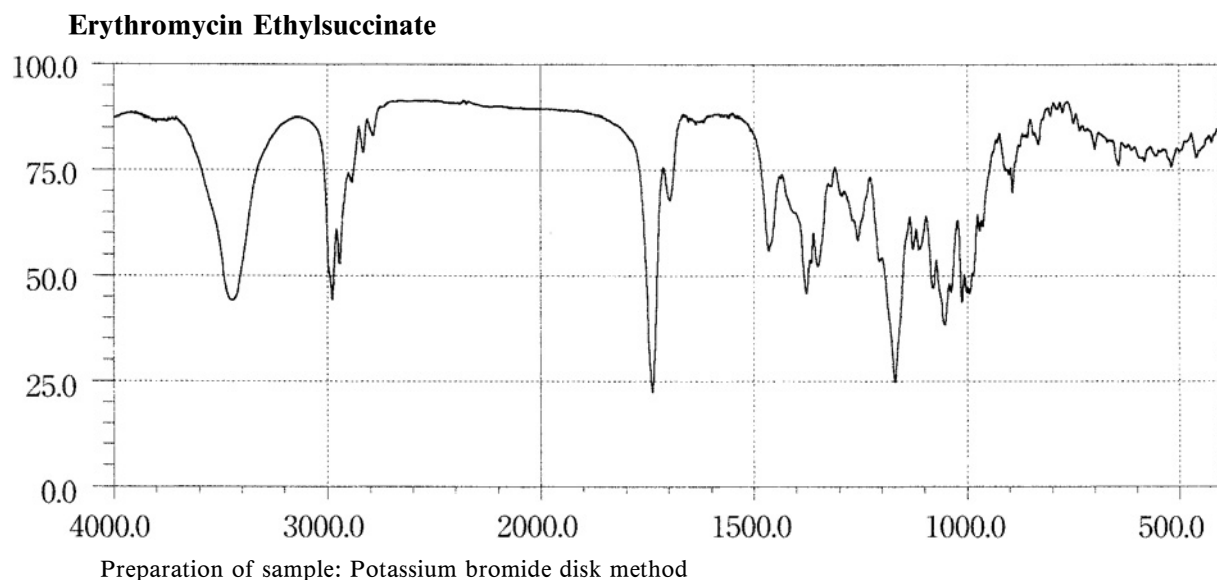
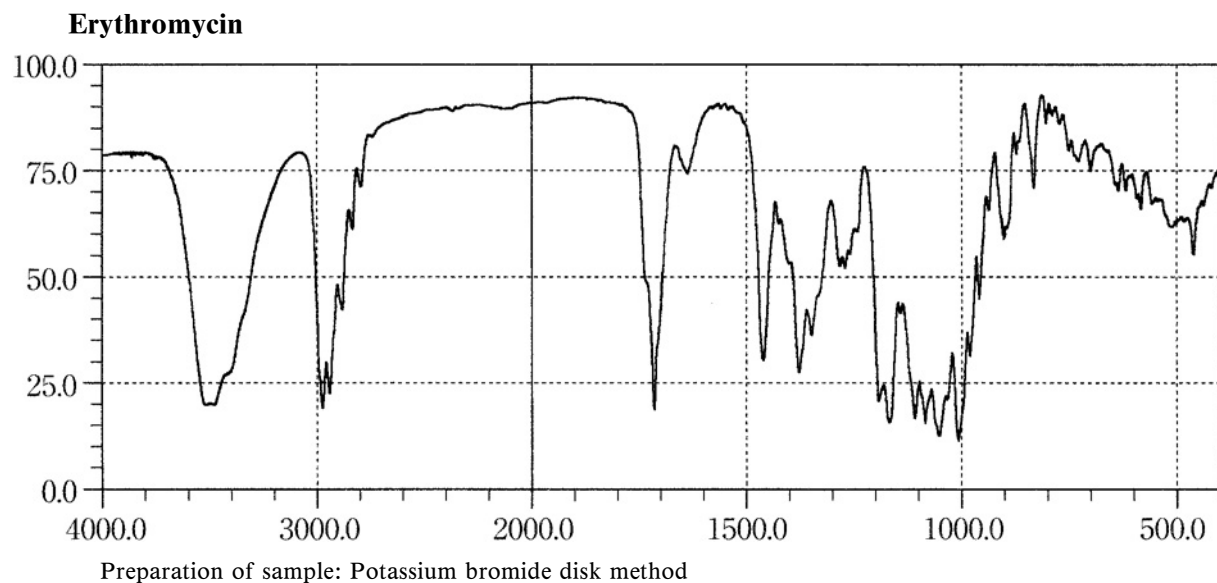
Preparation of sample: Potassium chloride disk method

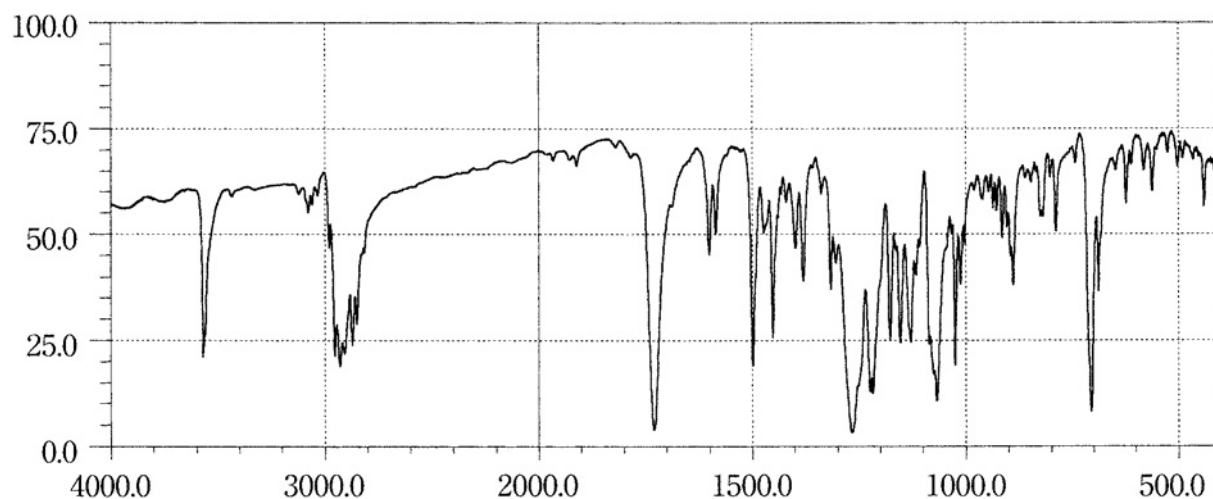
Ephedrine Hydrochloride

Preparation of sample: Potassium chloride disk method

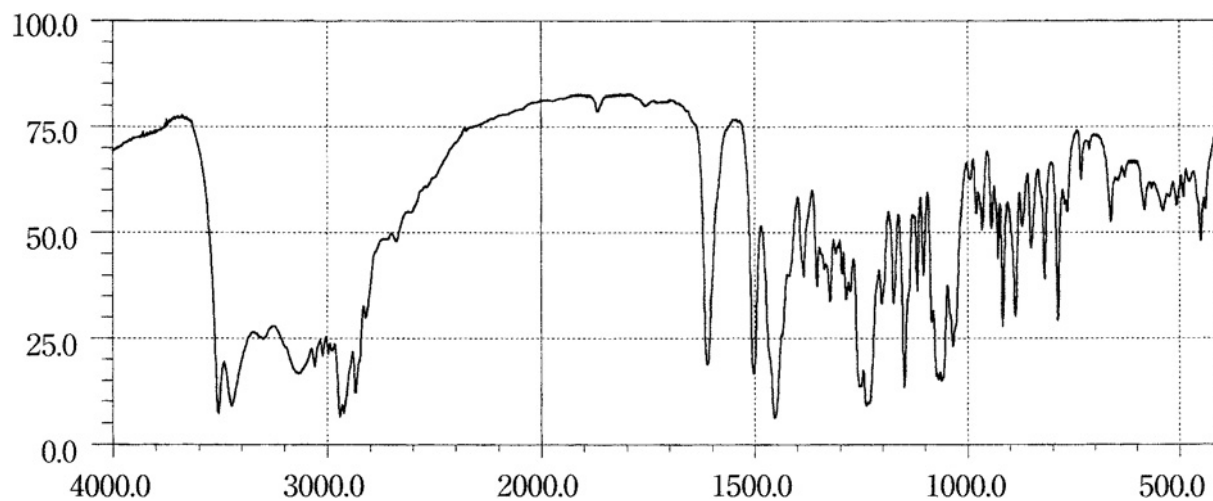
Ergocalciferol

Preparation of sample: Potassium bromide disk method

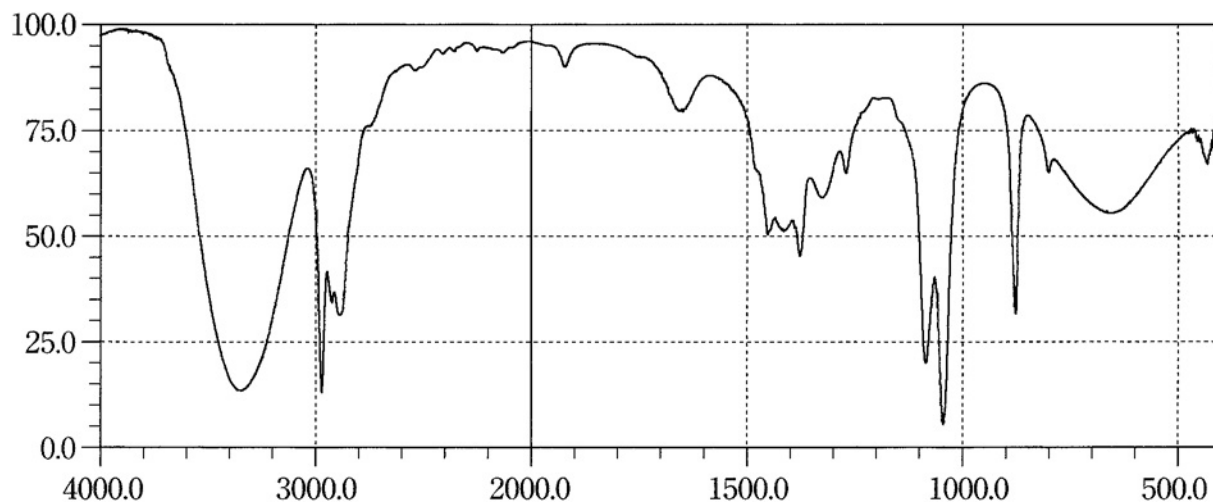


Estradiol Benzoate

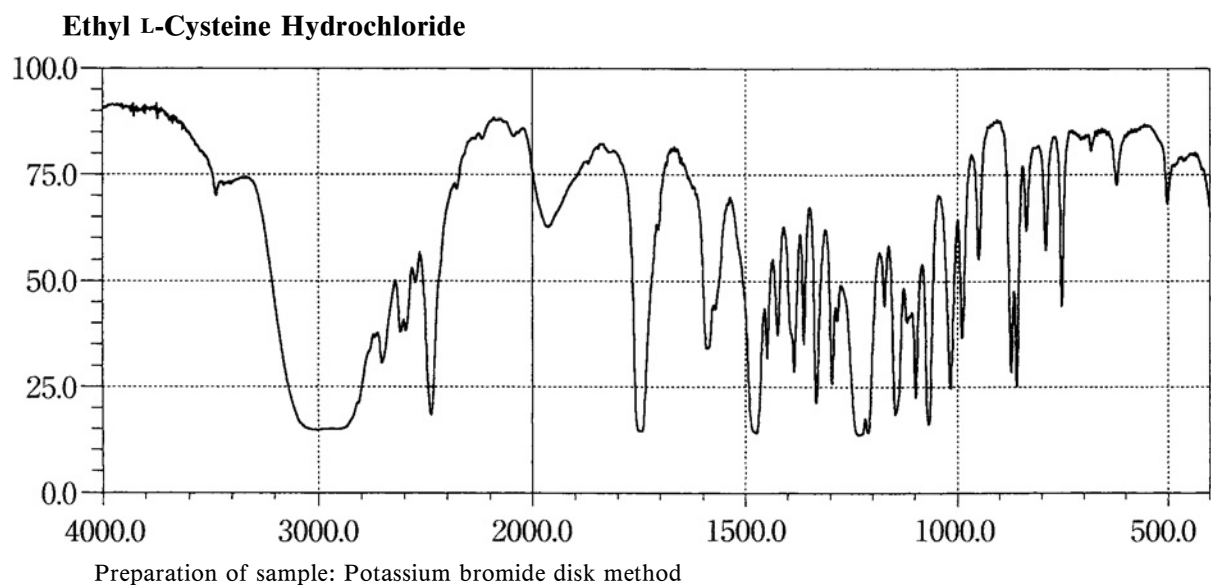
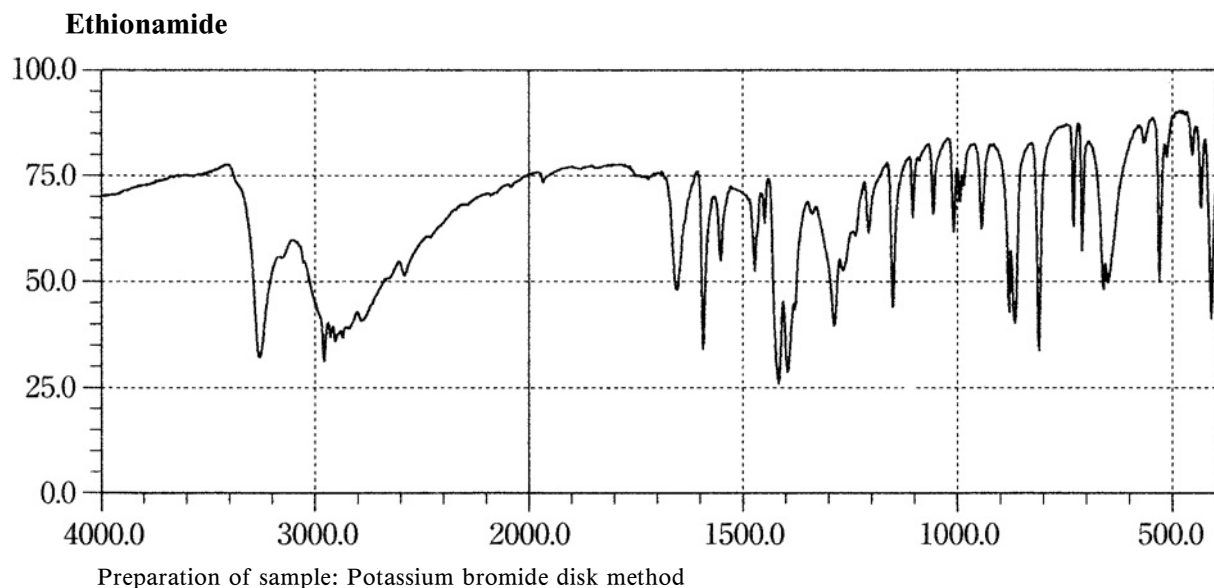
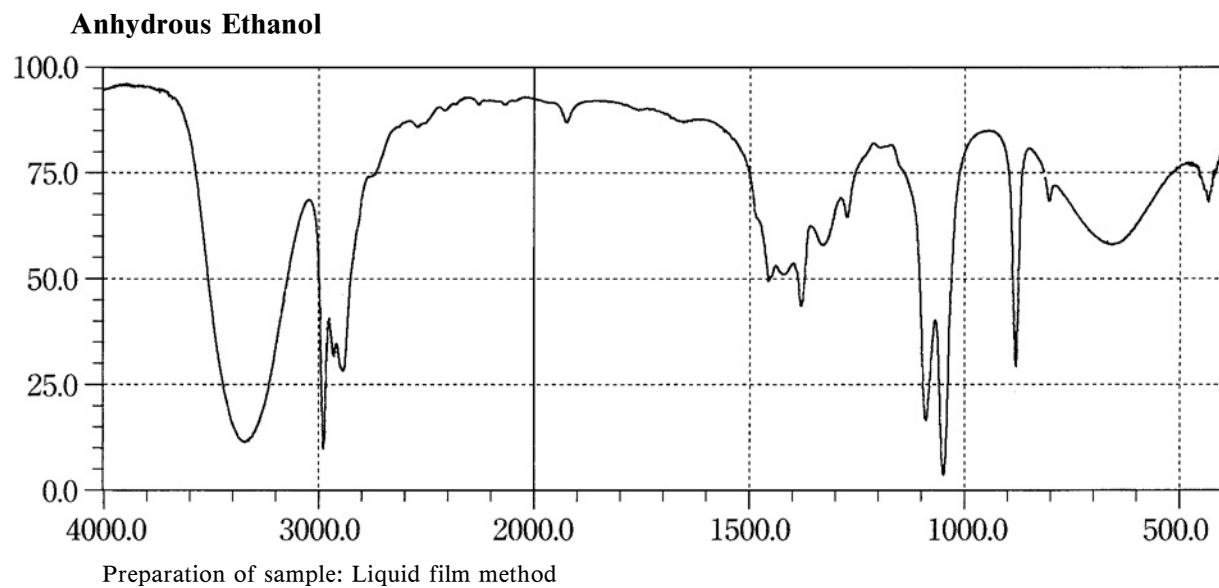
Preparation of sample: Potassium bromide disk method

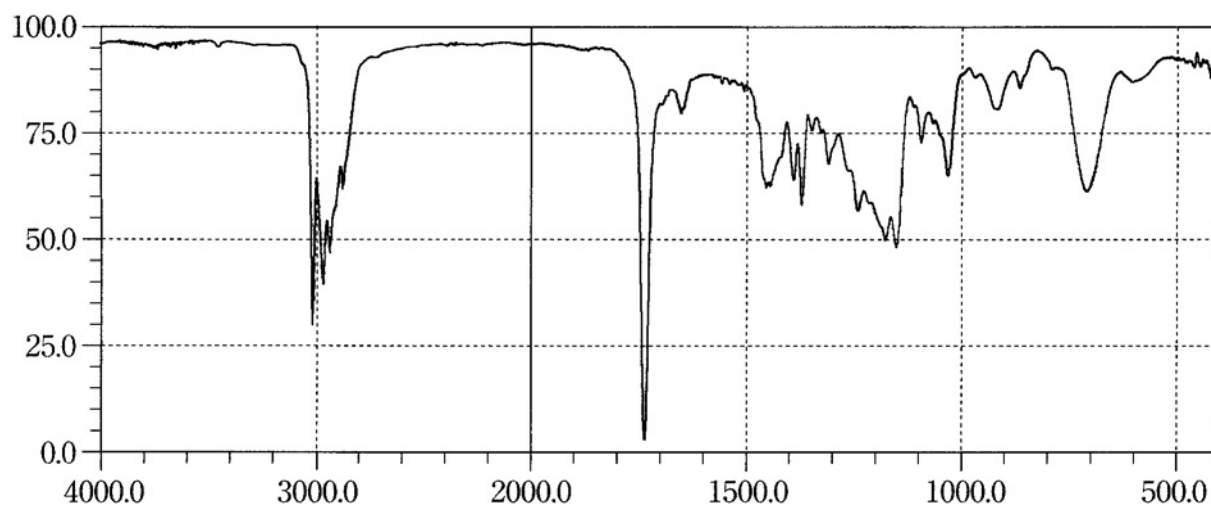
Estriol

Preparation of sample: Potassium bromide disk method

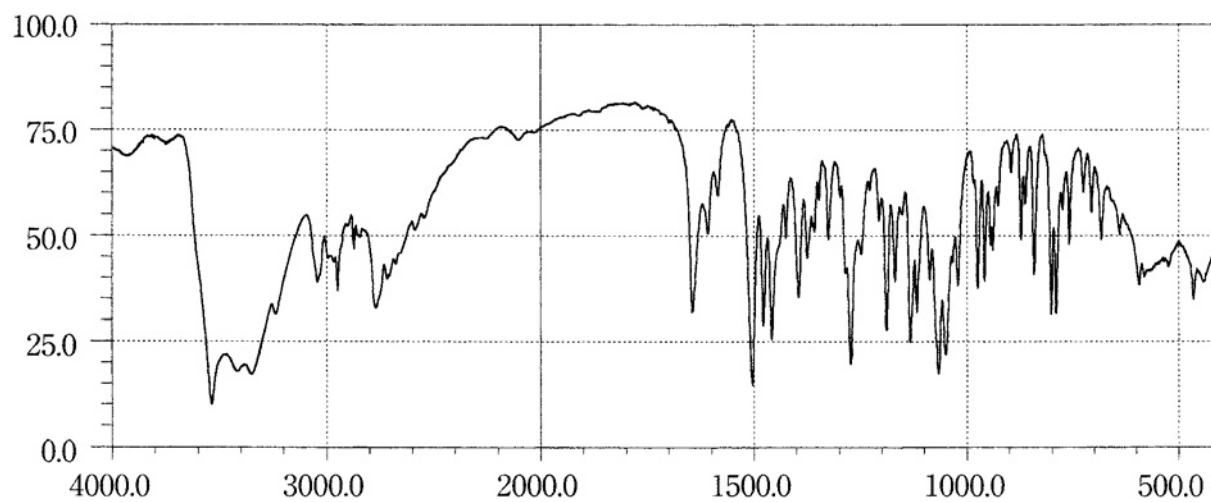
Ethanol

Preparation of sample: Liquid film method

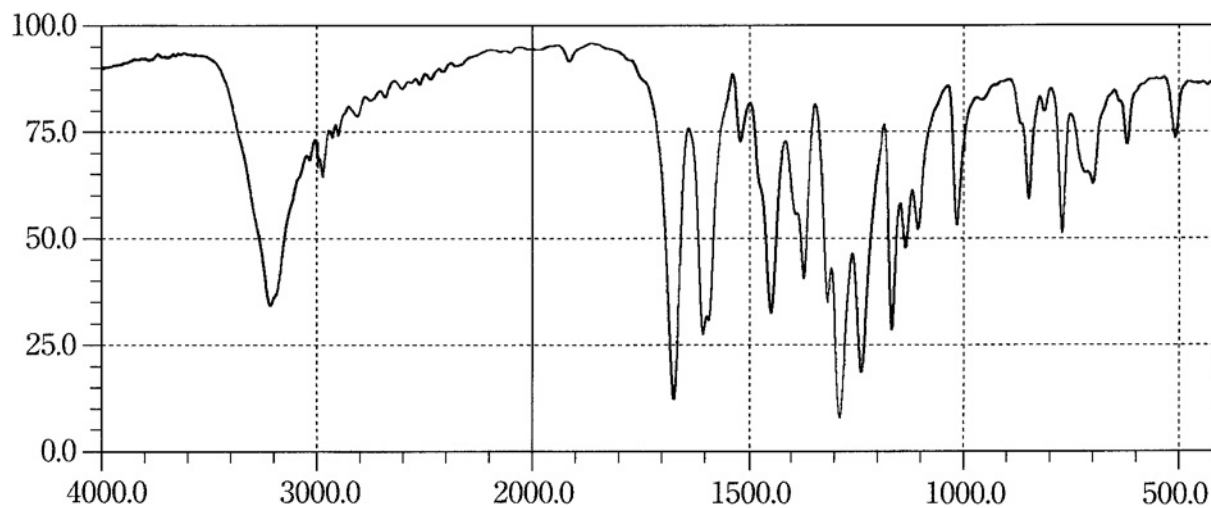


Ethyl Icosapentate

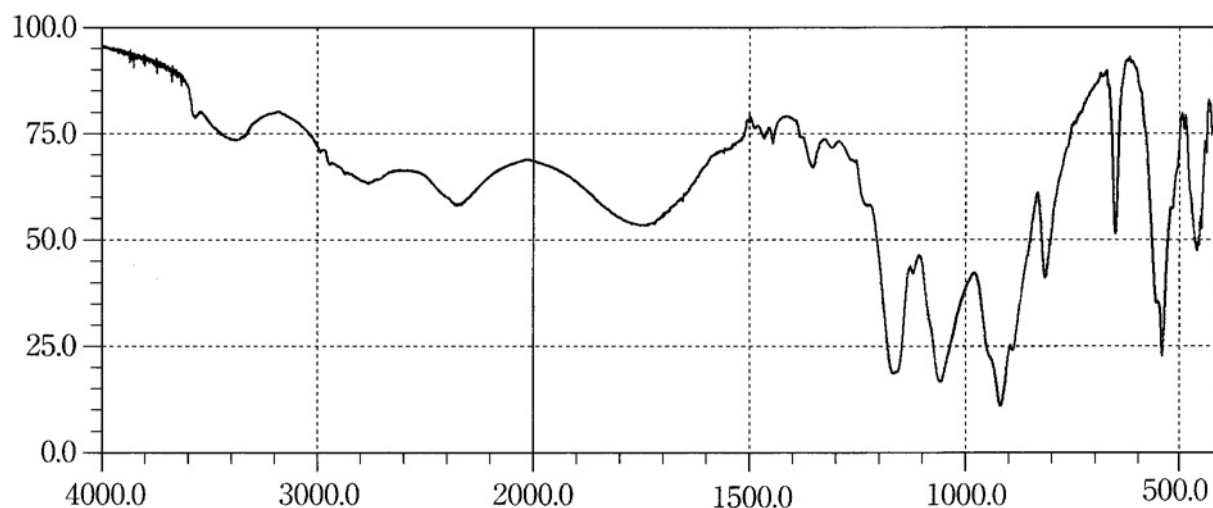
Preparation of sample: Liquid film method

Ethylmorphine Hydrochloride Hydrate

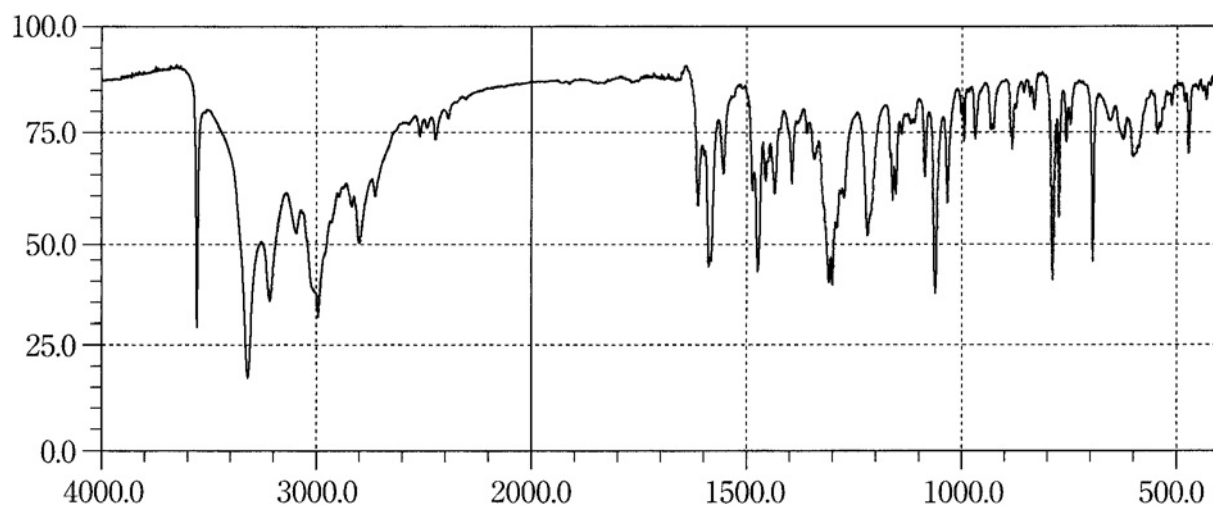
Preparation of sample: Potassium bromide disk method

Ethyl Parahydroxybenzoate

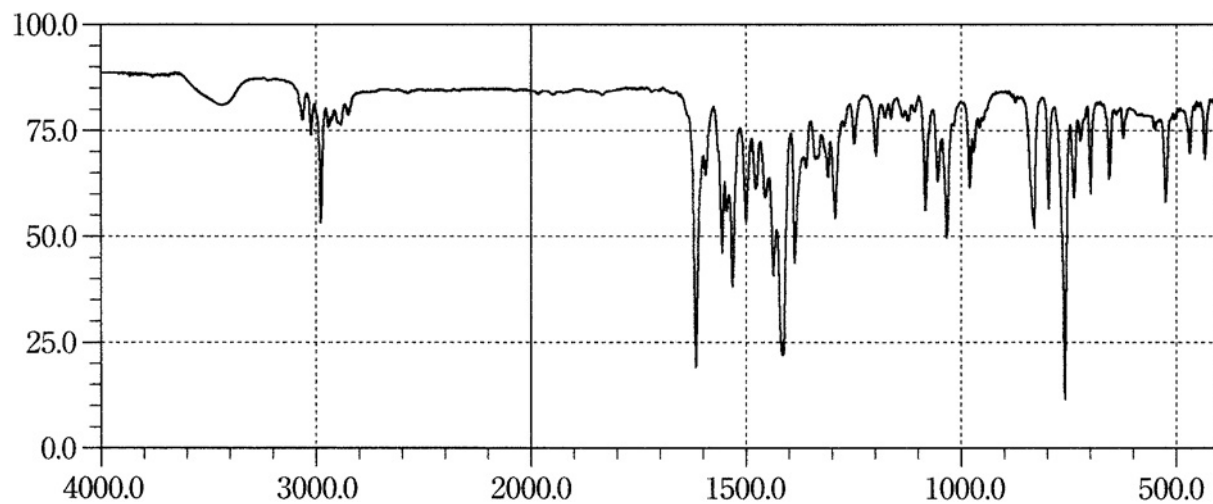
Preparation of sample: Potassium bromide disk method

Etidronate Disodium

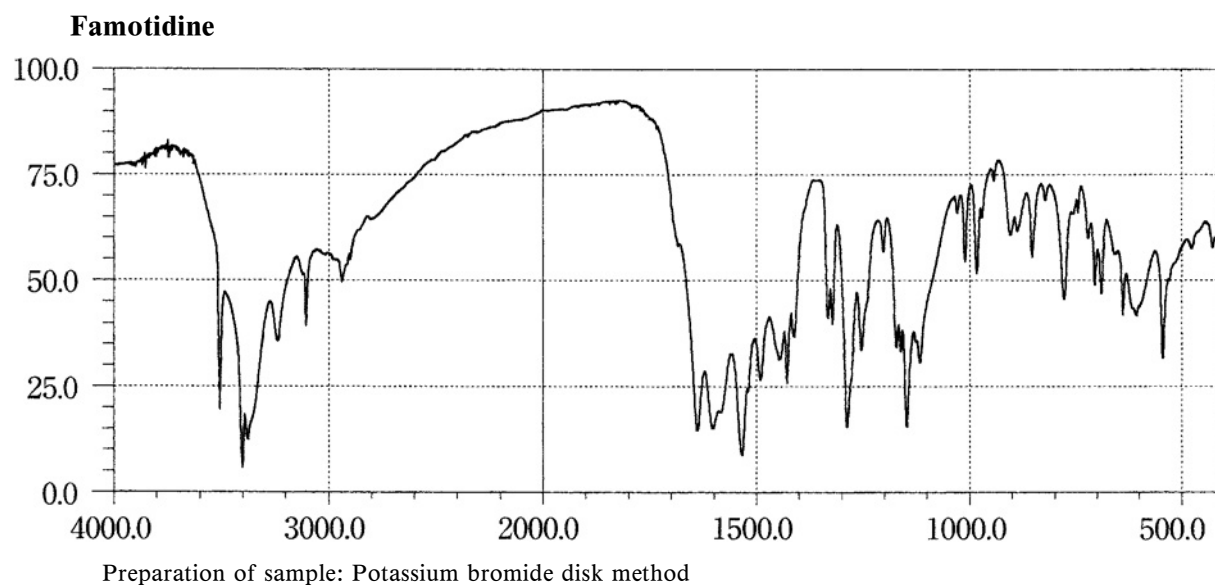
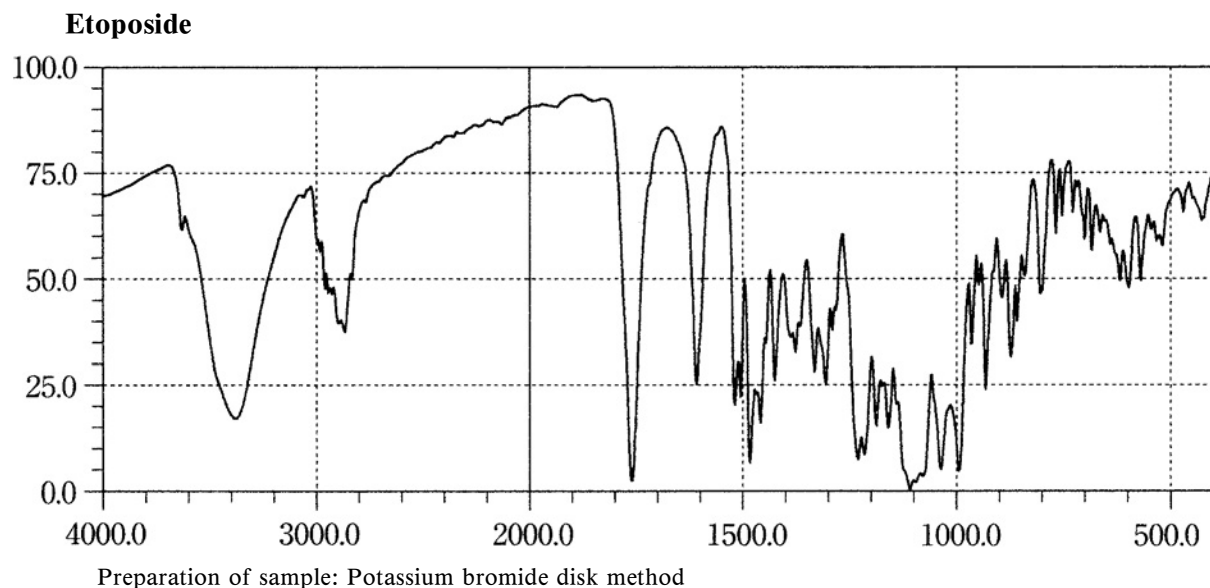
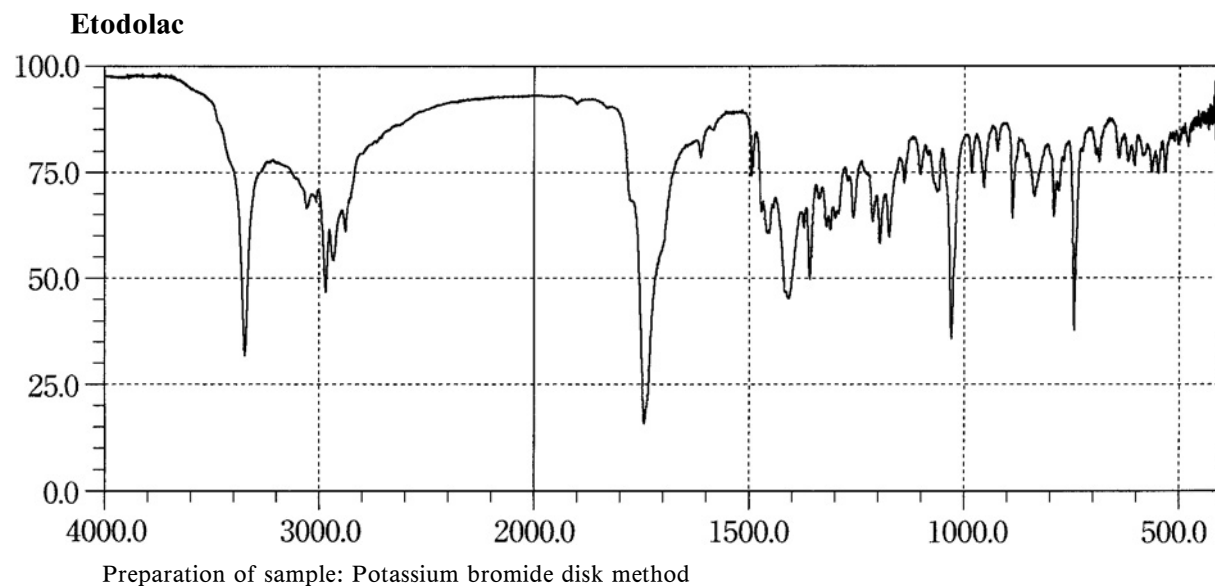
Preparation of sample: Potassium bromide disk method

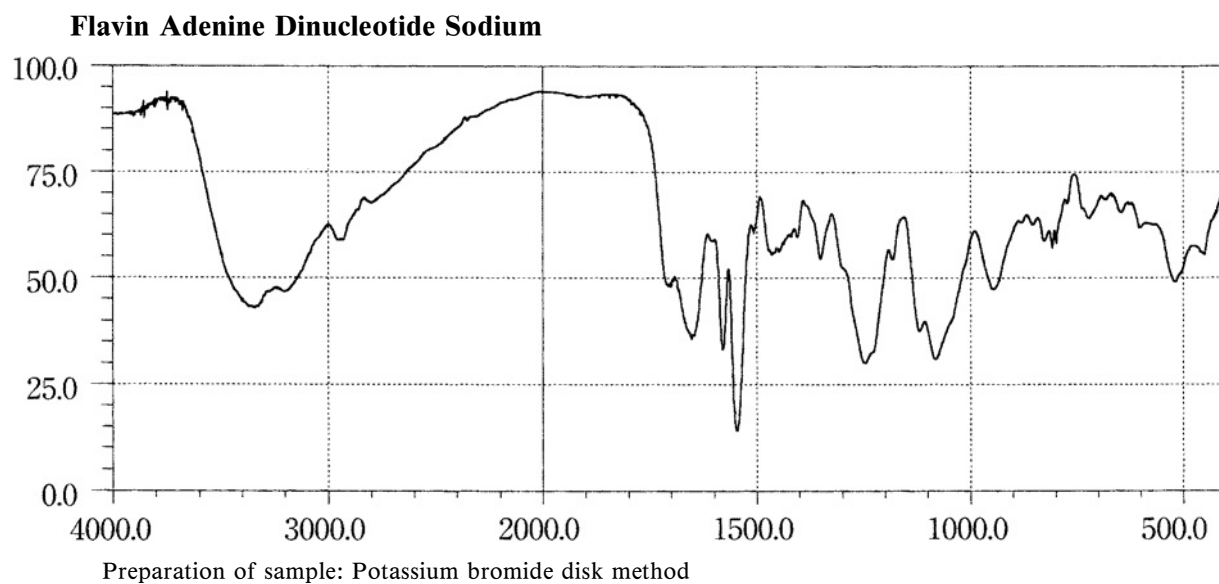
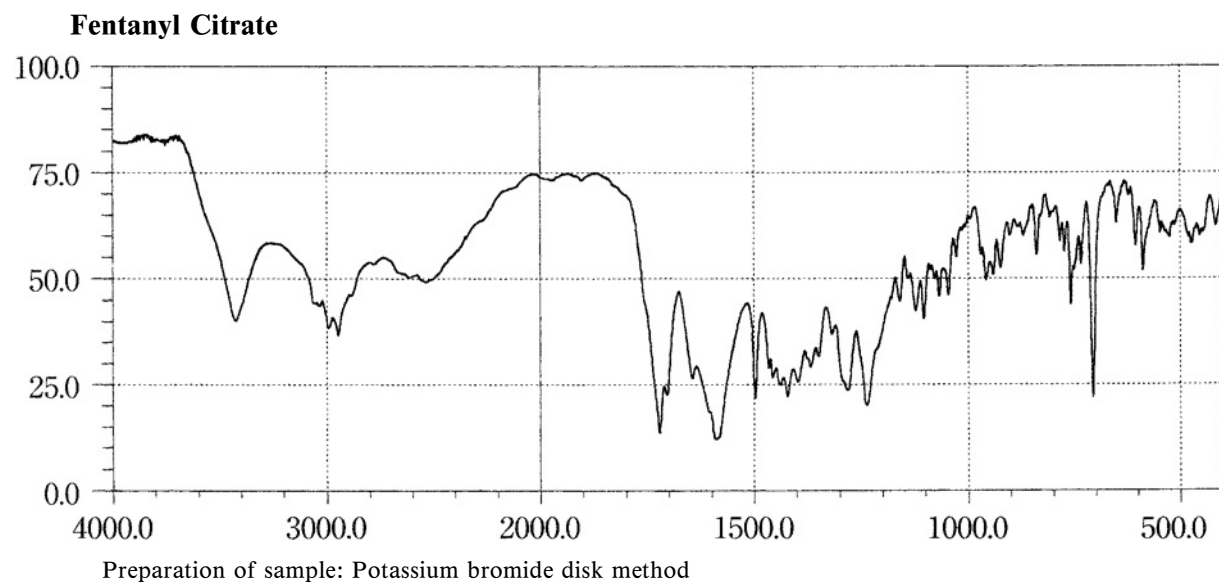
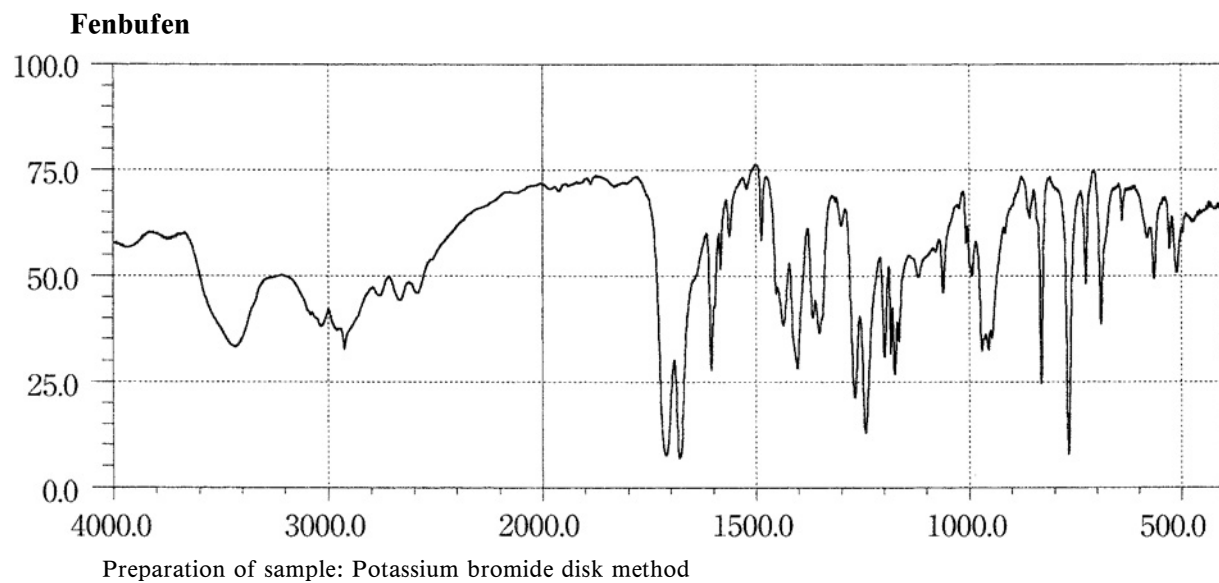
Etilefrine Hydrochloride

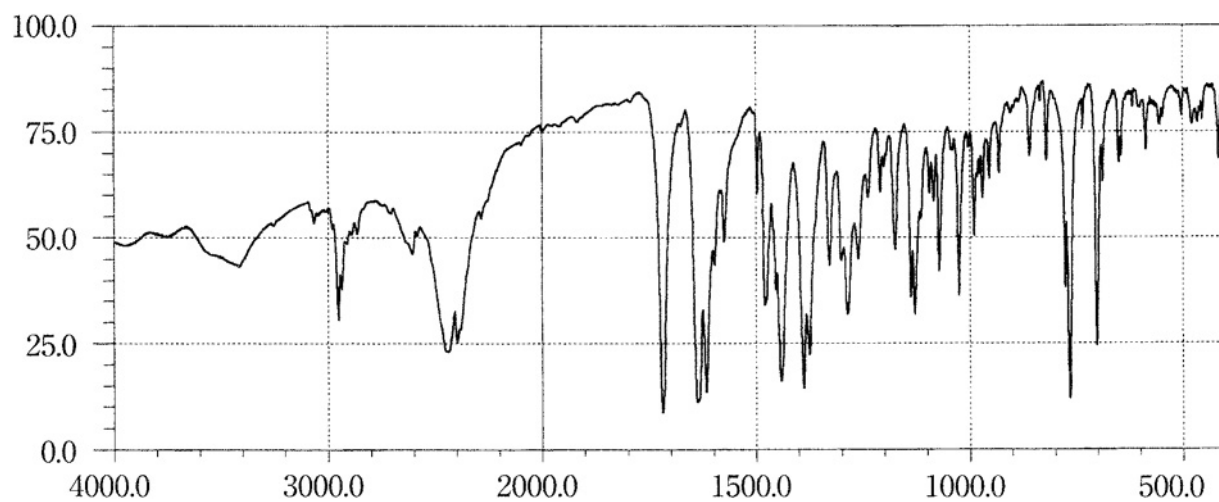
Preparation of sample: Potassium chloride disk method

Etizolam

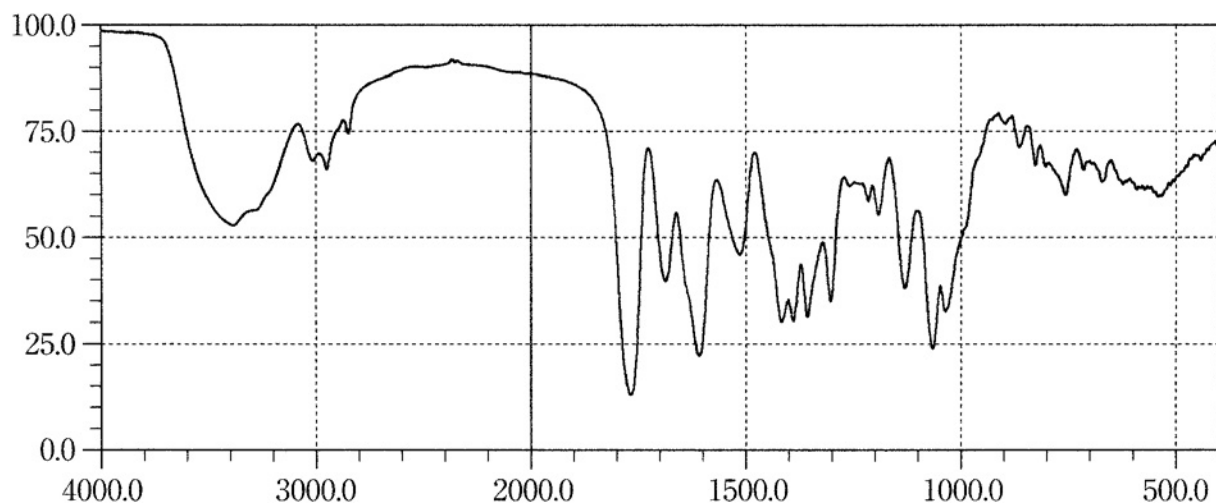
Preparation of sample: Potassium bromide disk method



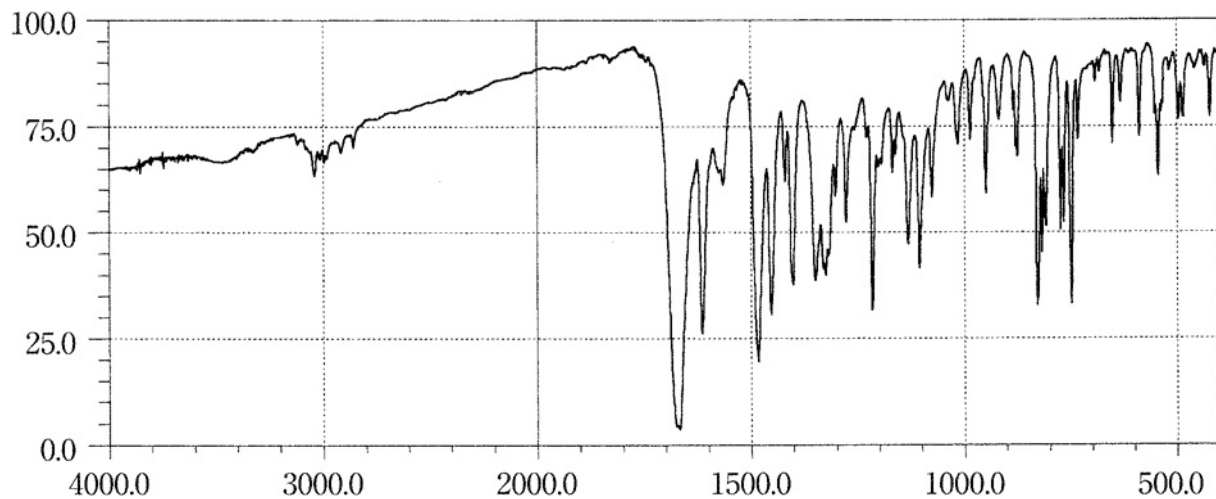


Flavoxate Hydrochloride

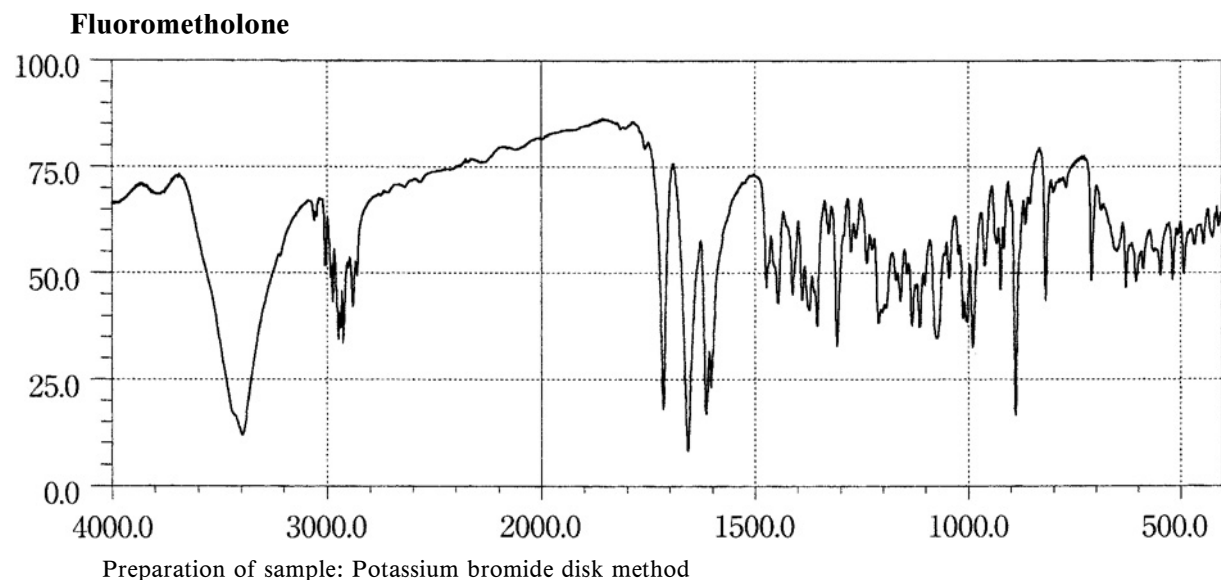
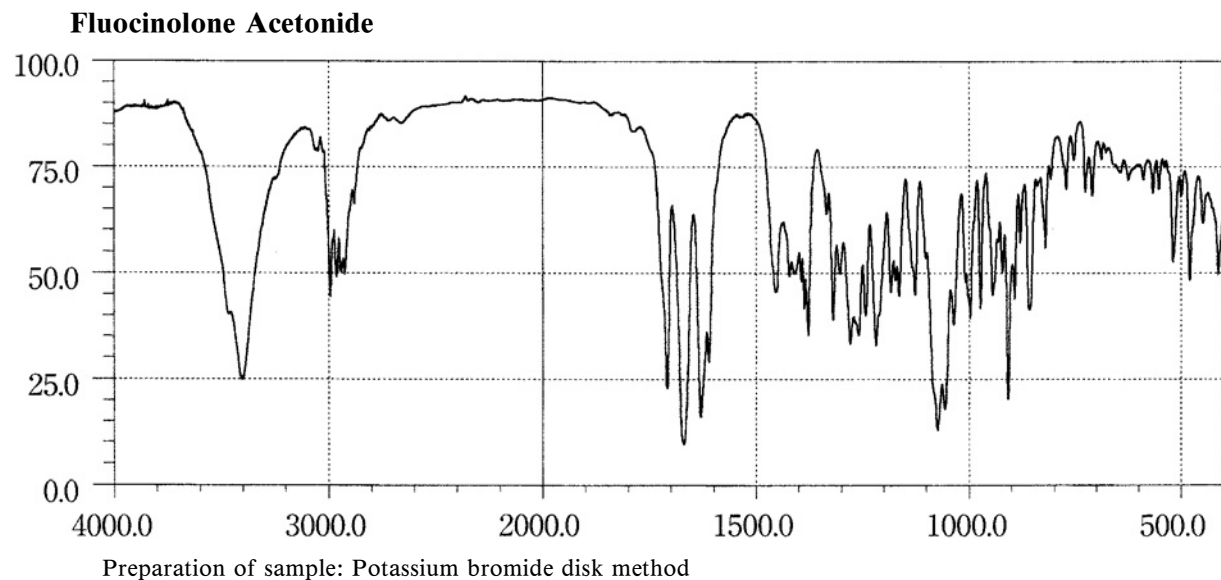
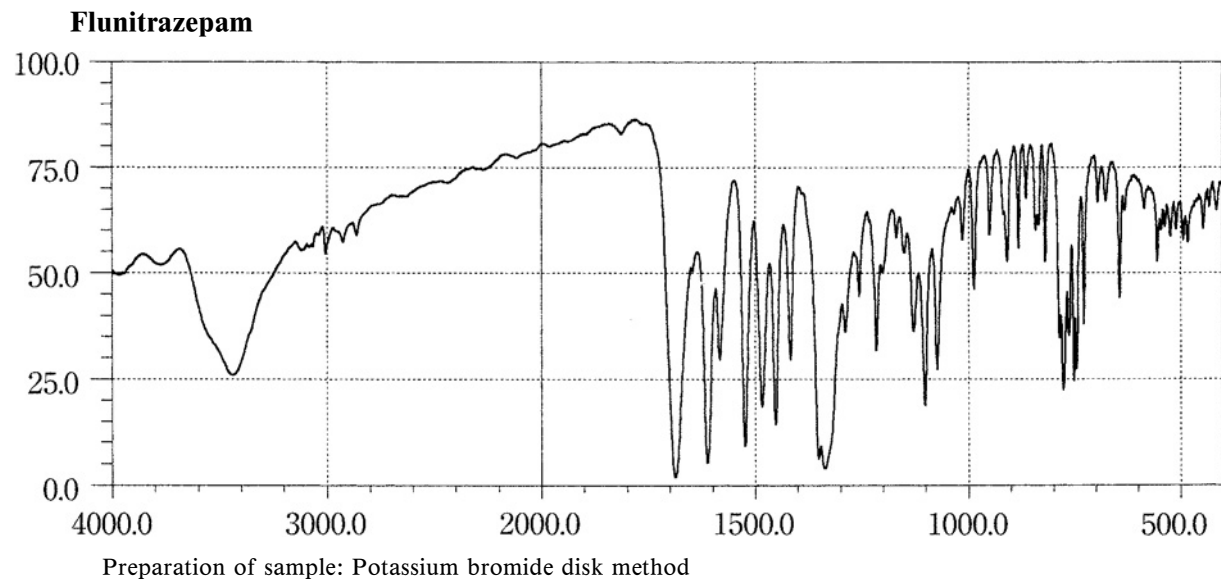
Preparation of sample: Potassium bromide disk method

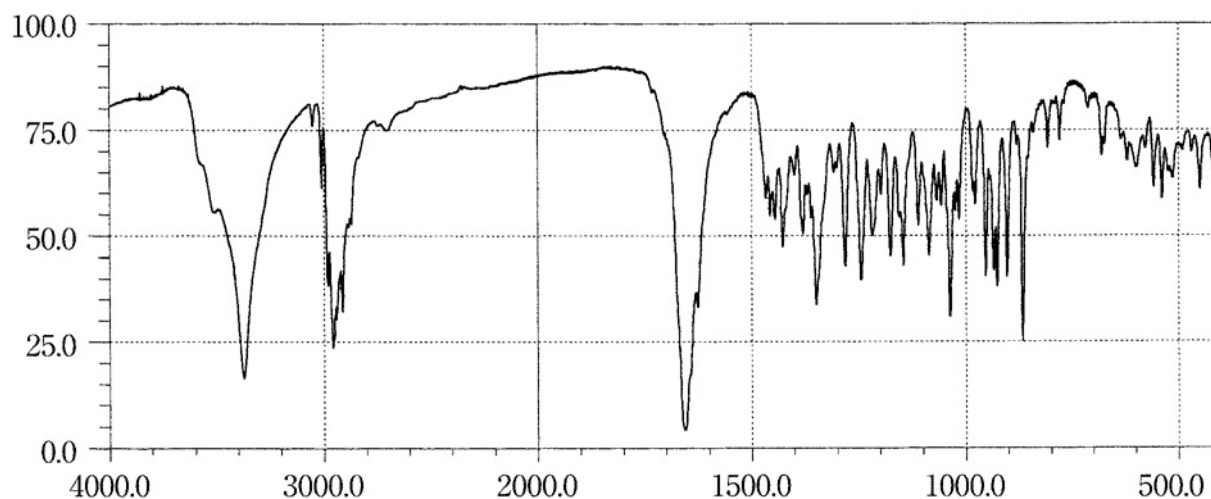
Flomoxef Sodium

Preparation of sample: Potassium bromide disk method

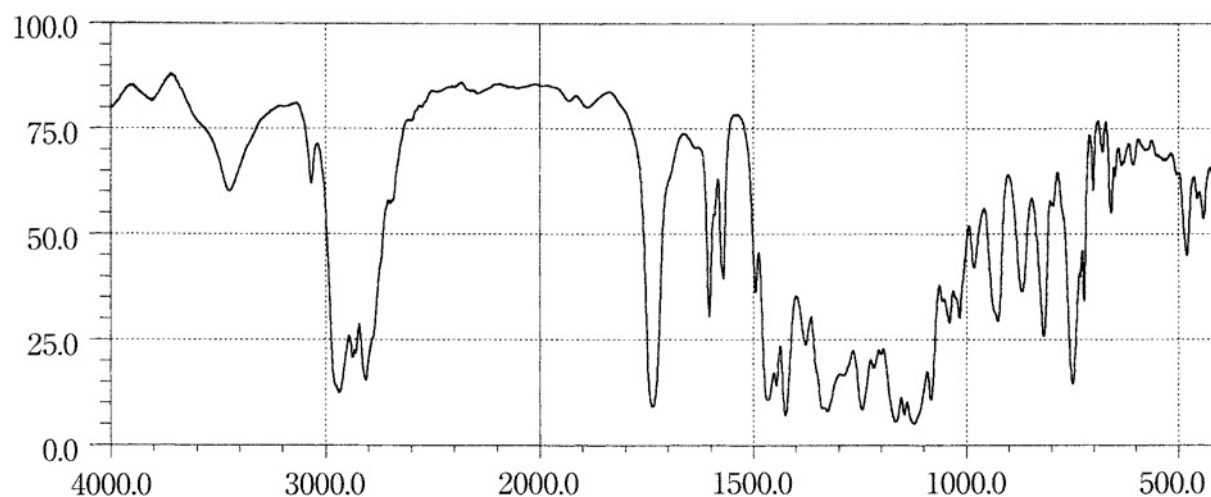
Fludiazepam

Preparation of sample: Potassium bromide disk method

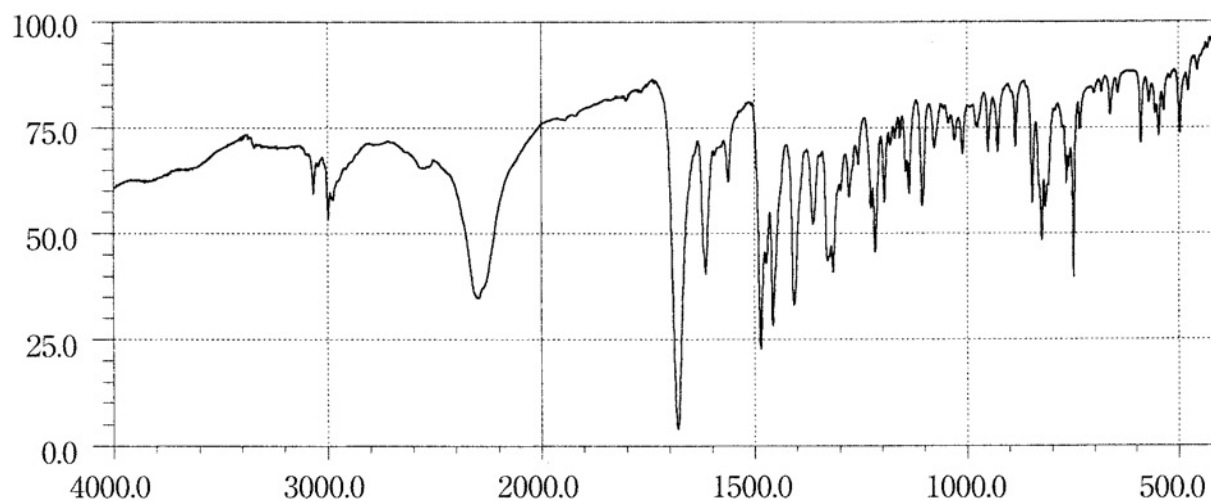


Fluoxymesterone

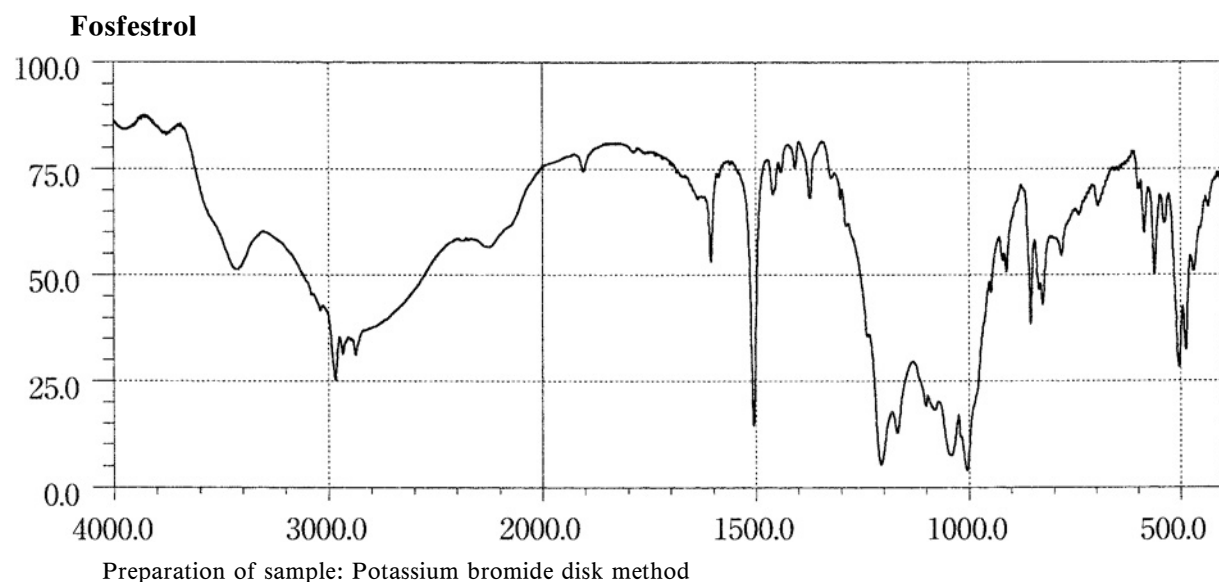
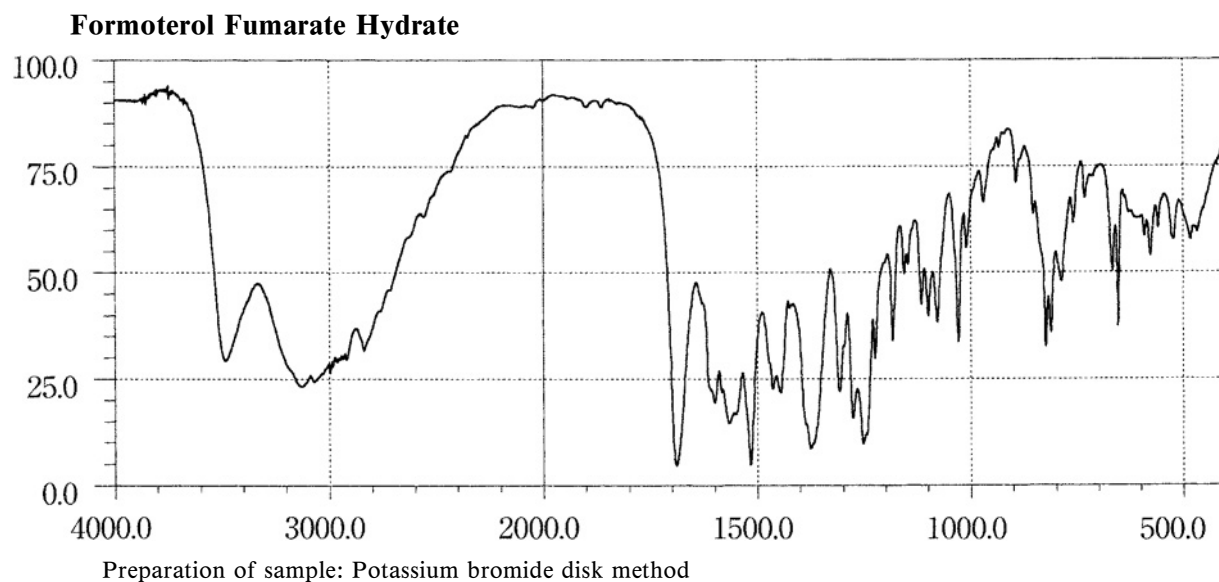
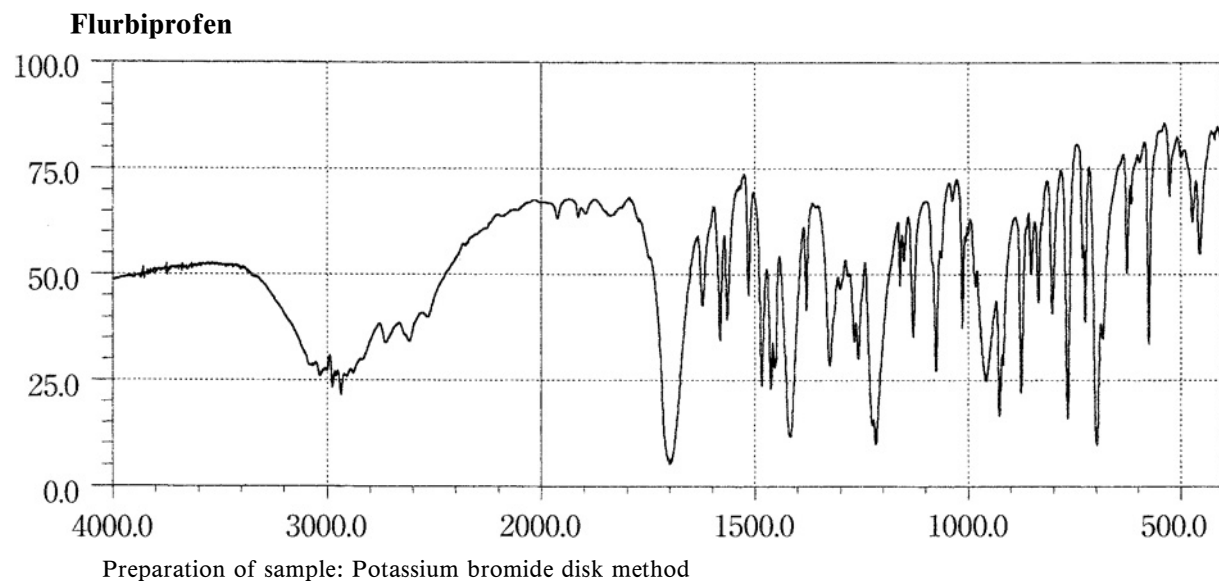
Preparation of sample: Potassium bromide disk method

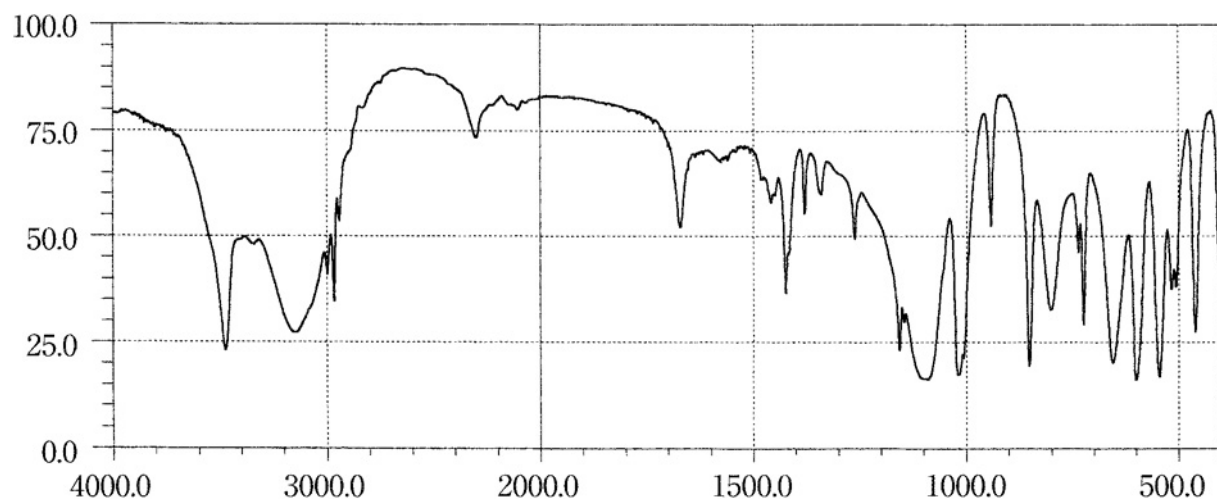
Fluphenazine Enanthate

Preparation of sample: Liquid film method

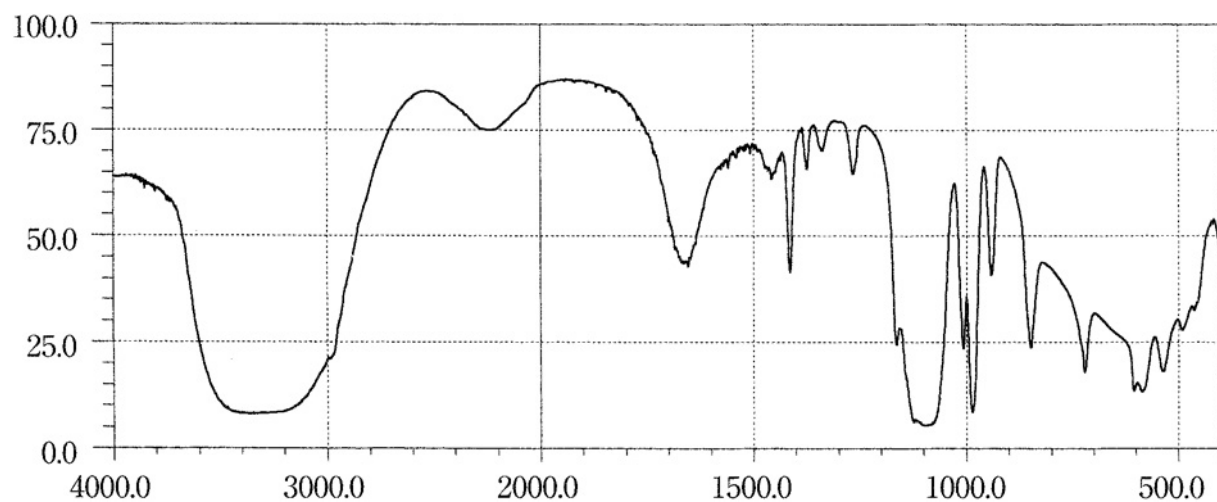
Flurazepam Hydrochloride

Preparation of sample: Potassium chloride disk method

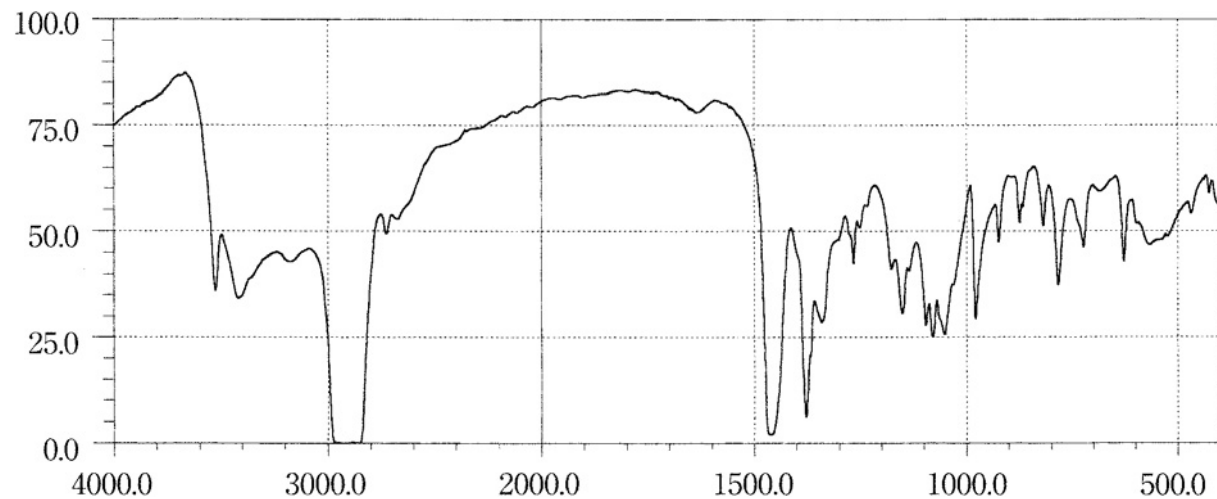


Fosfomycin Calcium Hydrate

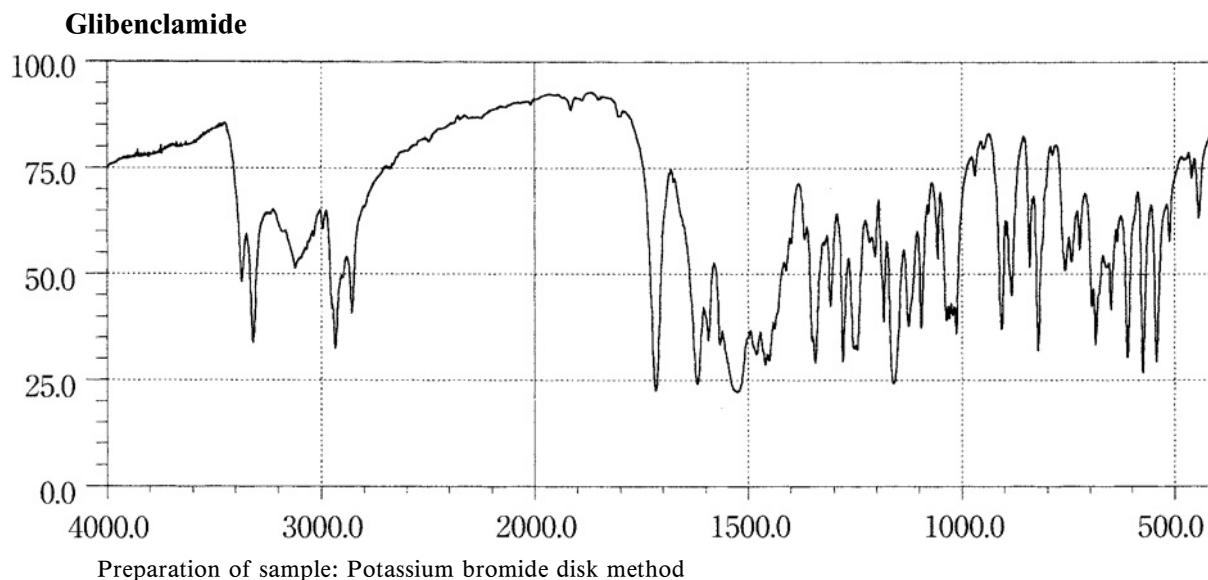
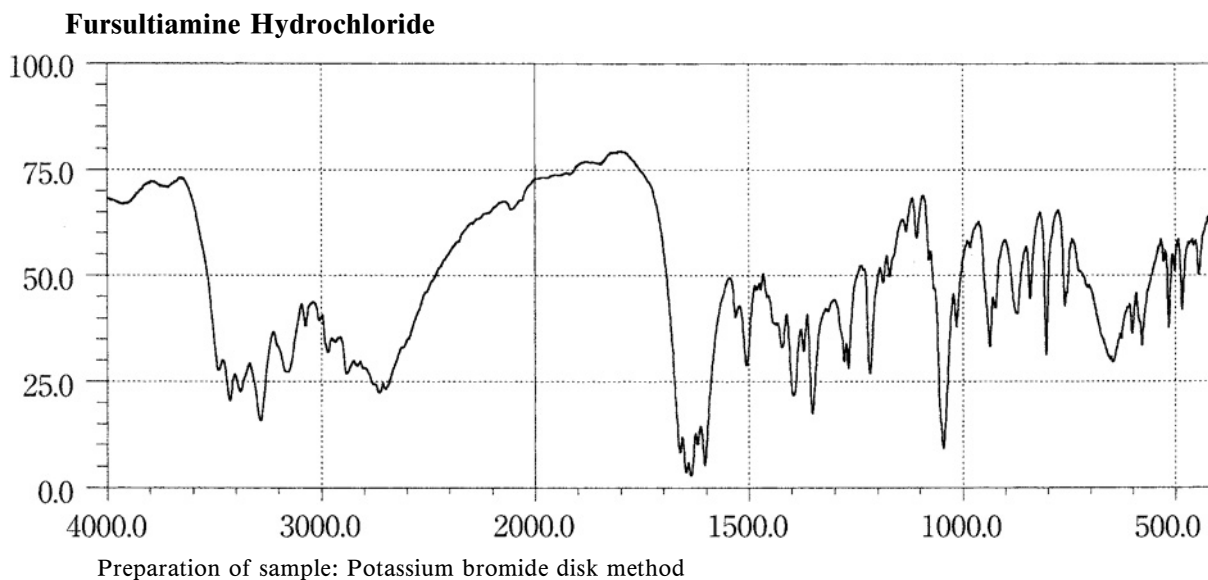
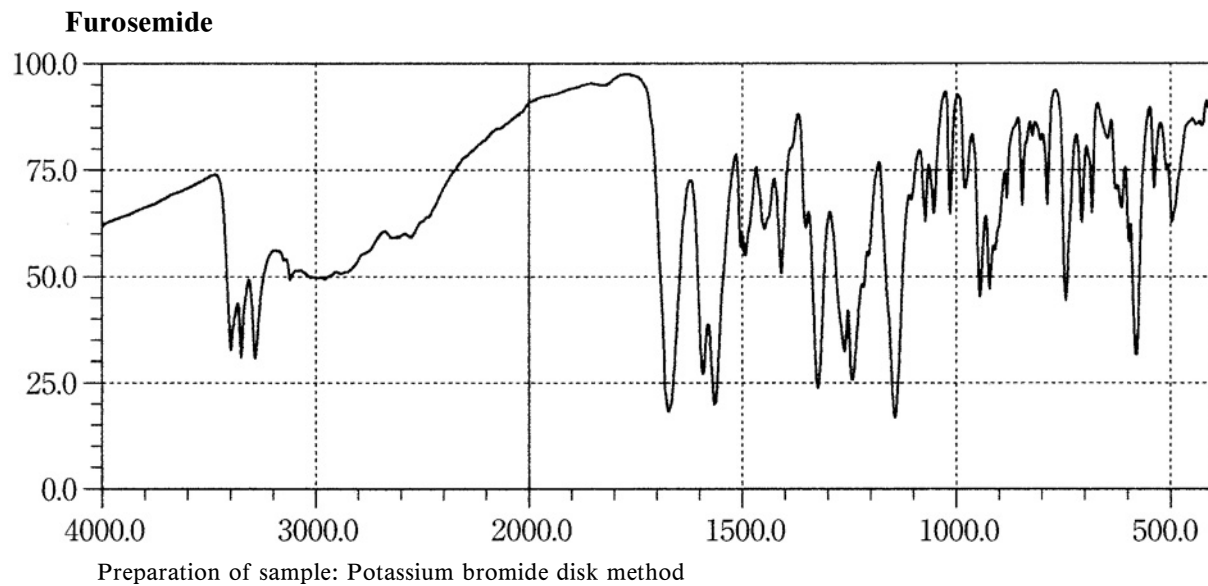
Preparation of sample: Potassium bromide disk method

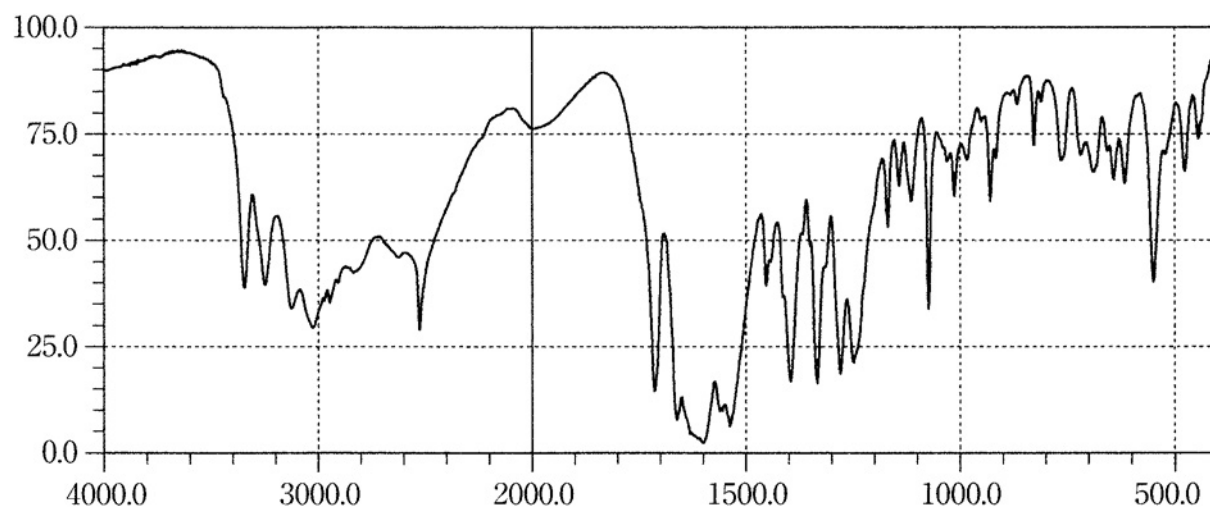
Fosfomycin Sodium

Preparation of sample: Potassium bromide disk method

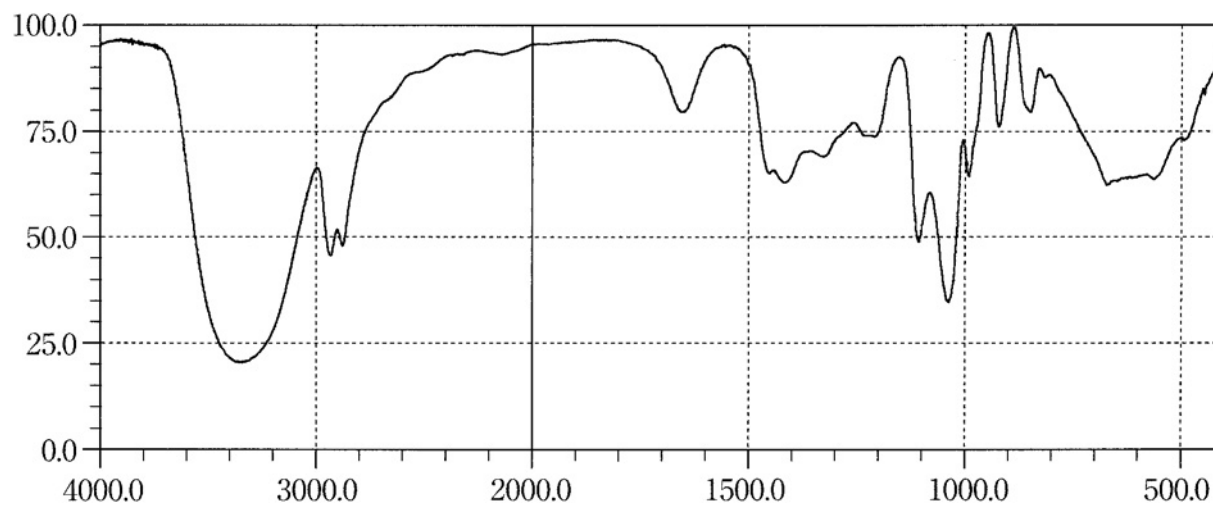
Fructose

Preparation of sample: Paste method

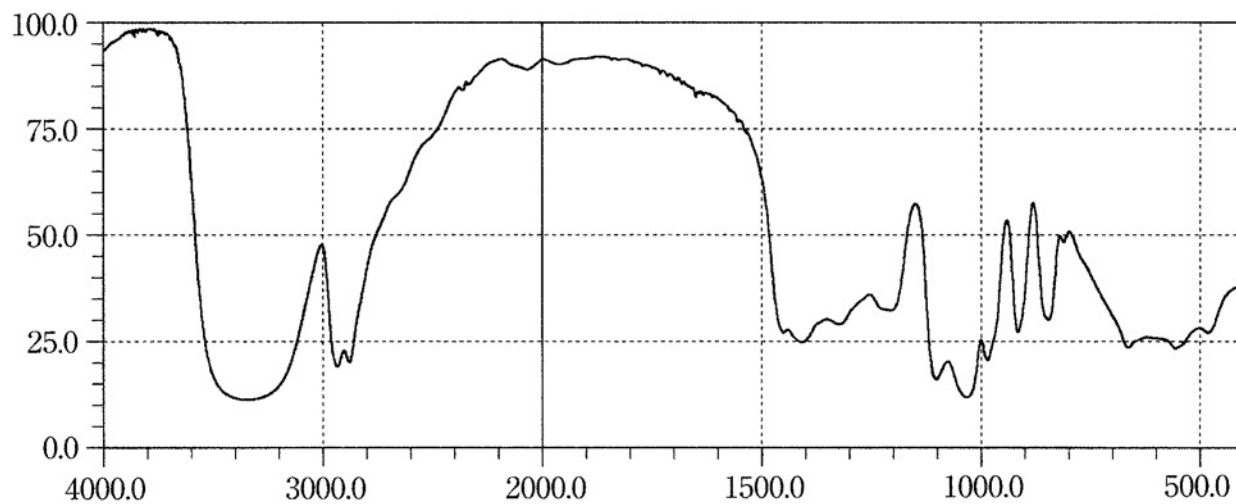


Glutathione

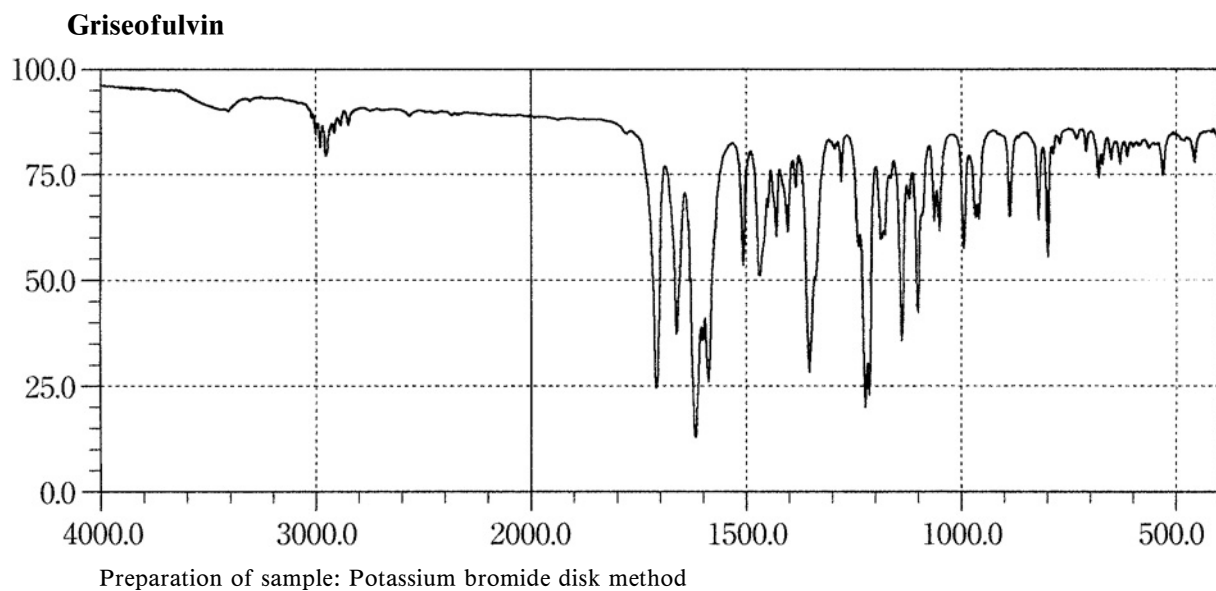
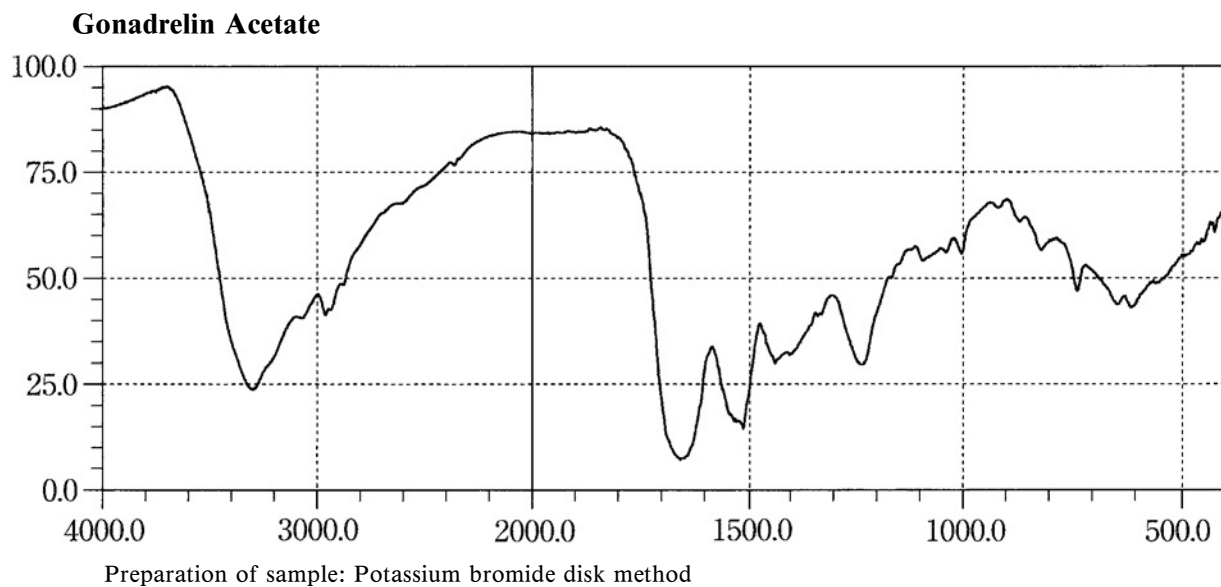
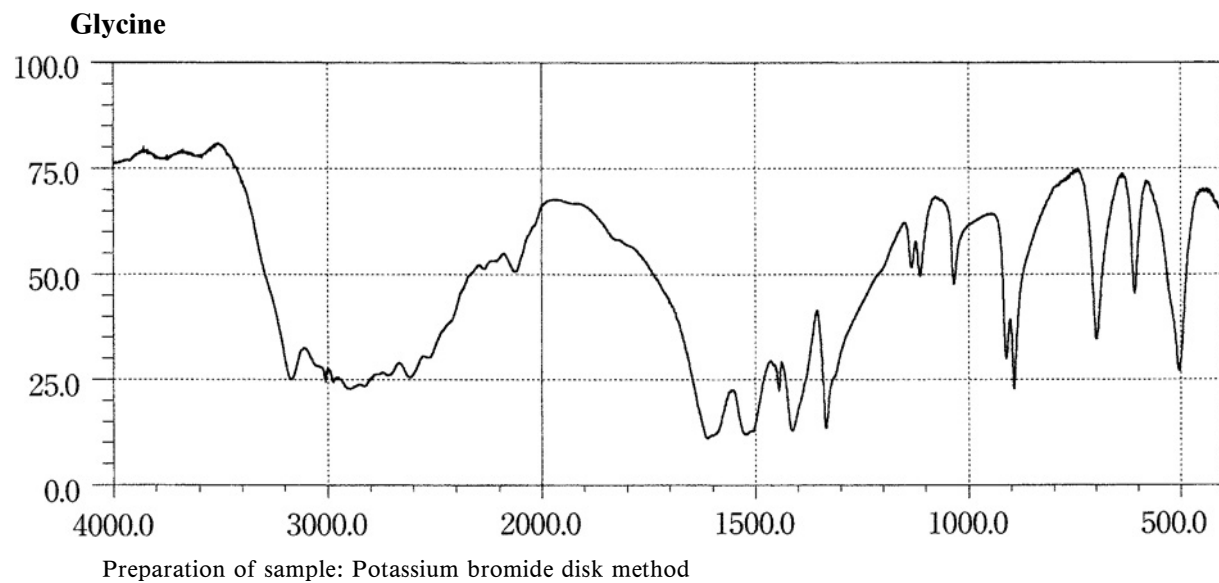
Preparation of sample: Potassium bromide disk method

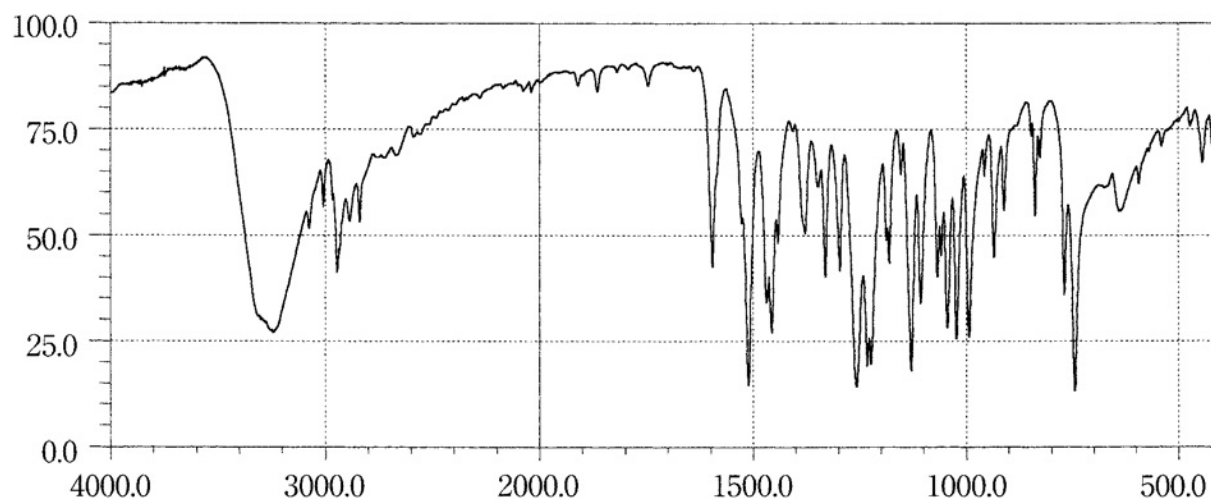
Glycerin

Preparation of sample: Liquid film method

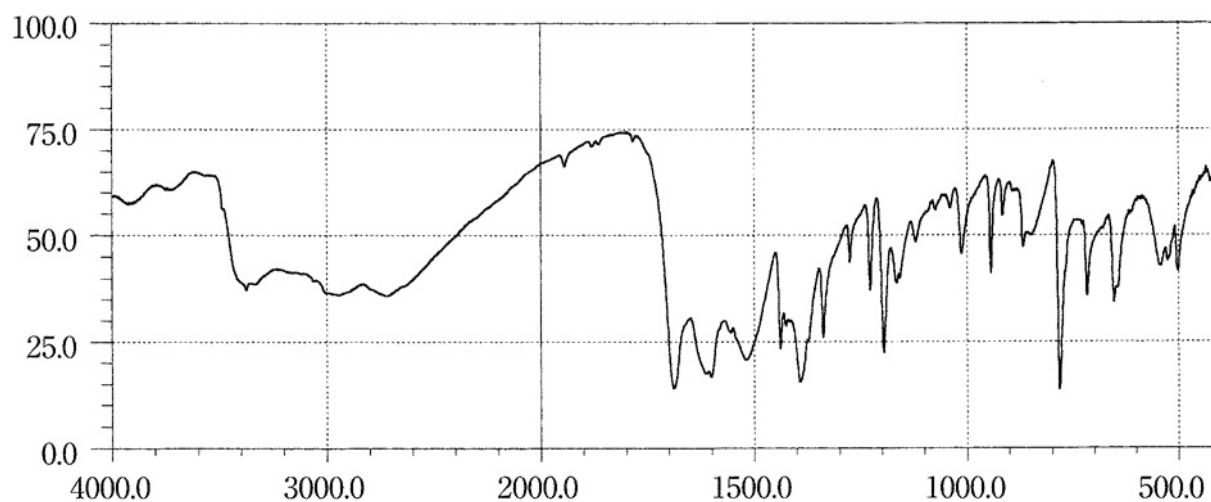
Concentrated Glycerin

Preparation of sample: Liquid film method

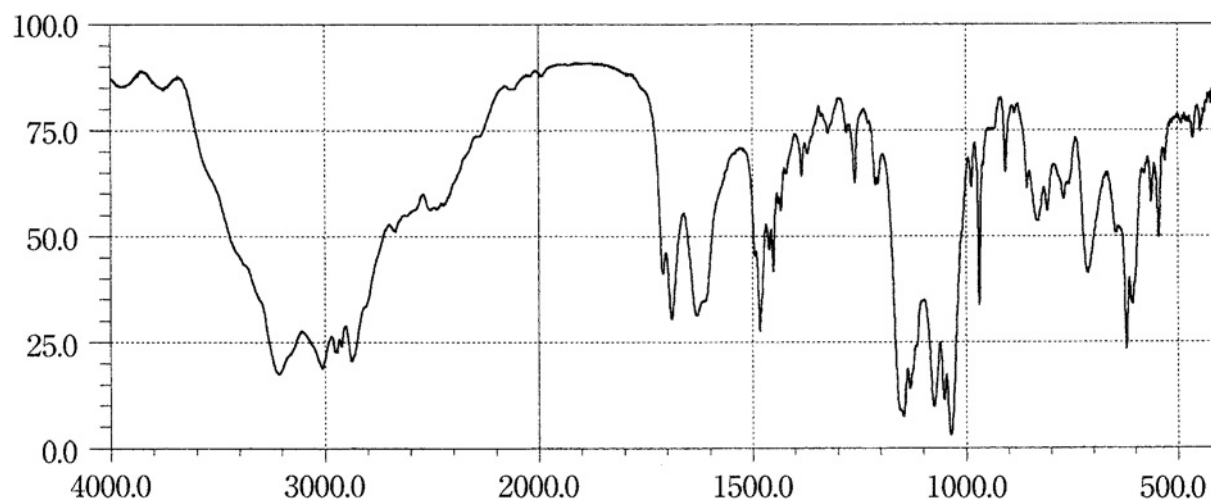


Guaifenesin

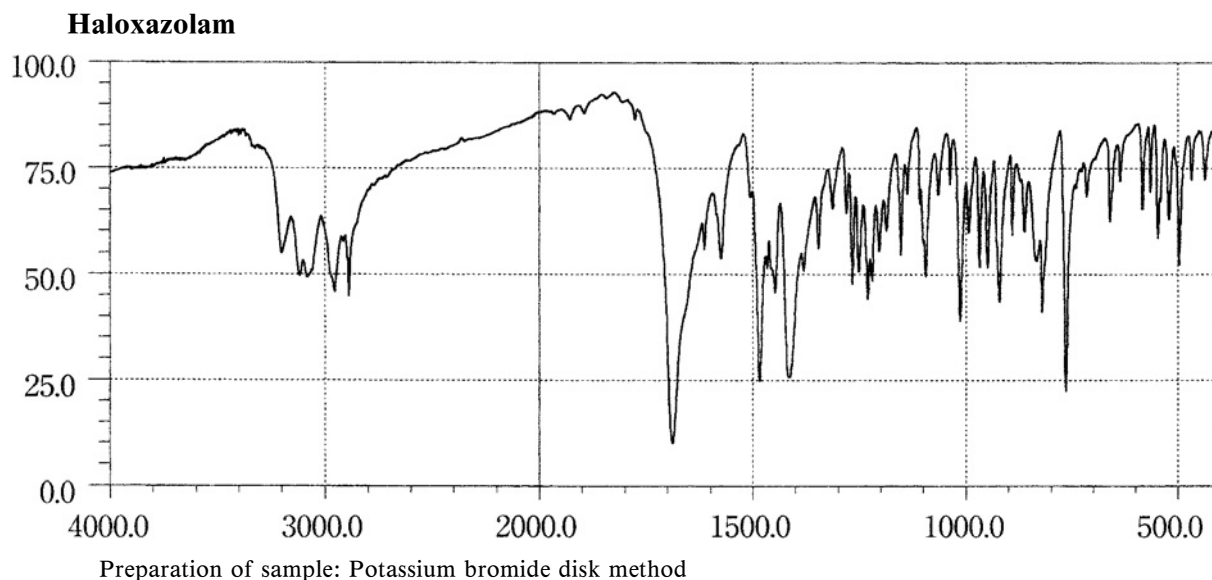
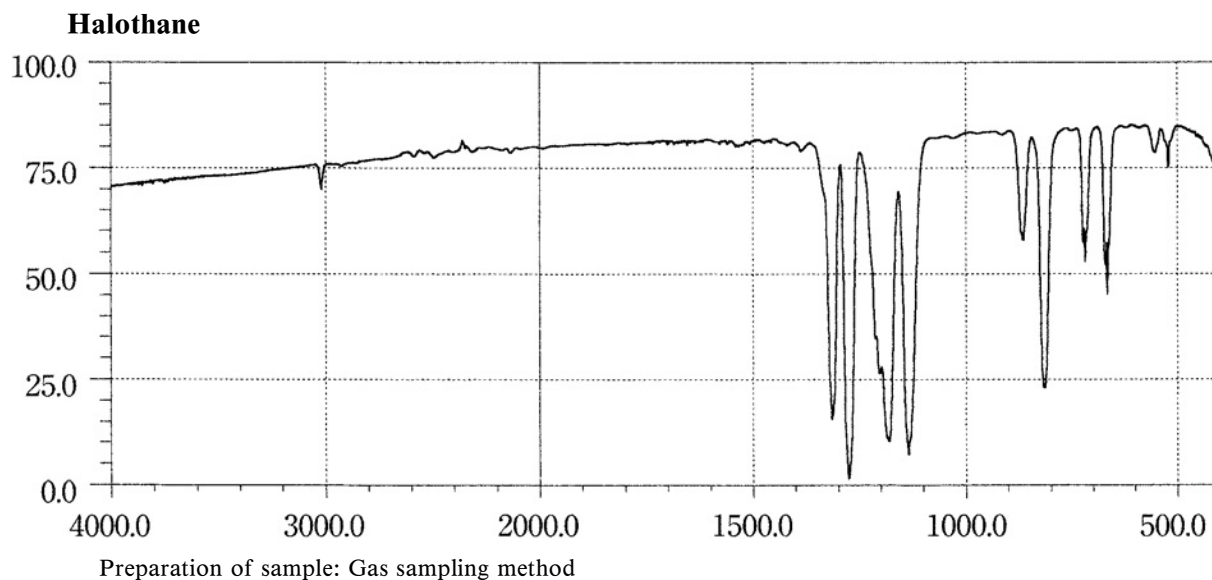
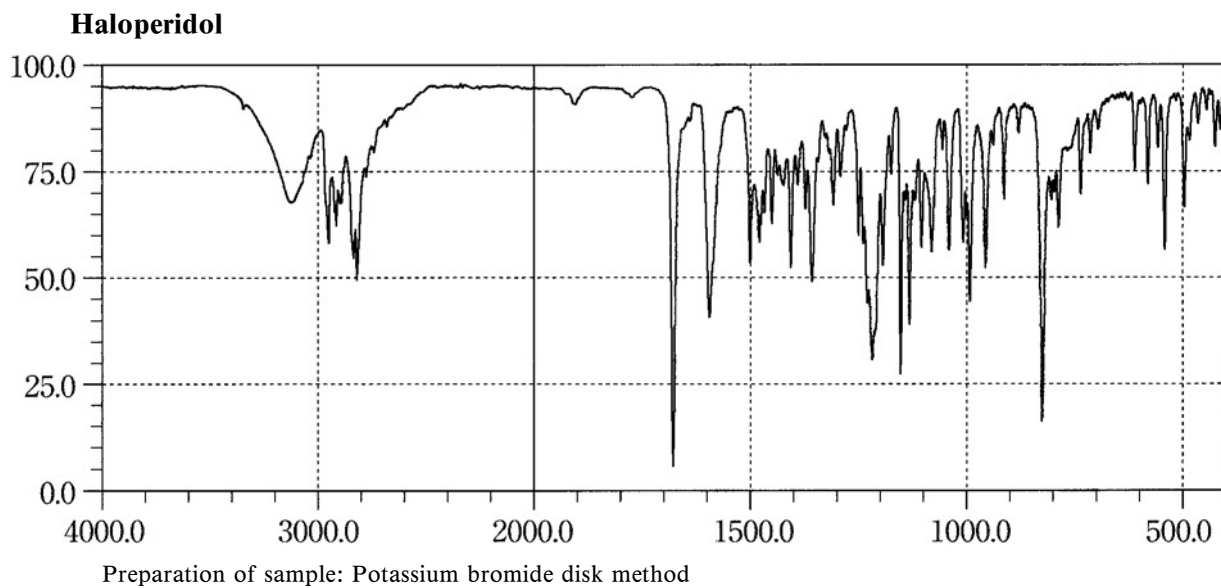
Preparation of sample: Potassium bromide disk method

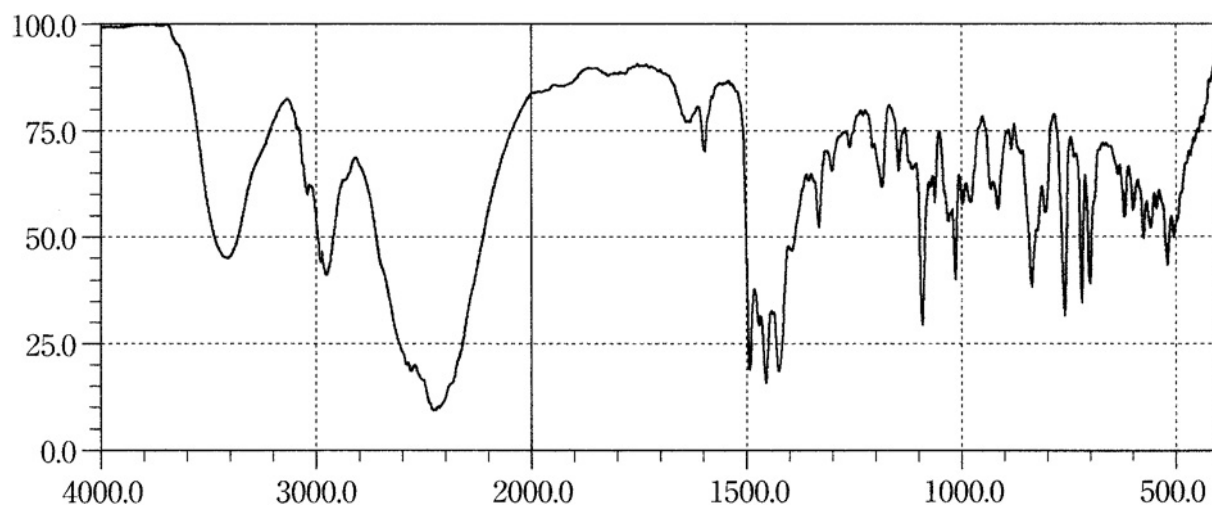
Guanabenz Acetate

Preparation of sample: Potassium bromide disk method

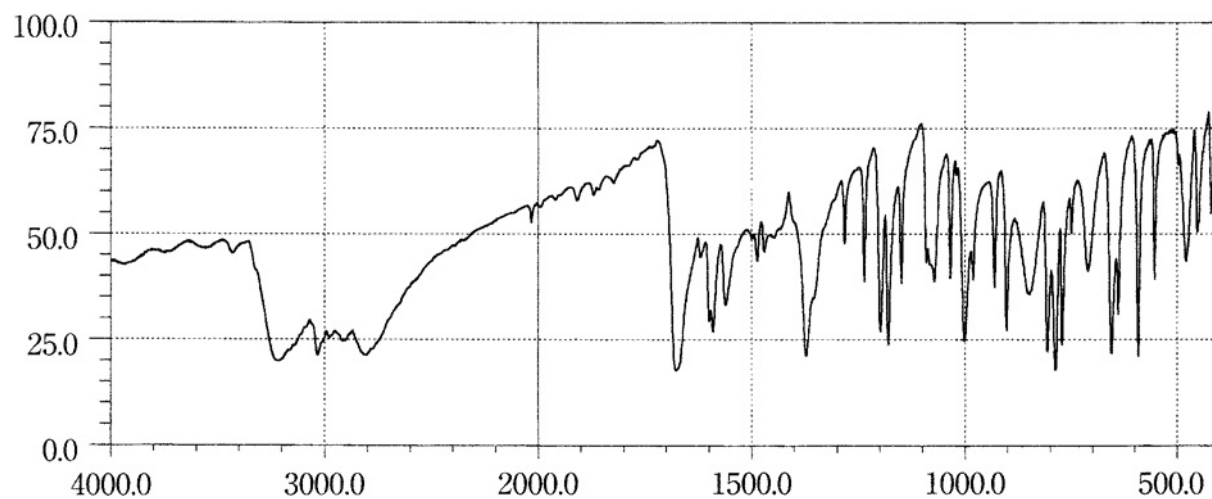
Guanethidine Sulfate

Preparation of sample: Potassium bromide disk method

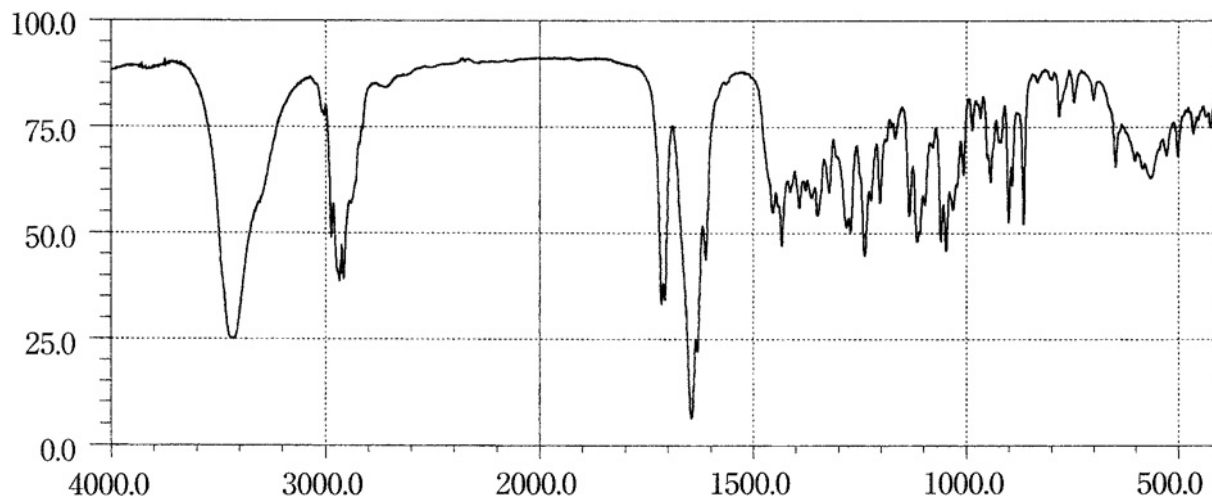


Homochlorcyclizine Hydrochloride

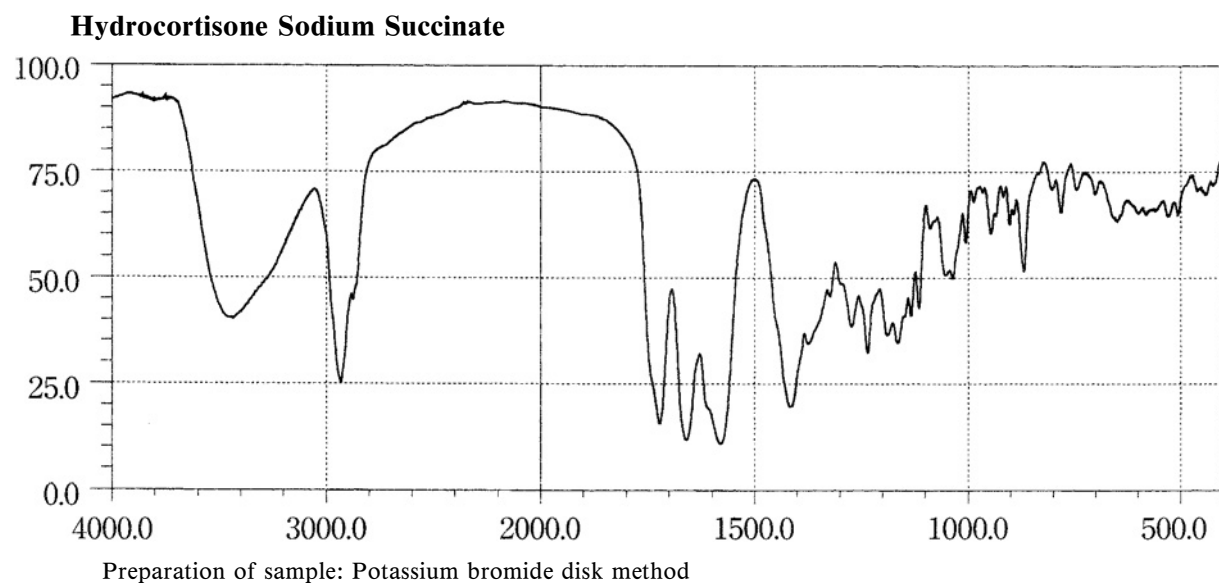
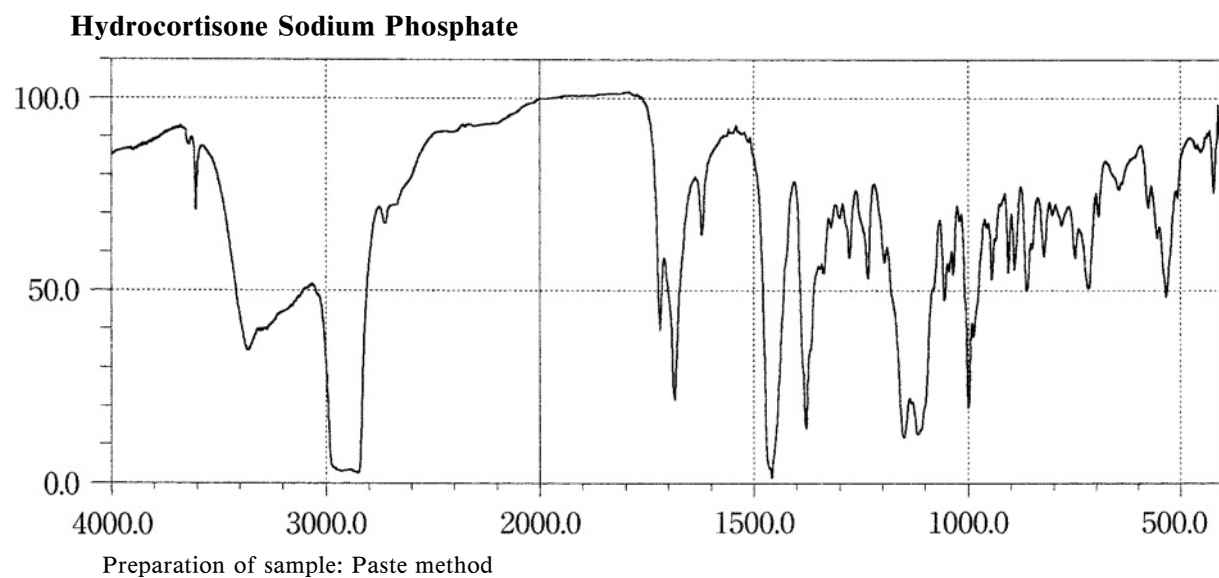
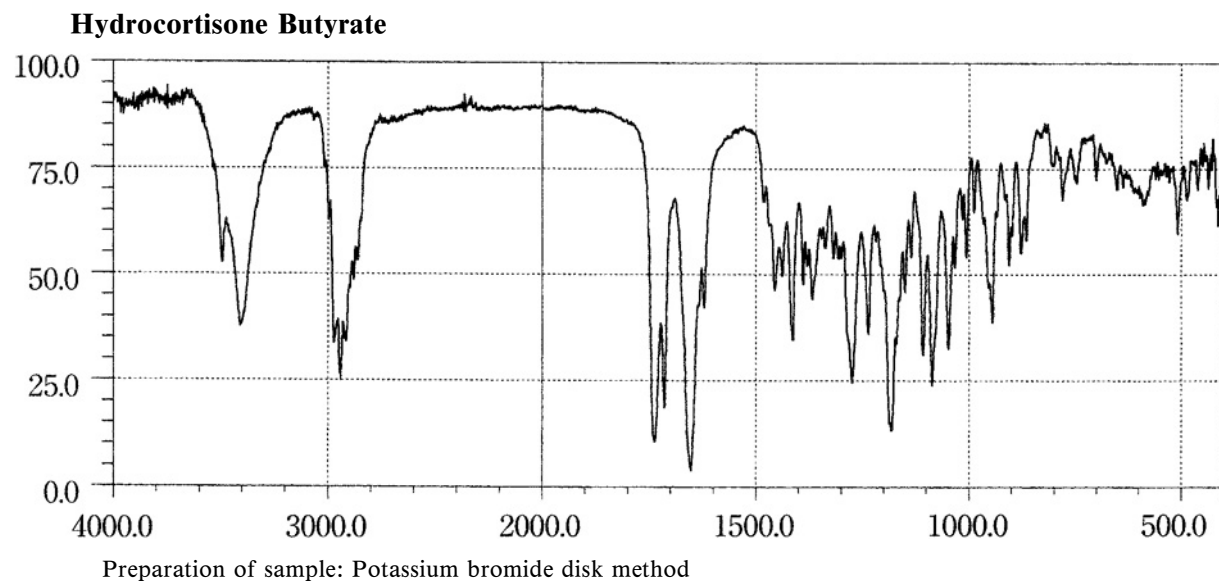
Preparation of sample: Potassium chloride disk method

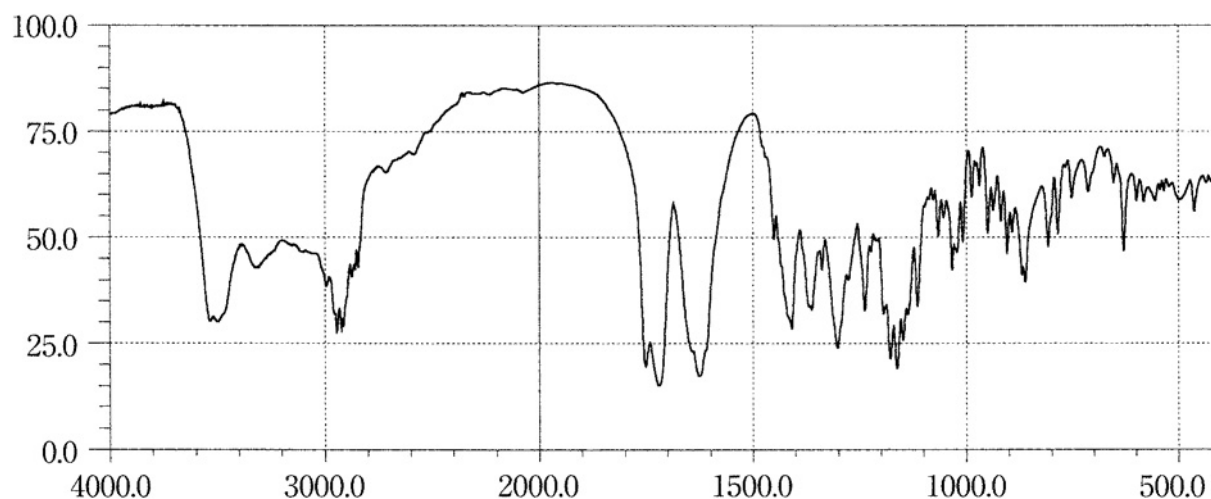
Hydralazine Hydrochloride

Preparation of sample: Potassium bromide disk method

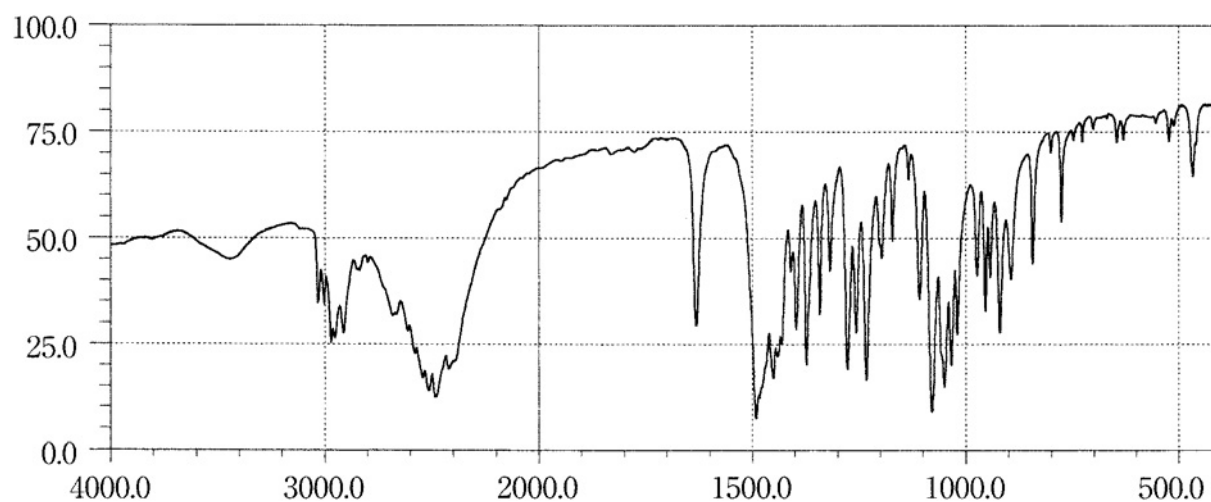
Hydrocortisone

Preparation of sample: Potassium bromide disk method

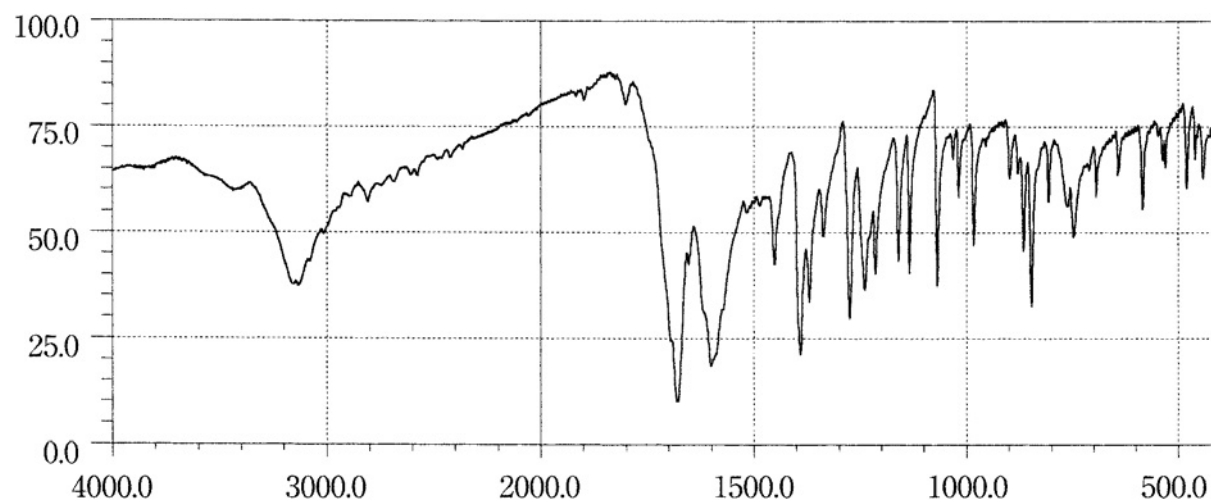


Hydrocortisone Succinate

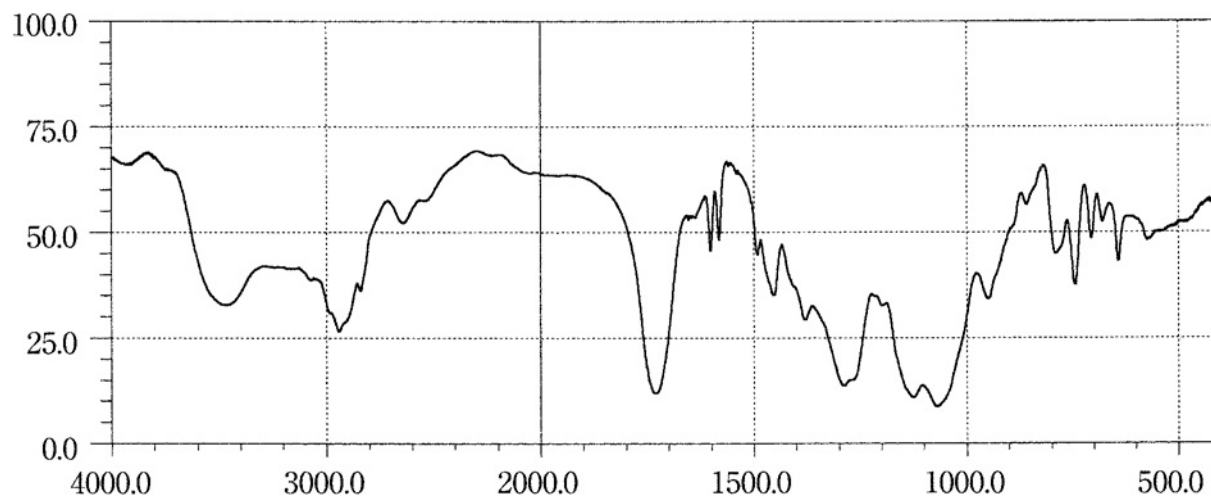
Preparation of sample: Potassium bromide disk method

Hydrocotarnine Hydrochloride Hydrate

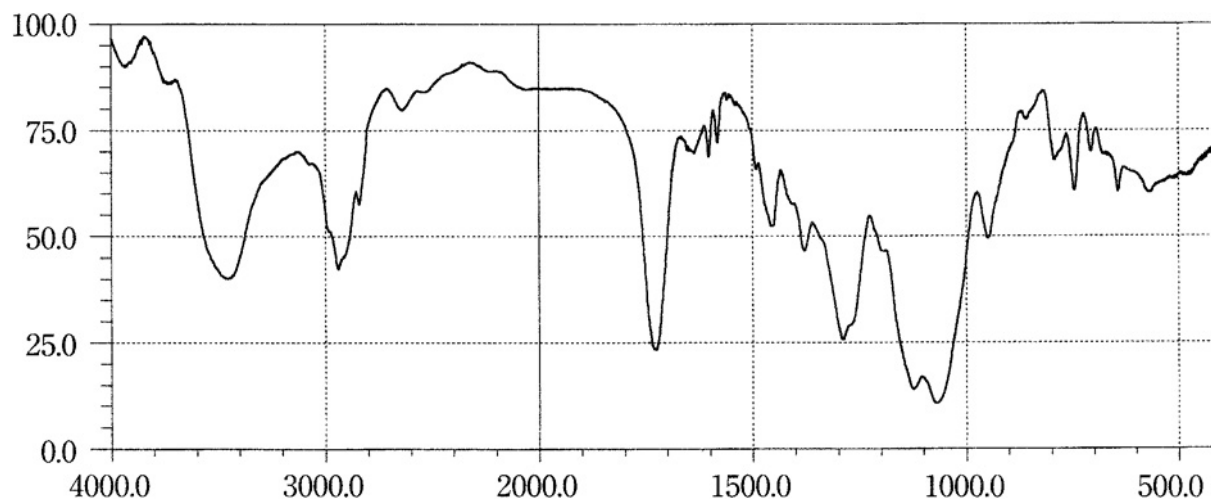
Preparation of sample: Potassium bromide disk method

Hymecromone

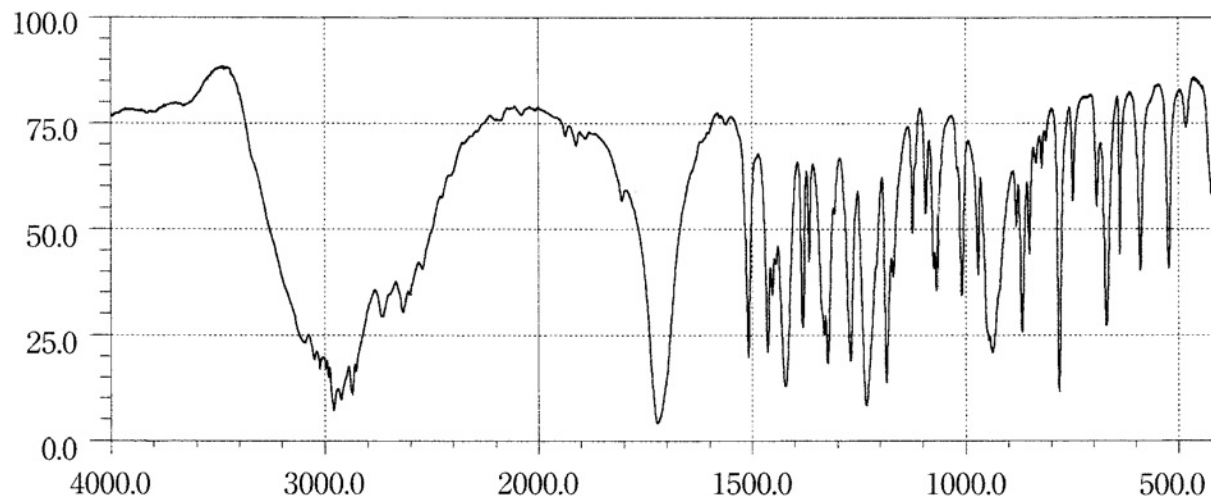
Preparation of sample: Potassium bromide disk method

Hypromellose Phthalate (200713)

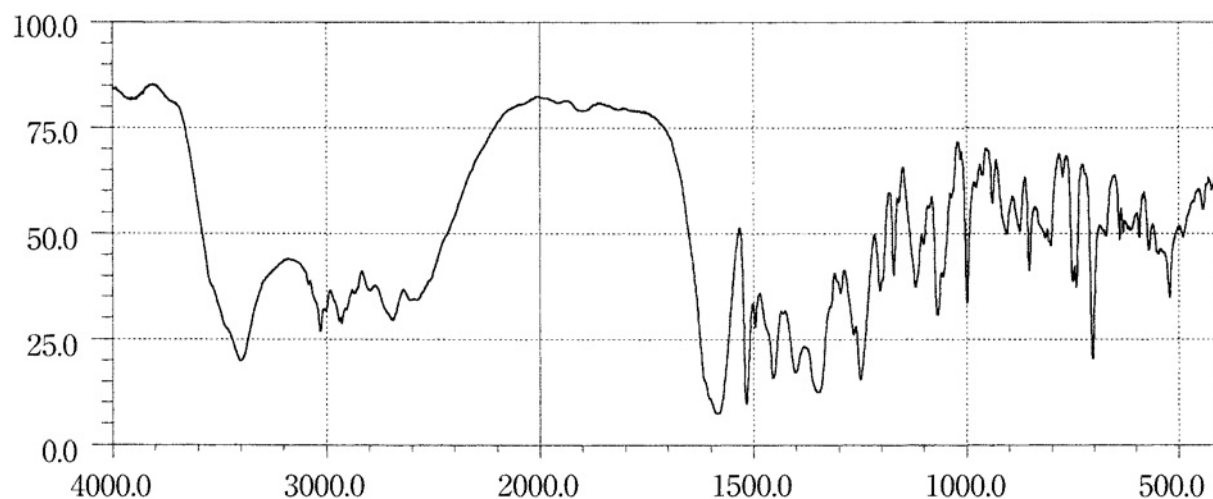
Preparation of sample: Potassium bromide disk method

Hypromellose Phthalate (220824)

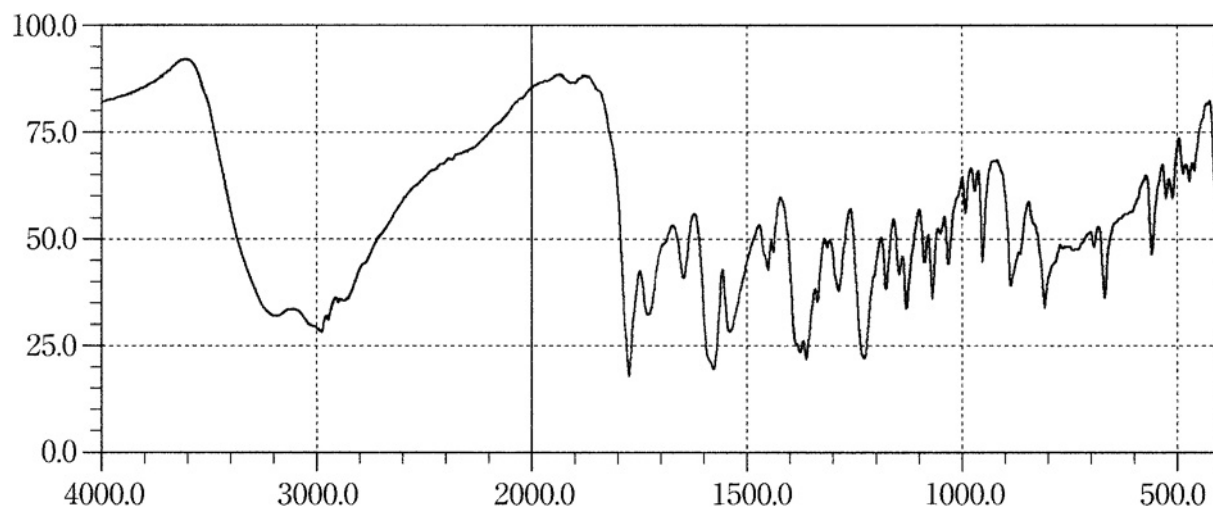
Preparation of sample: Potassium bromide disk method

Ibuprofen

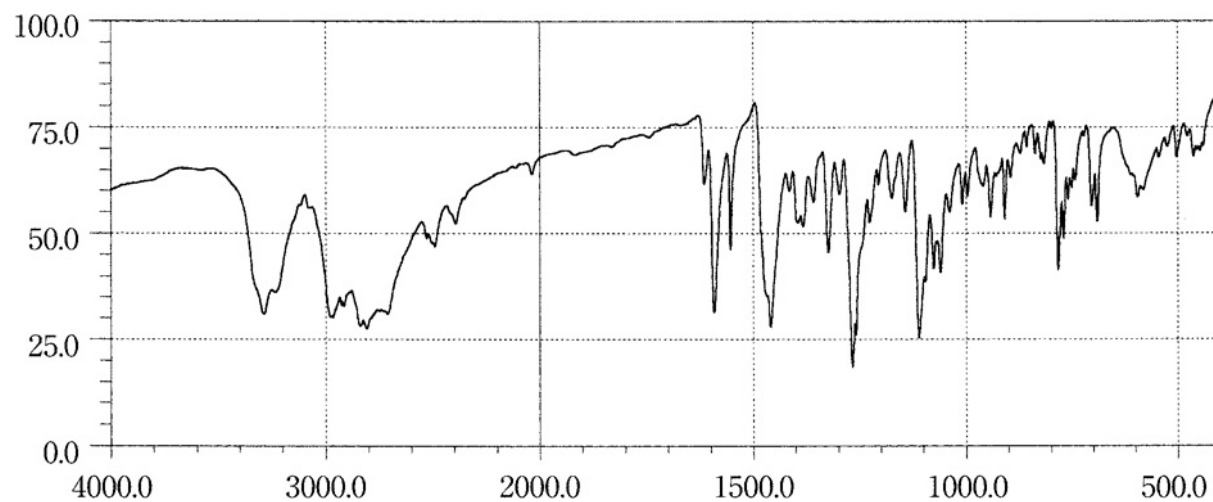
Preparation of sample: Potassium bromide disk method

Ifenprodil Tartrate

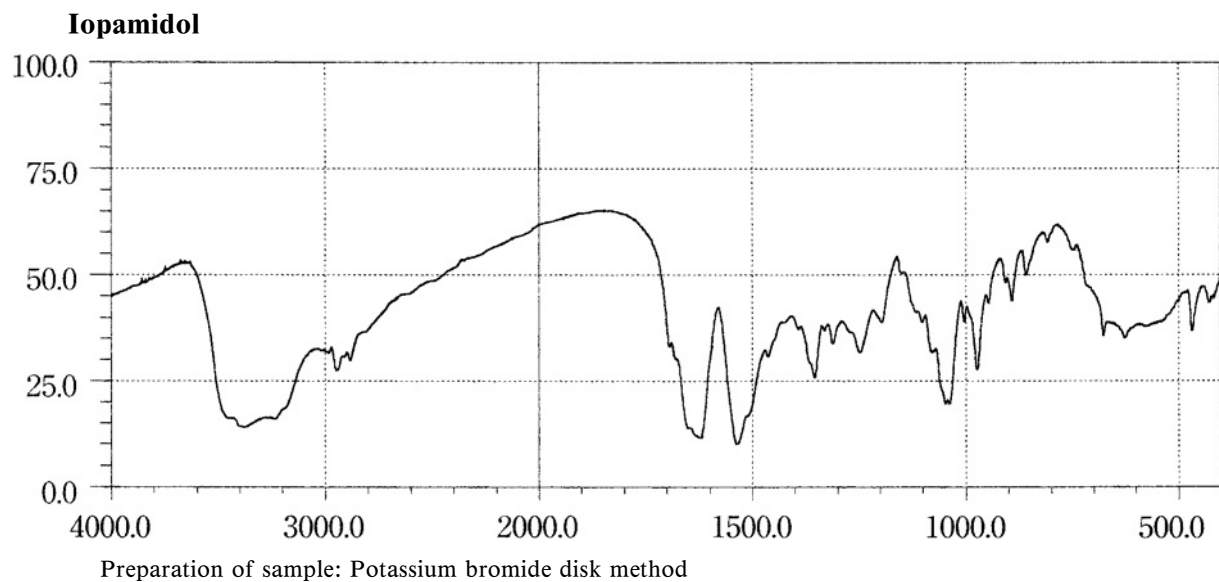
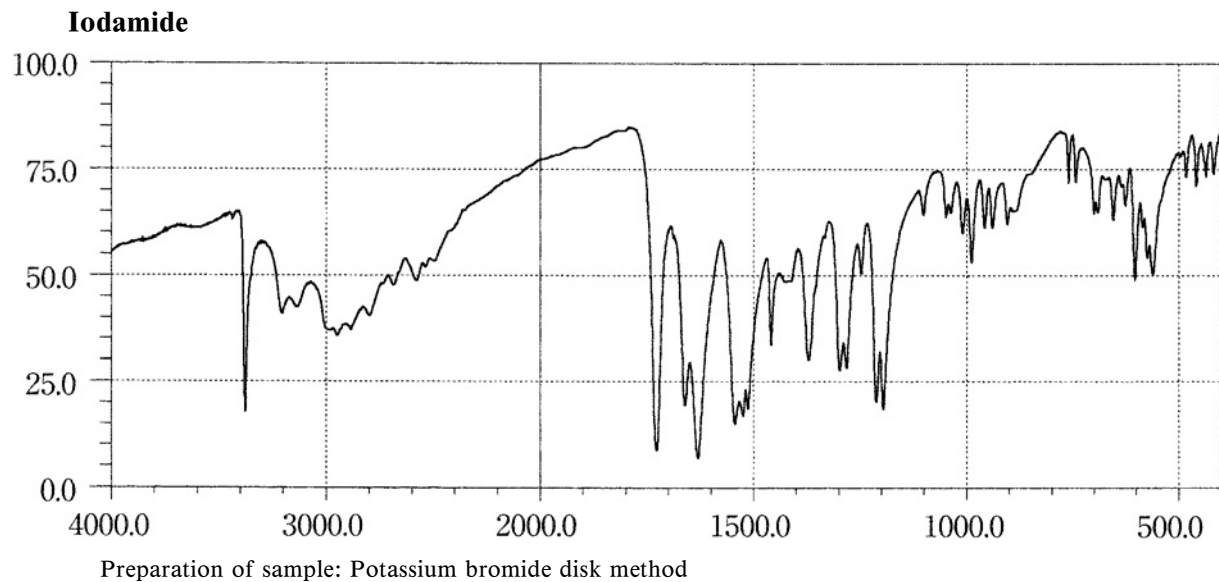
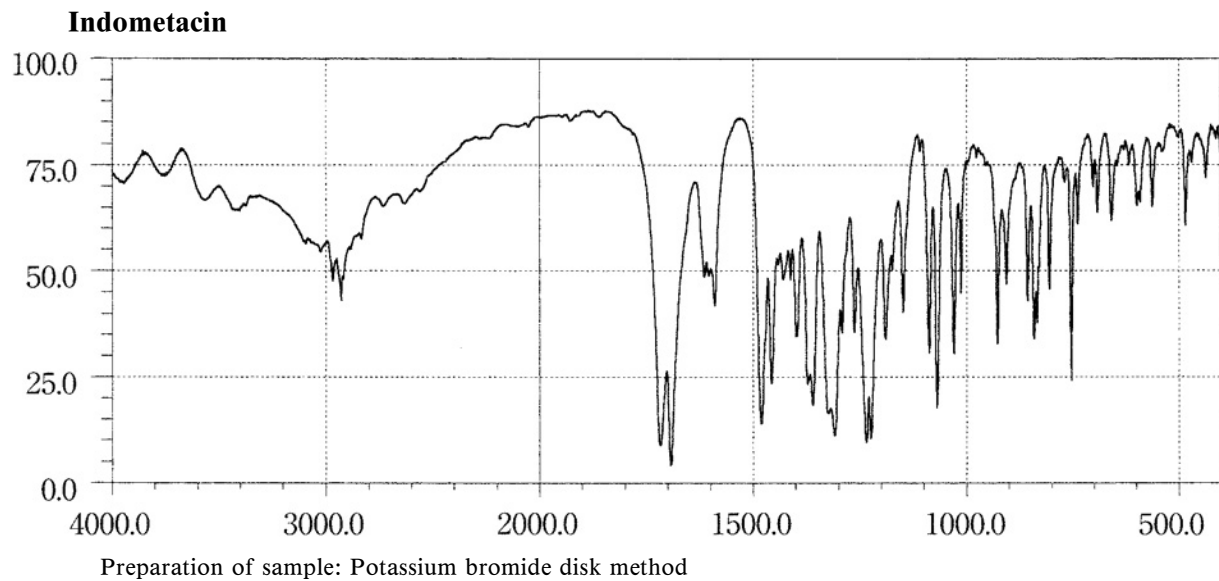
Preparation of sample: Potassium bromide disk method

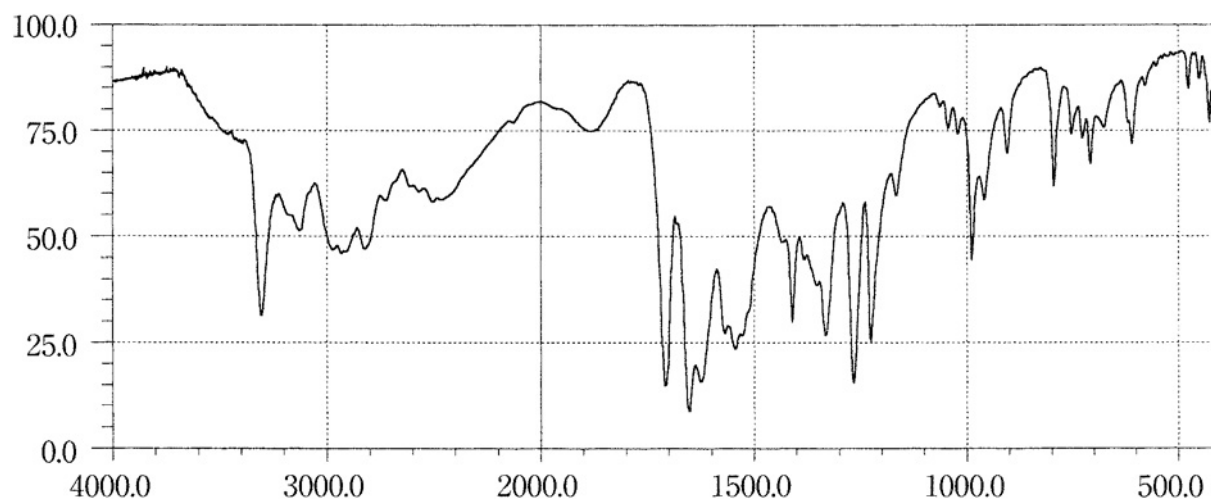
Imipenem Hydrate

Preparation of sample: Potassium bromide disk method

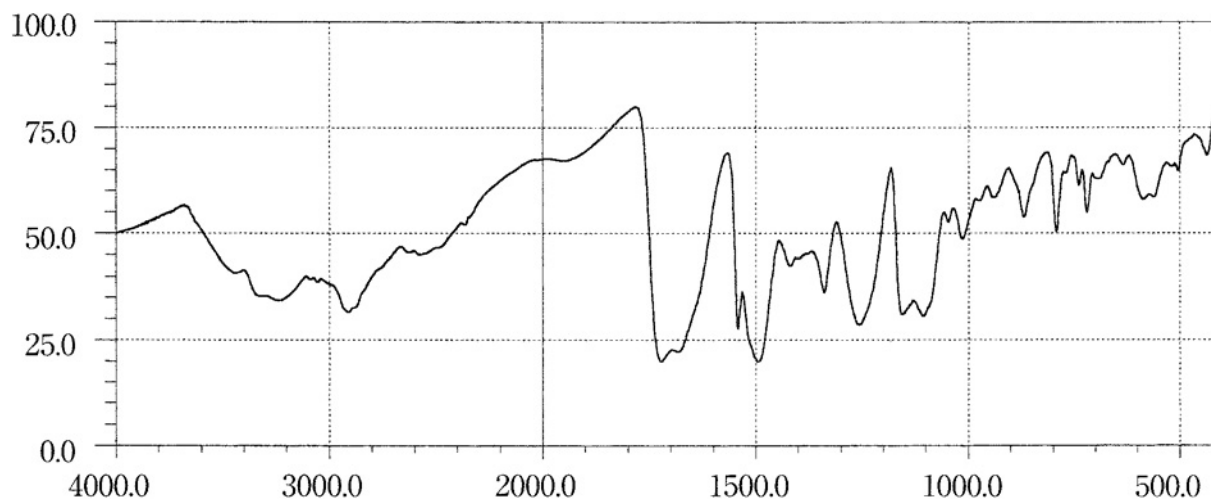
Indenolol Hydrochloride

Preparation of sample: Potassium chloride disk method

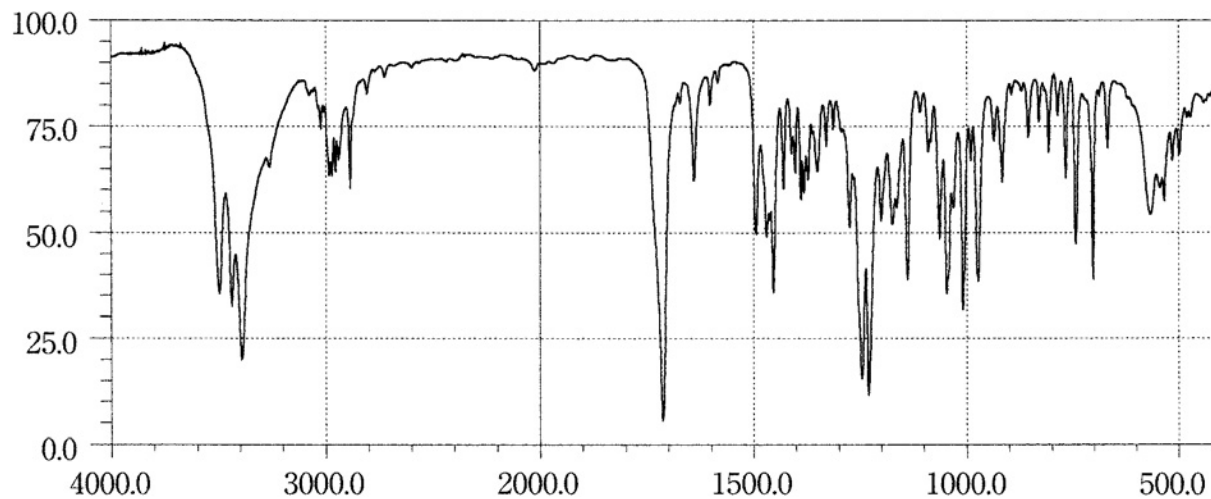


Iotalamic Acid

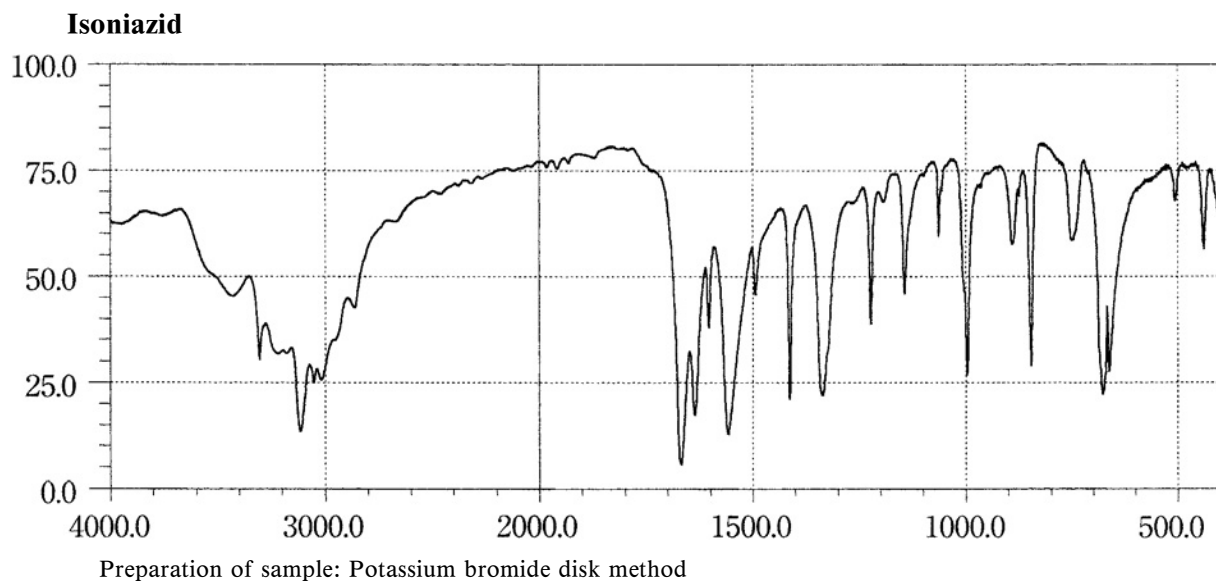
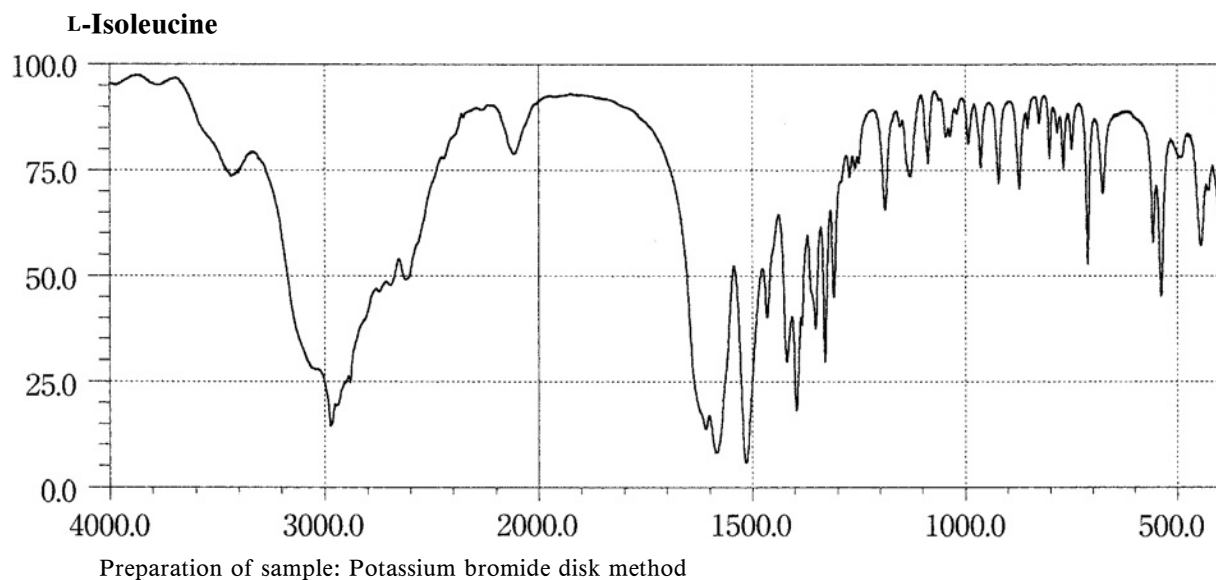
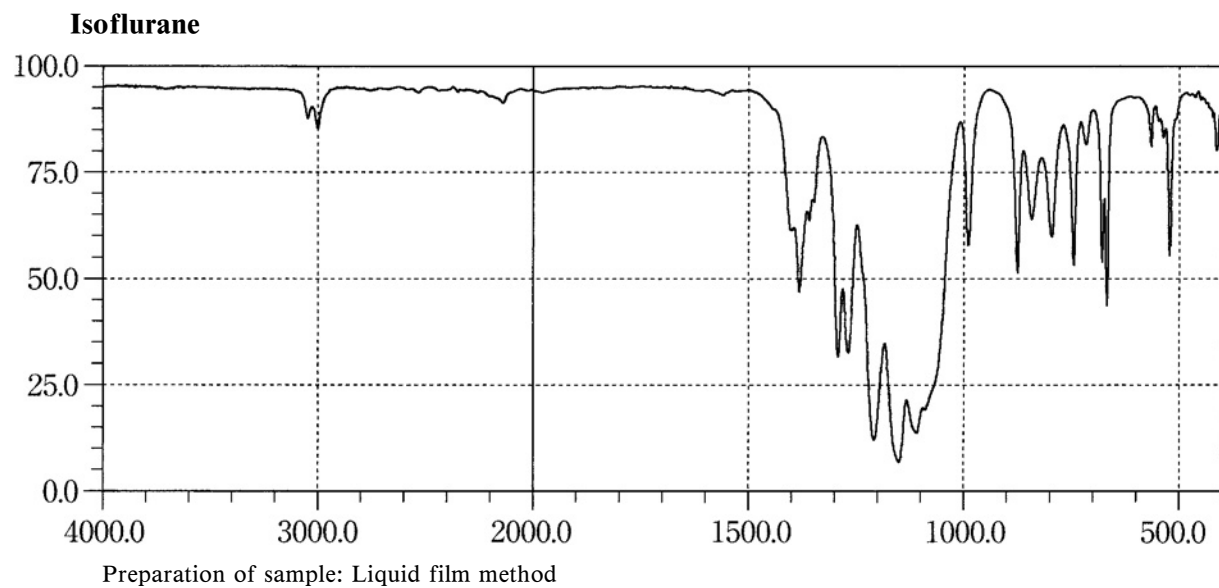
Preparation of sample: Potassium bromide disk method

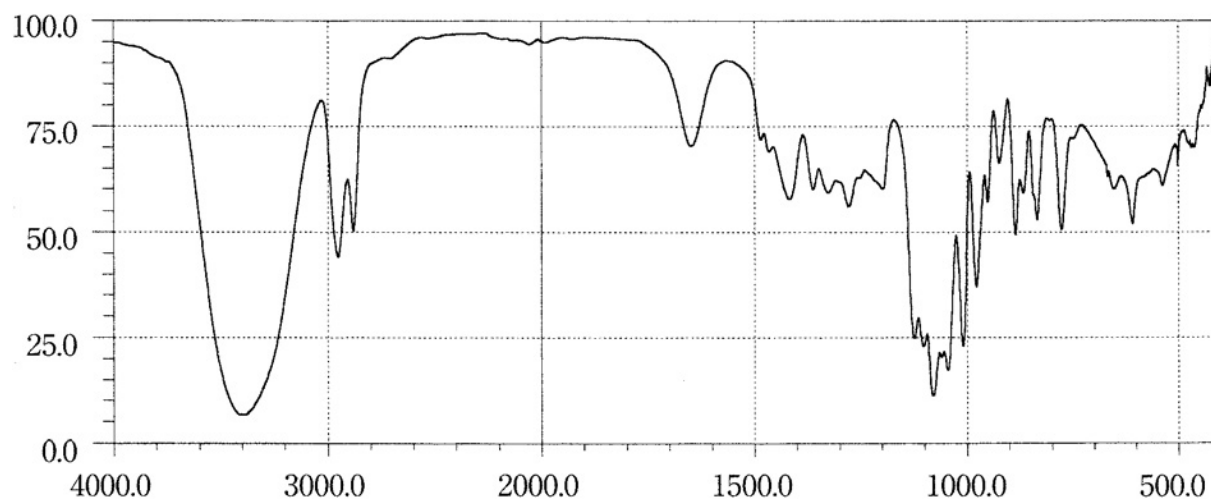
Ietroxic Acid

Preparation of sample: Potassium bromide disk method

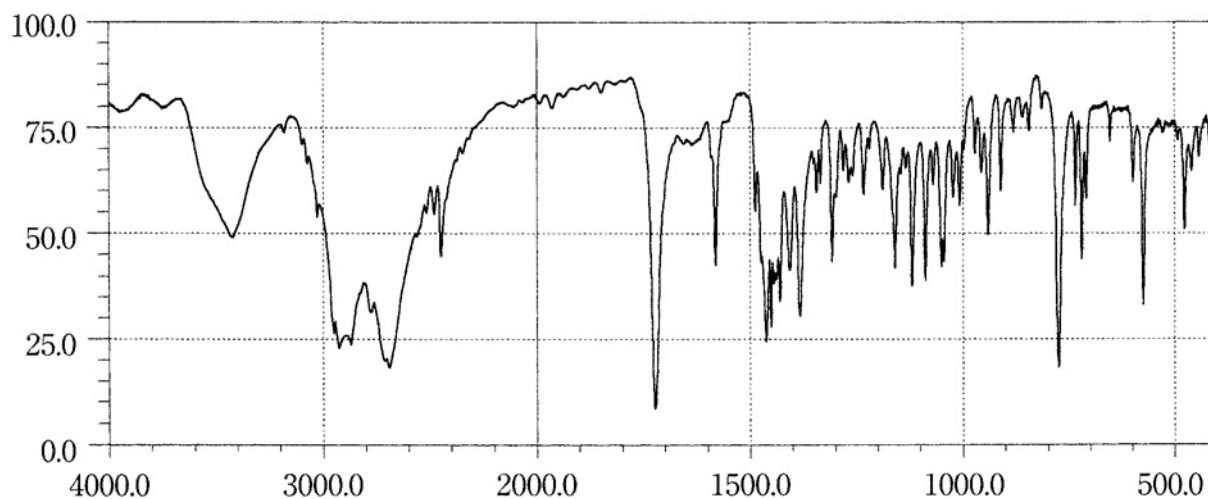
Ipratropium Bromide Hydrate

Preparation of sample: Potassium bromide disk method

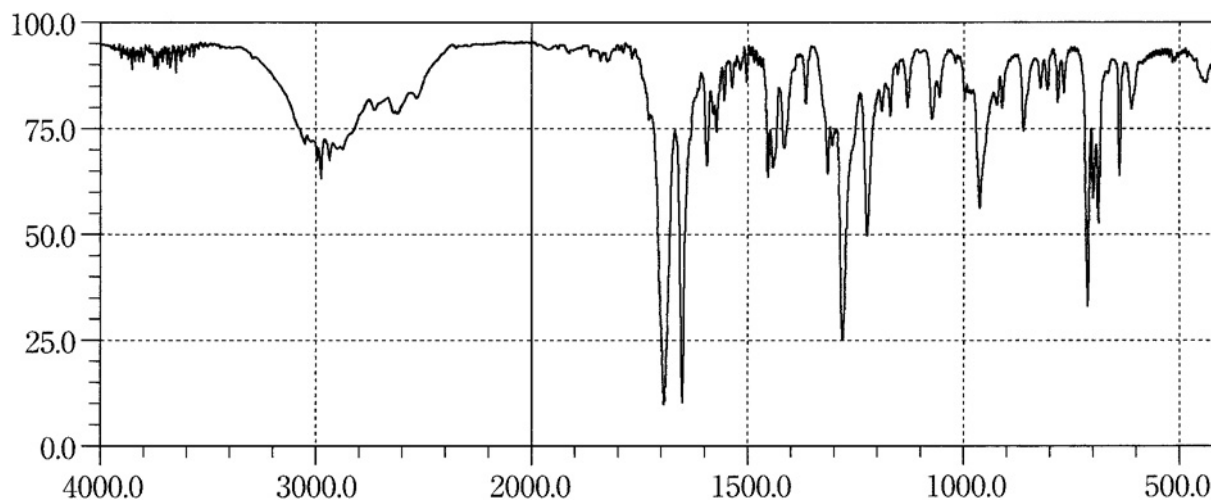


Isosorbide

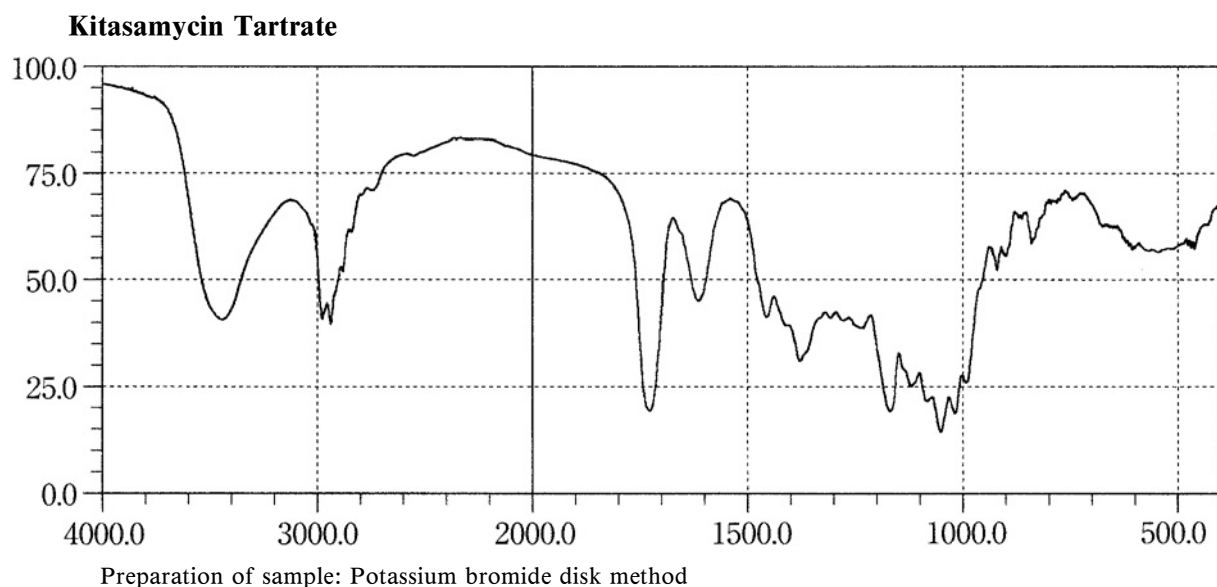
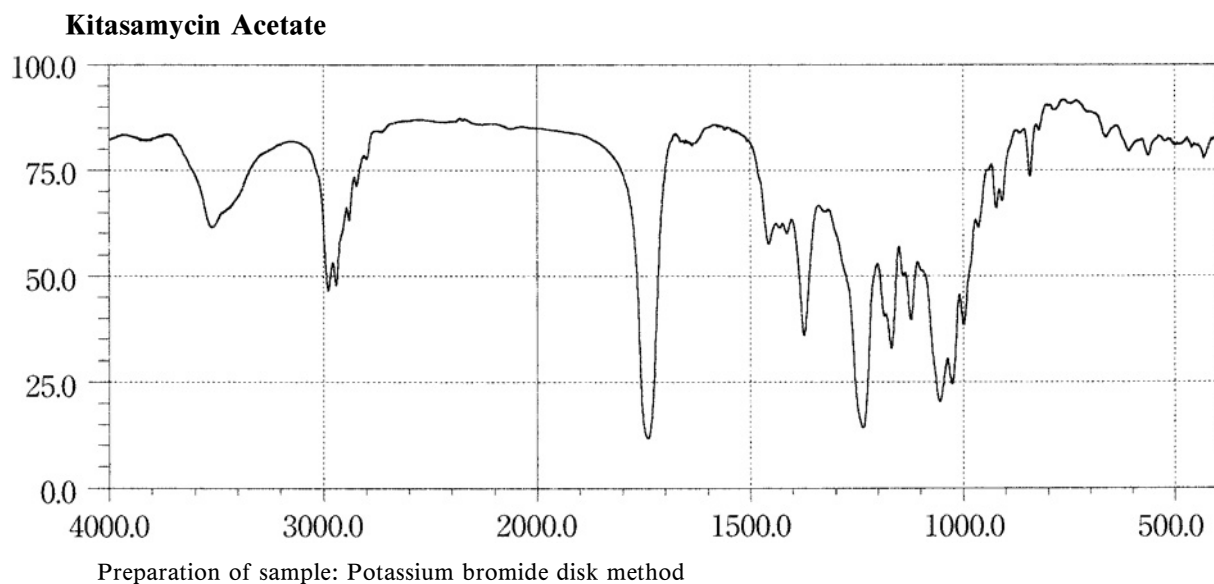
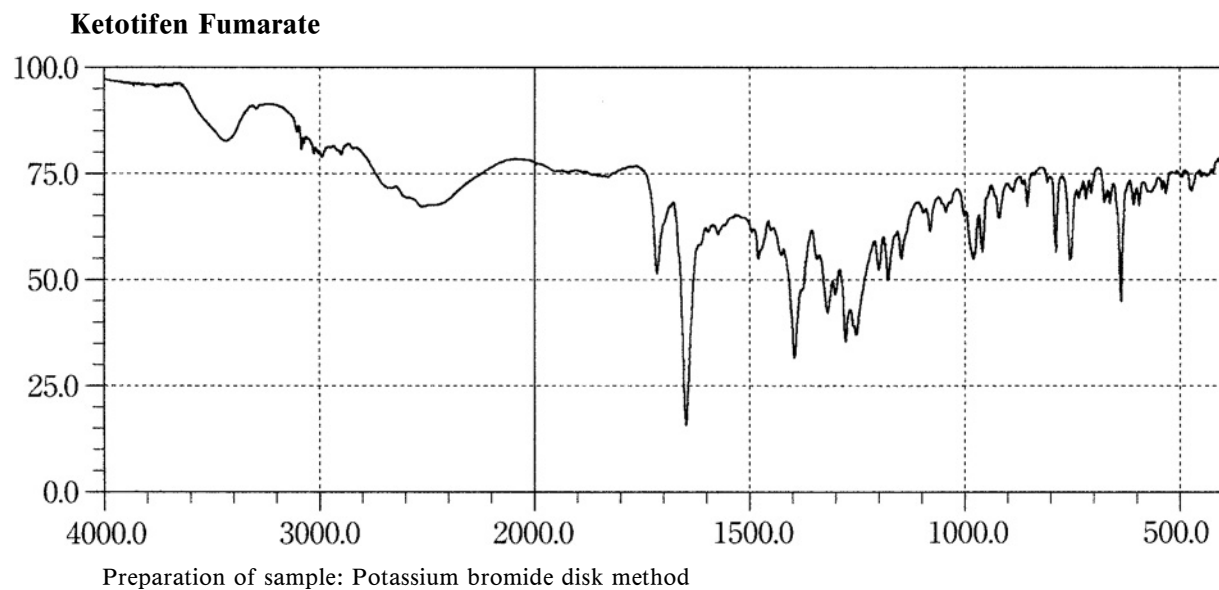
Preparation of sample: Potassium bromide disk method

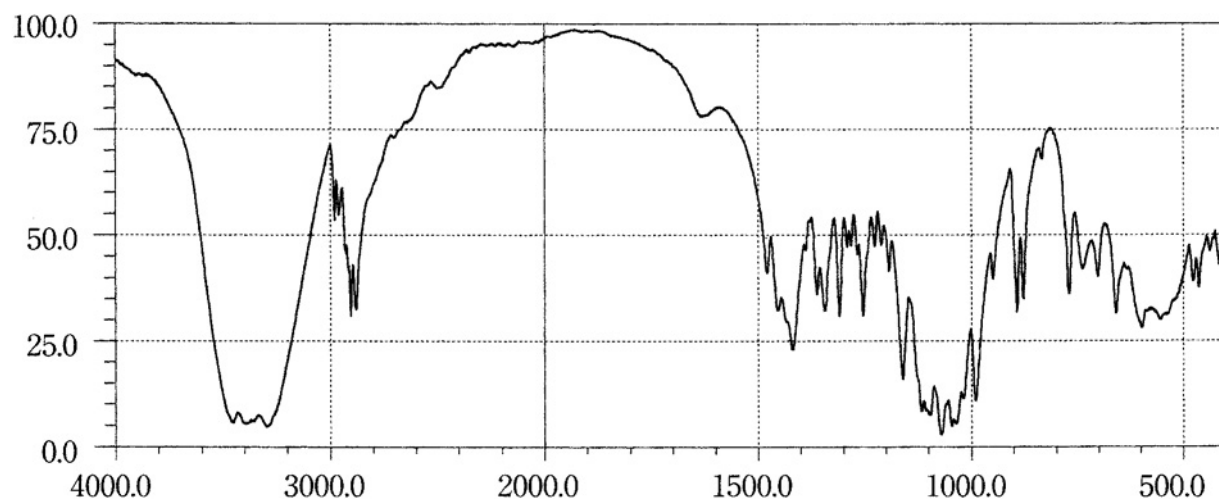
Ketamine Hydrochloride

Preparation of sample: Potassium bromide disk method

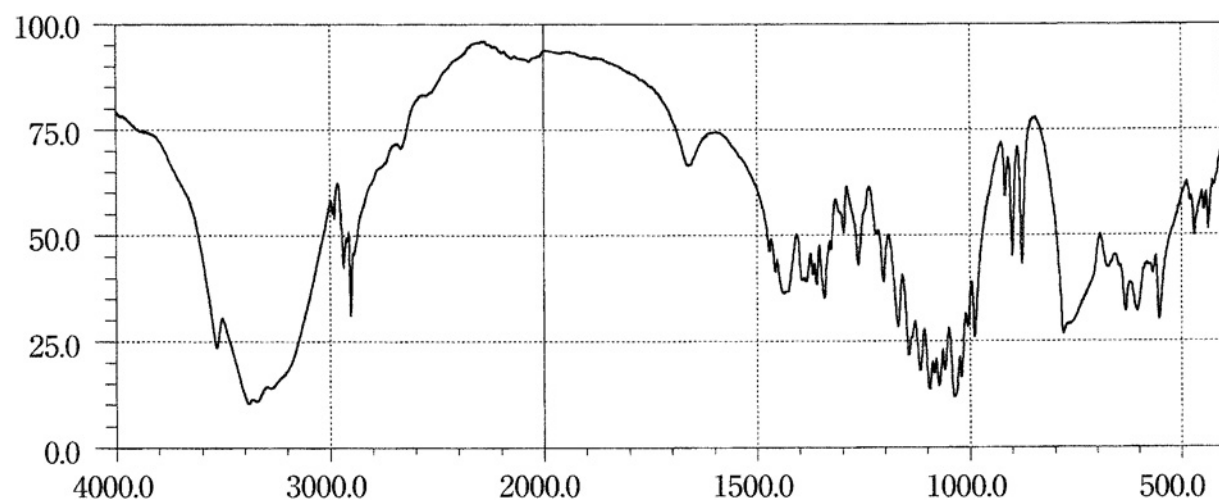
Ketoprofen

Preparation of sample: Potassium bromid disk method

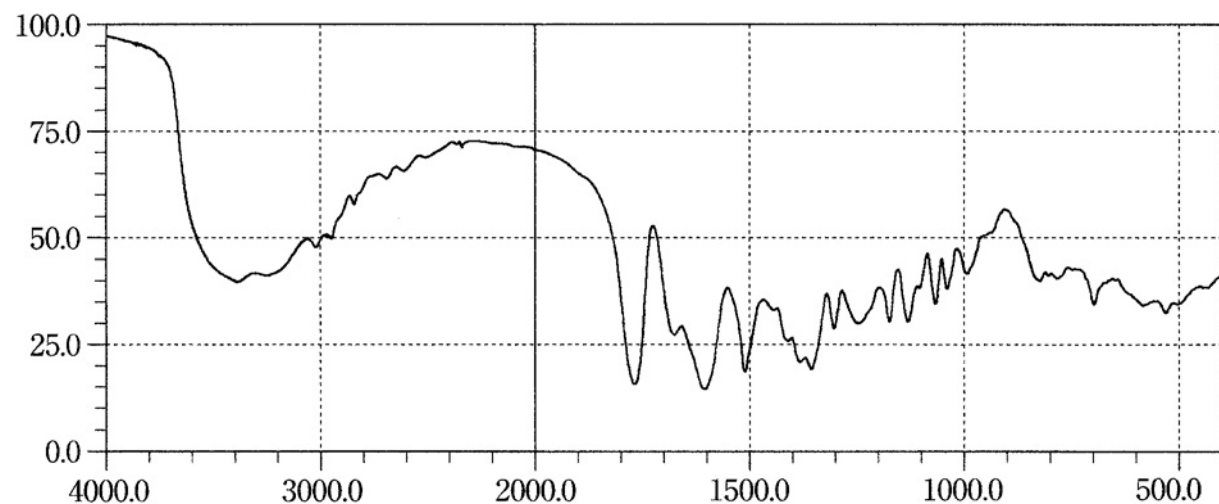


Anhydrous Lactose

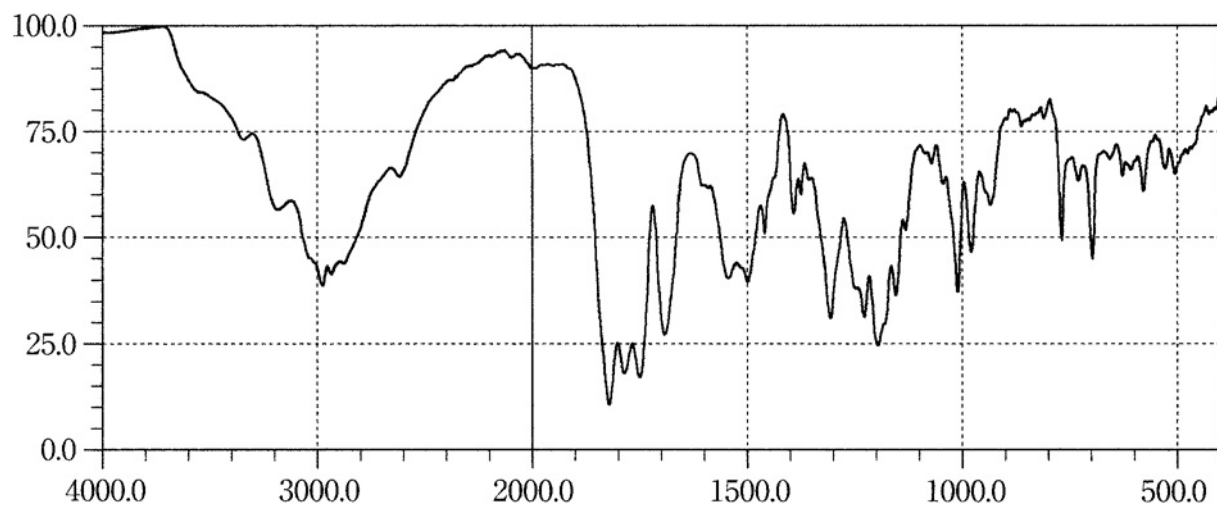
Preparation of sample: Potassium bromide disk method

Lactose Hydrate

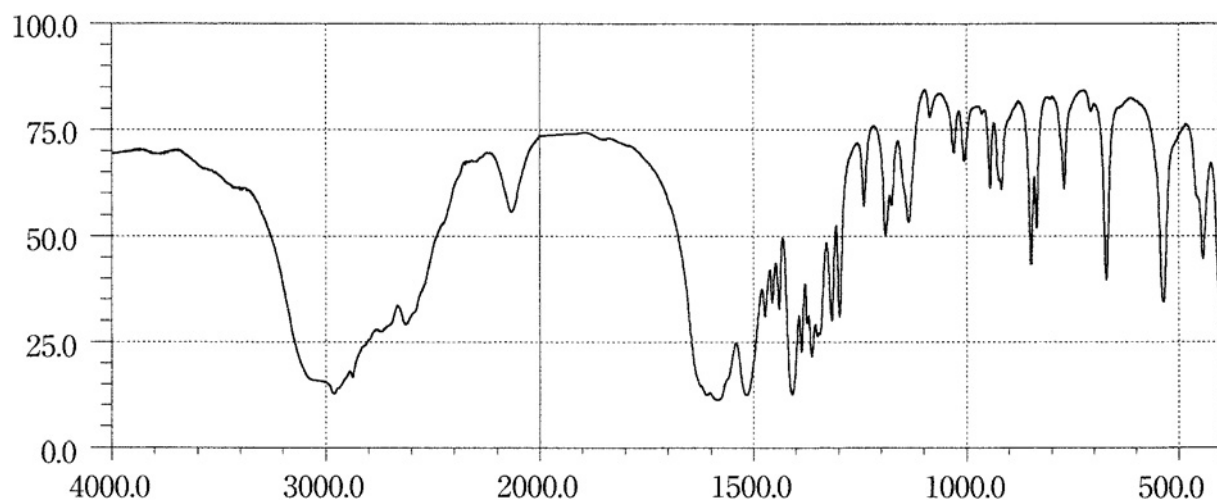
Preparation of sample: Potassium bromide disk method

Latamoxef Sodium

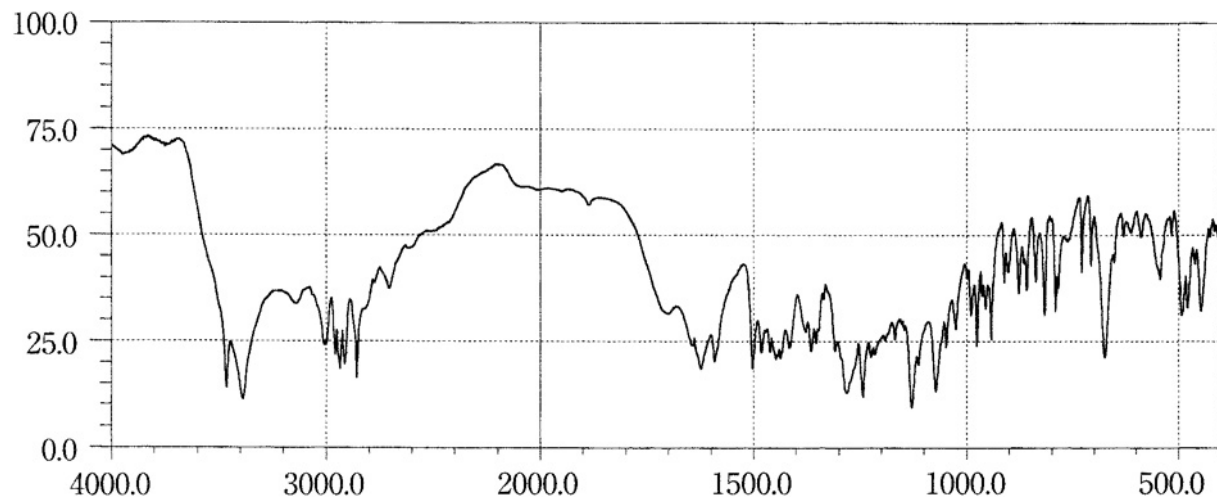
Preparation of sample: Potassium bromide disk method

Lenampicillin Hydrochloride

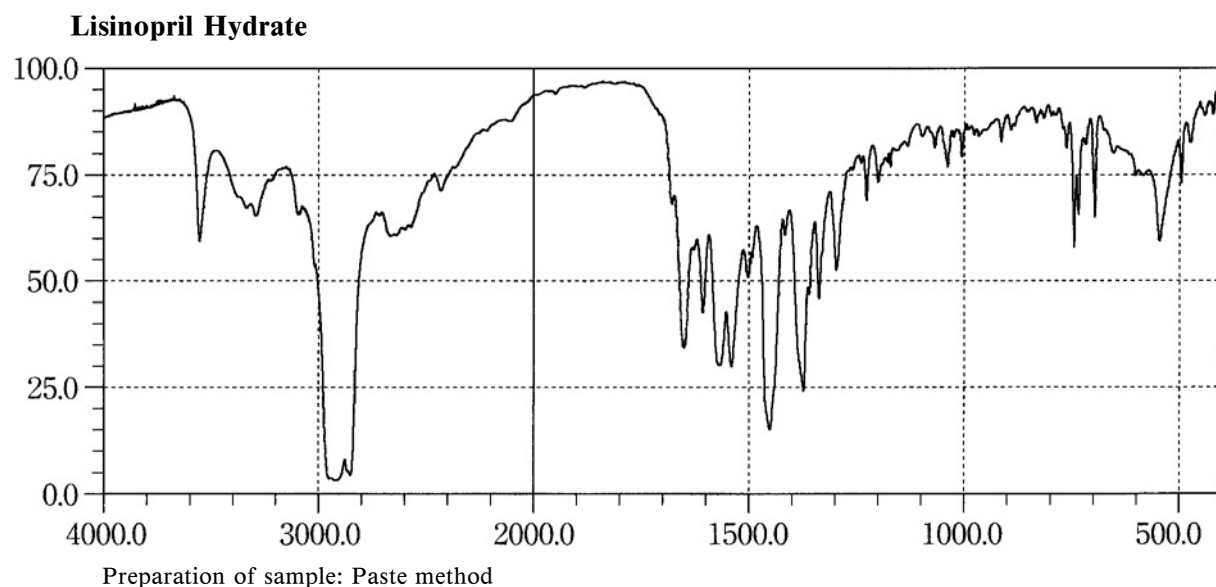
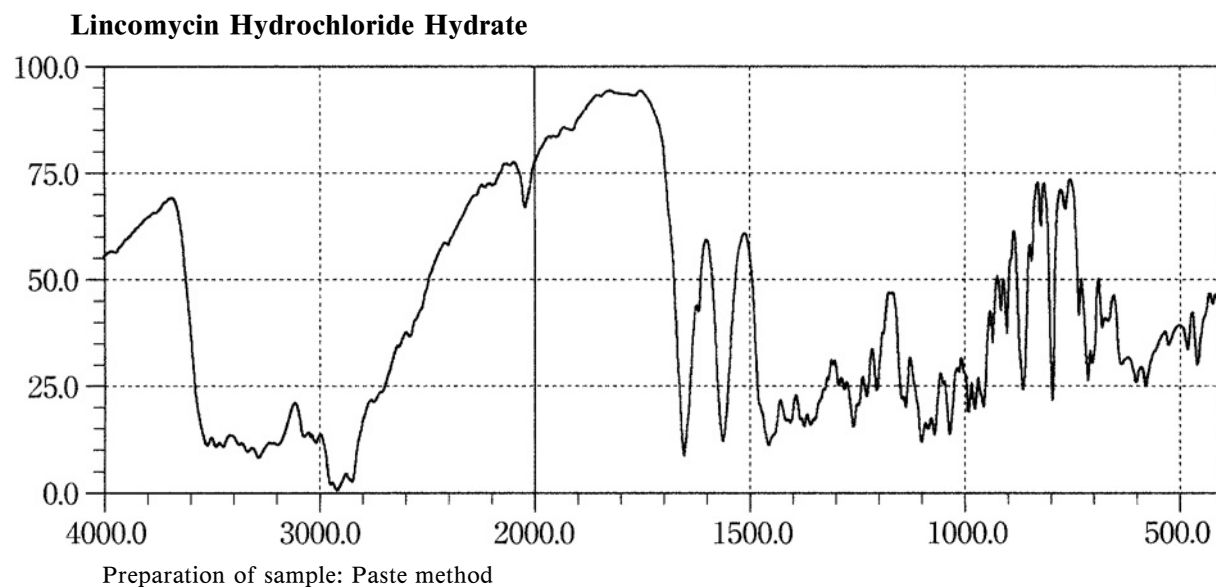
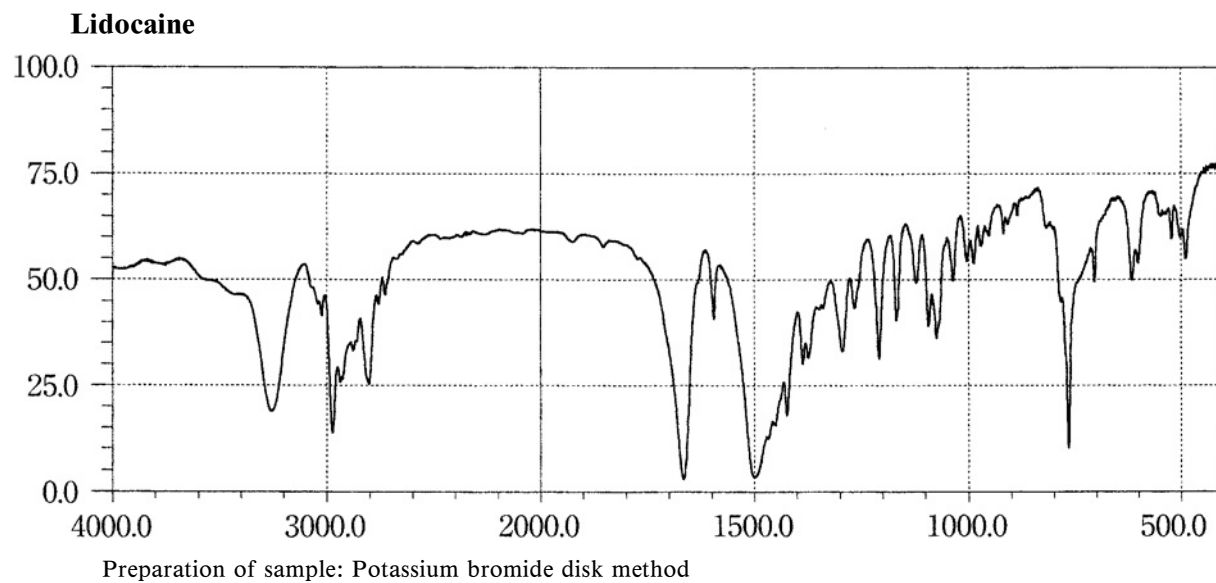
Preparation of sample: Potassium chloride disk method

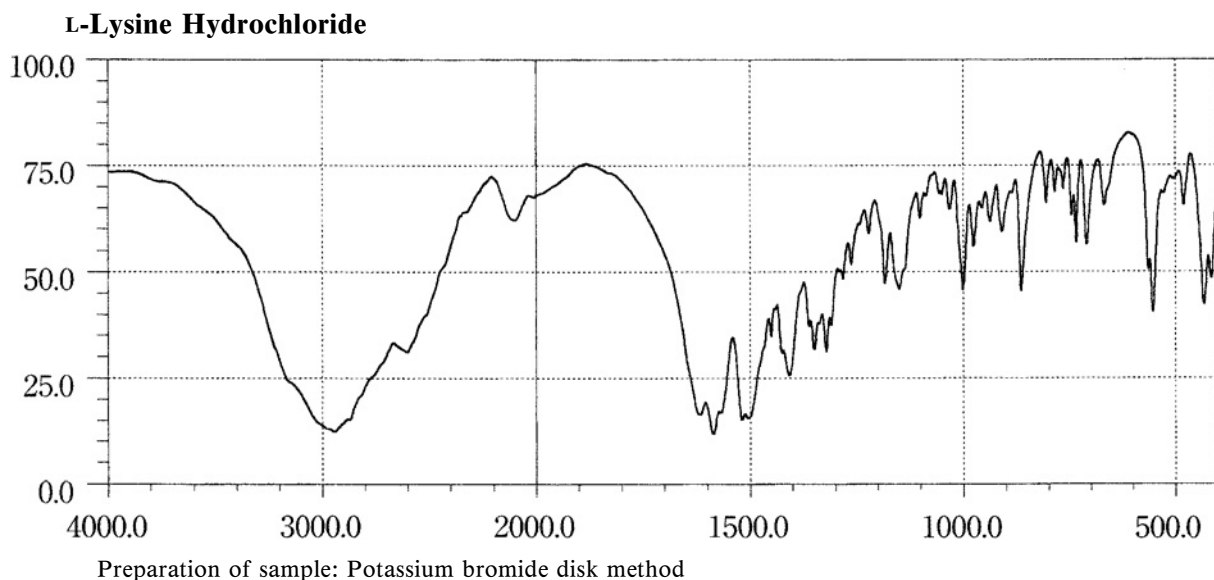
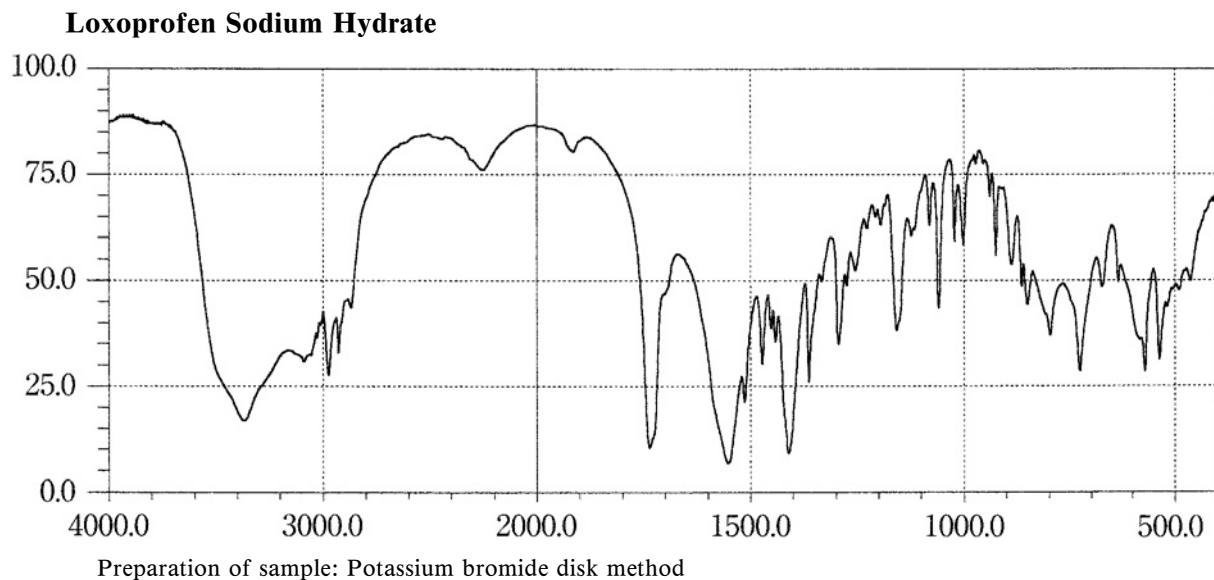
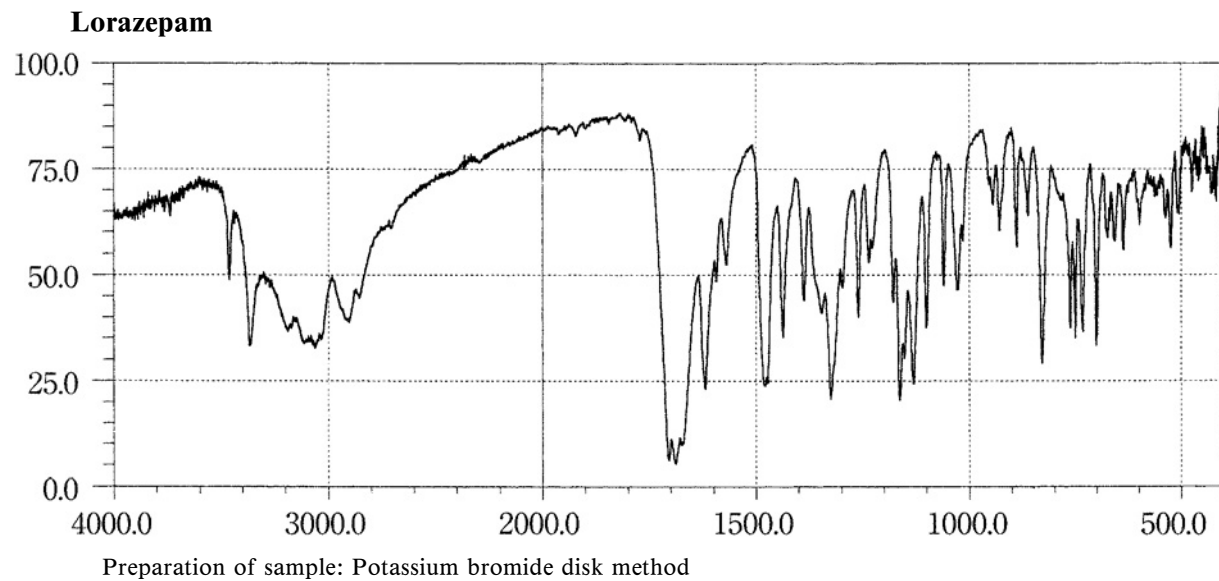
L-Leucine

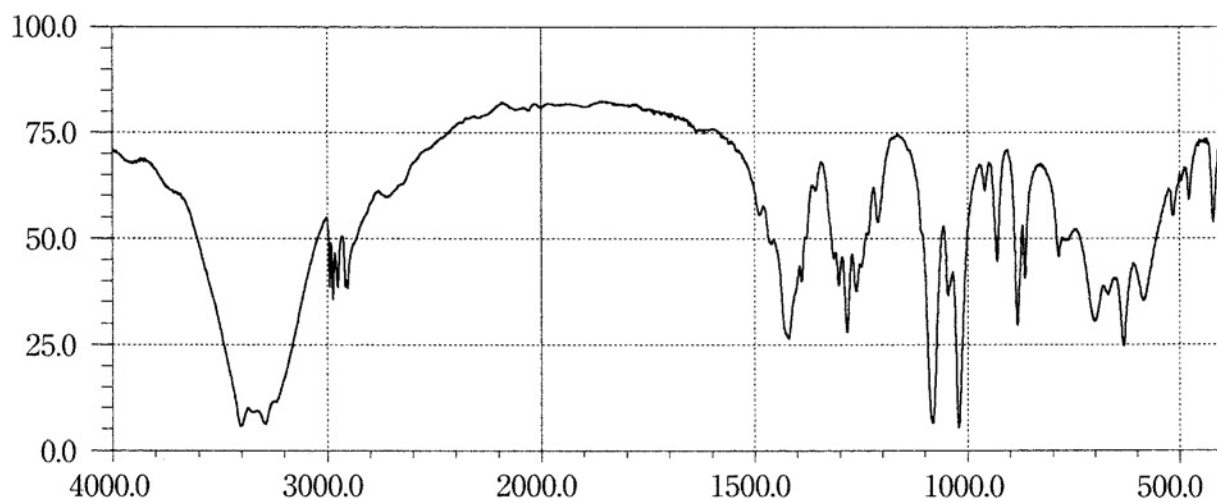
Preparation of sample: Potassium bromide disk method

Levallorphan Tartrate

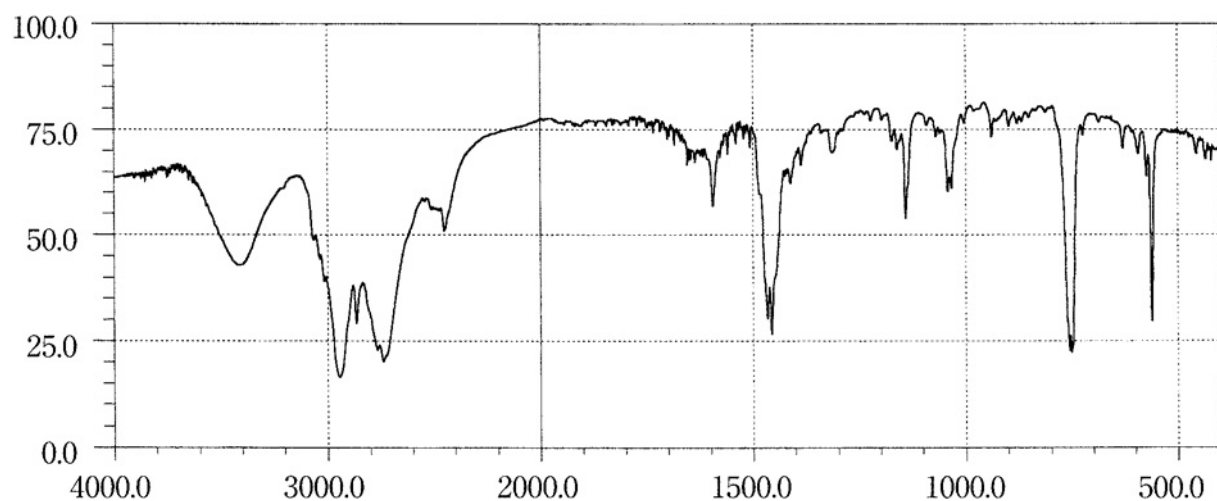
Preparation of sample: Potassium bromide disk method



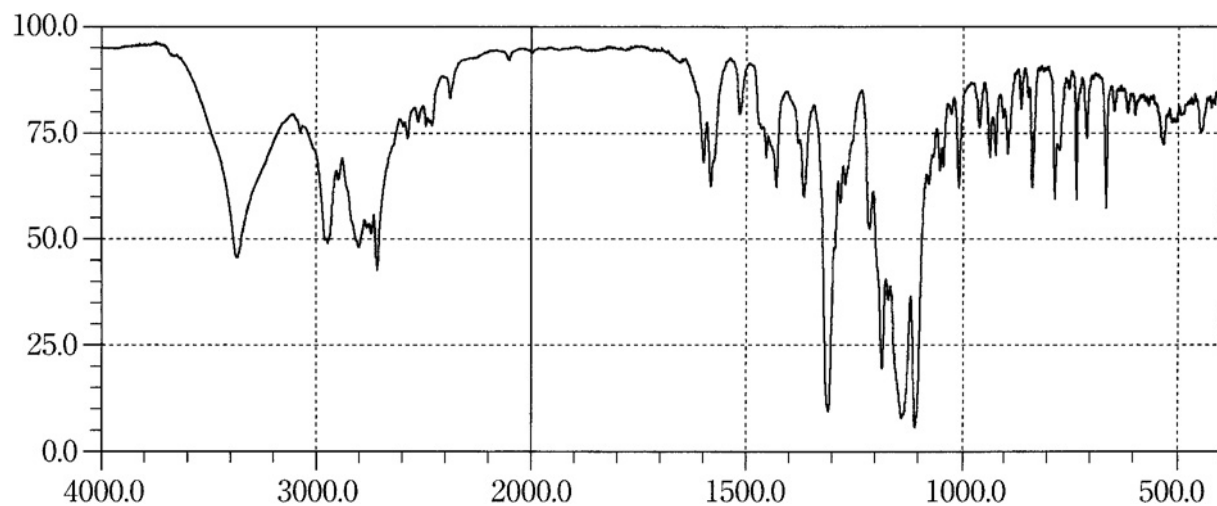


D-Mannitol

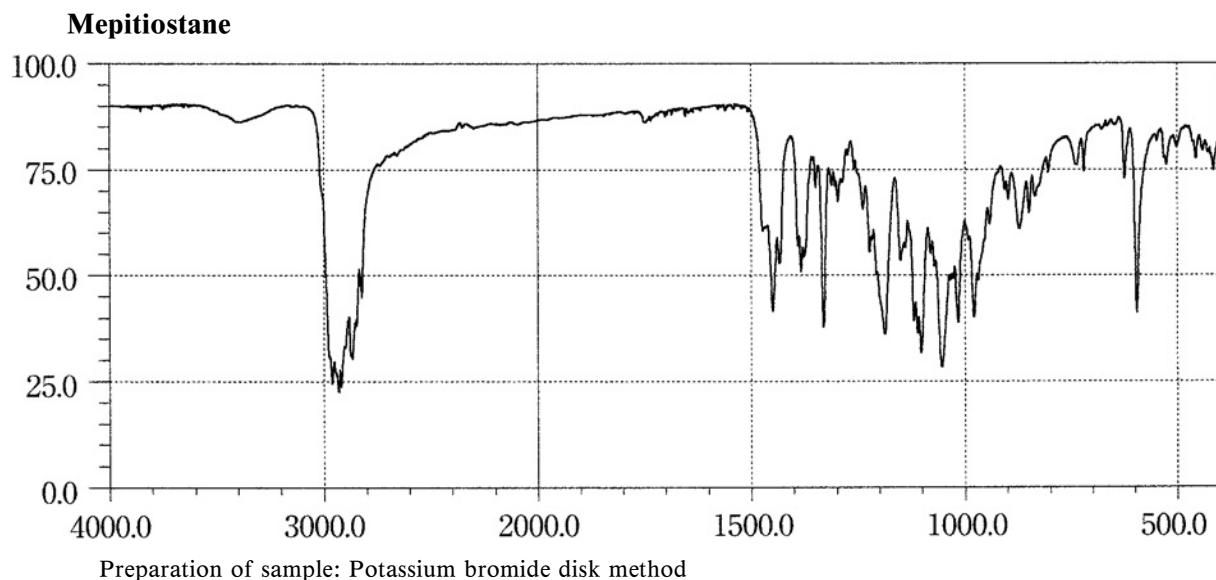
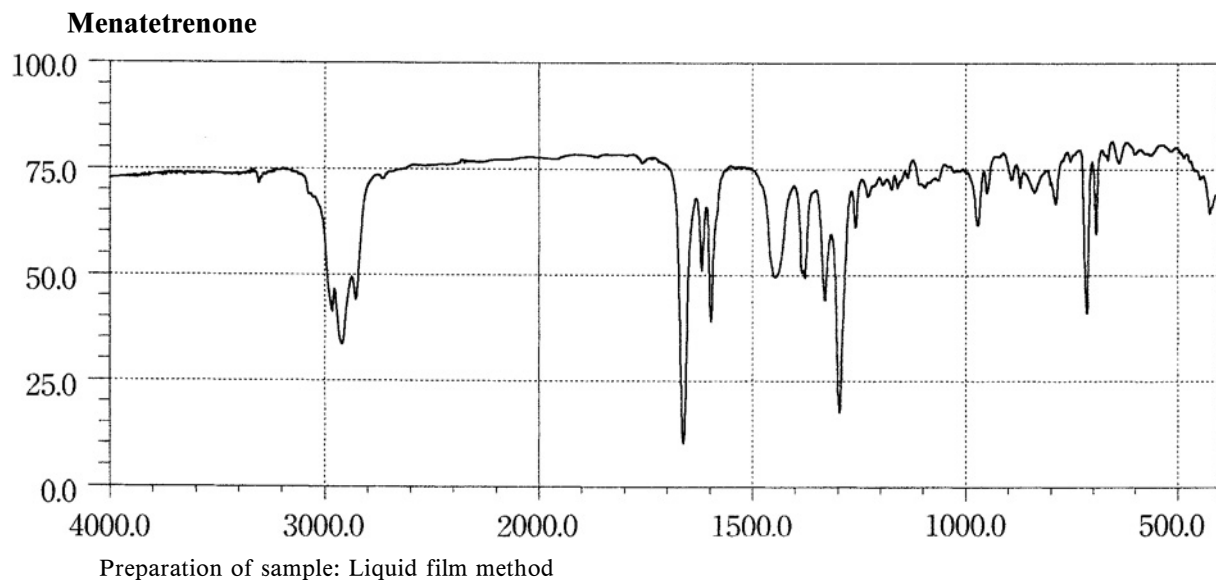
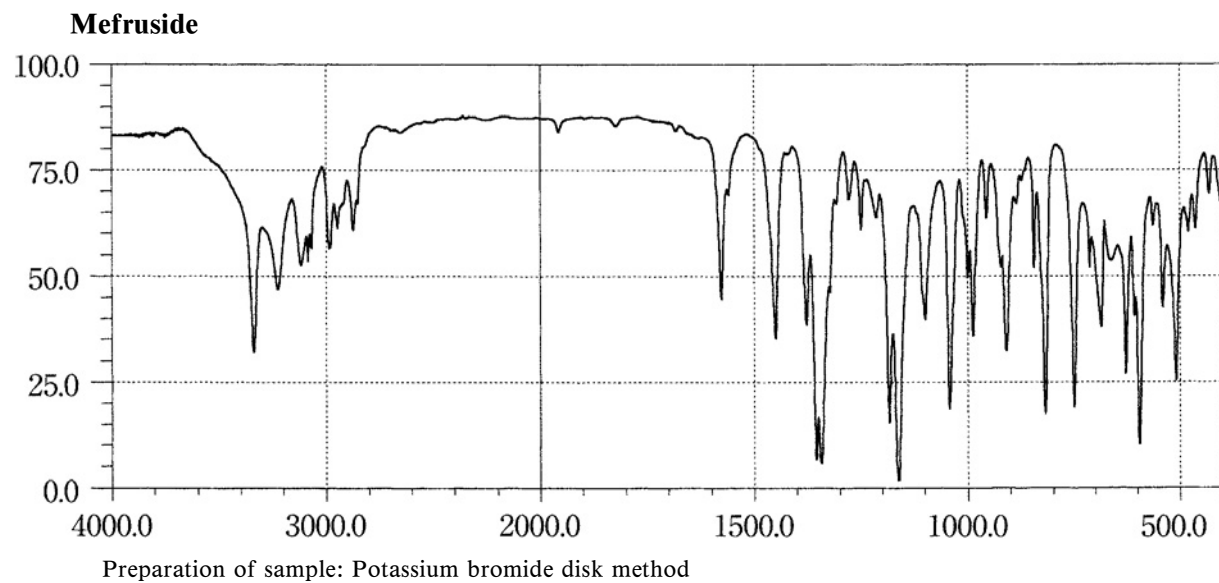
Preparation of sample: Potassium bromide disk method

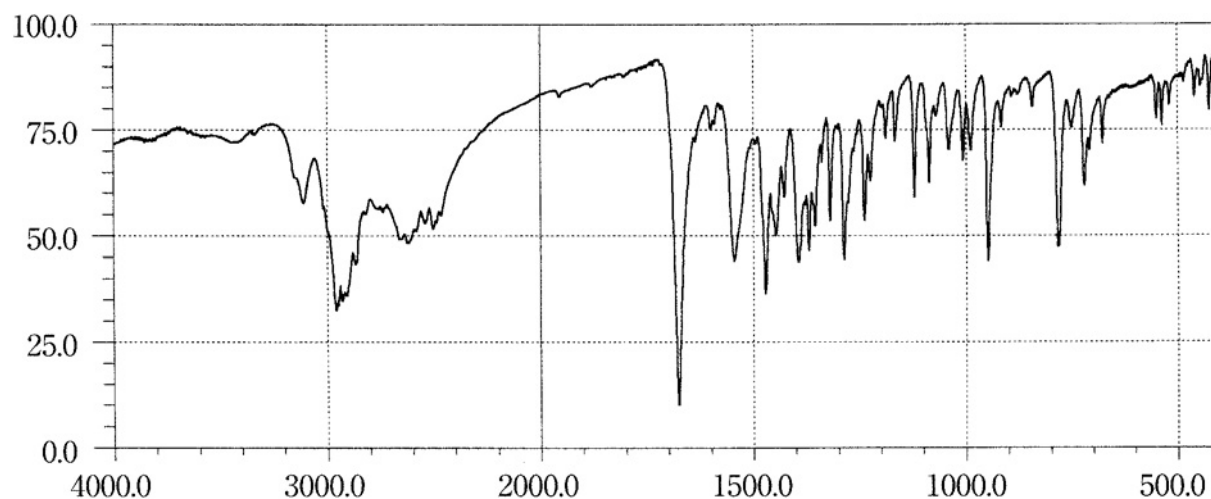
Maprotiline Hydrochloride

Preparation of sample: Potassium chloride disk method

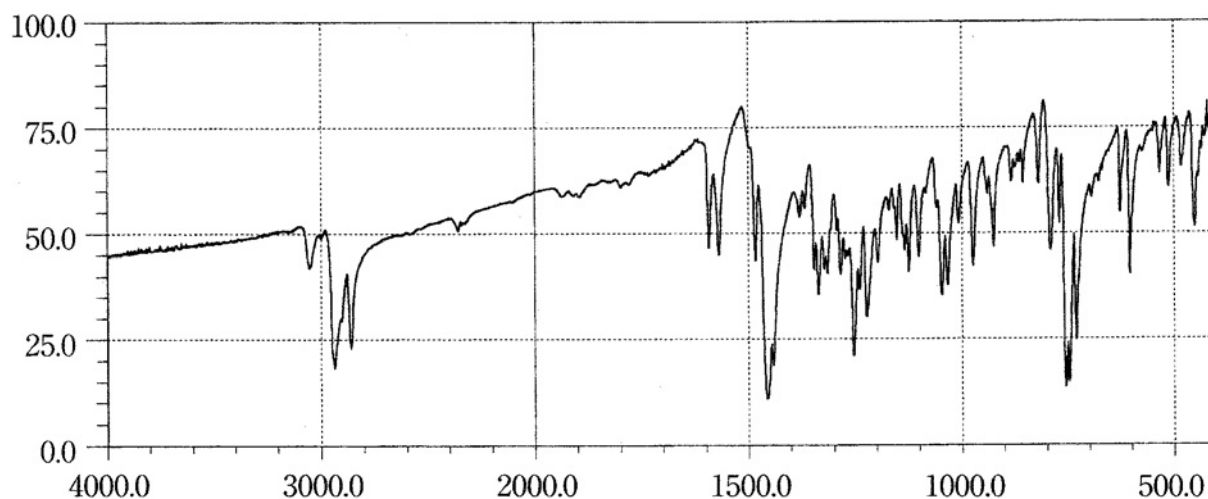
Mefloquine Hydrochloride

Preparation of sample: Potassium chloride disk method

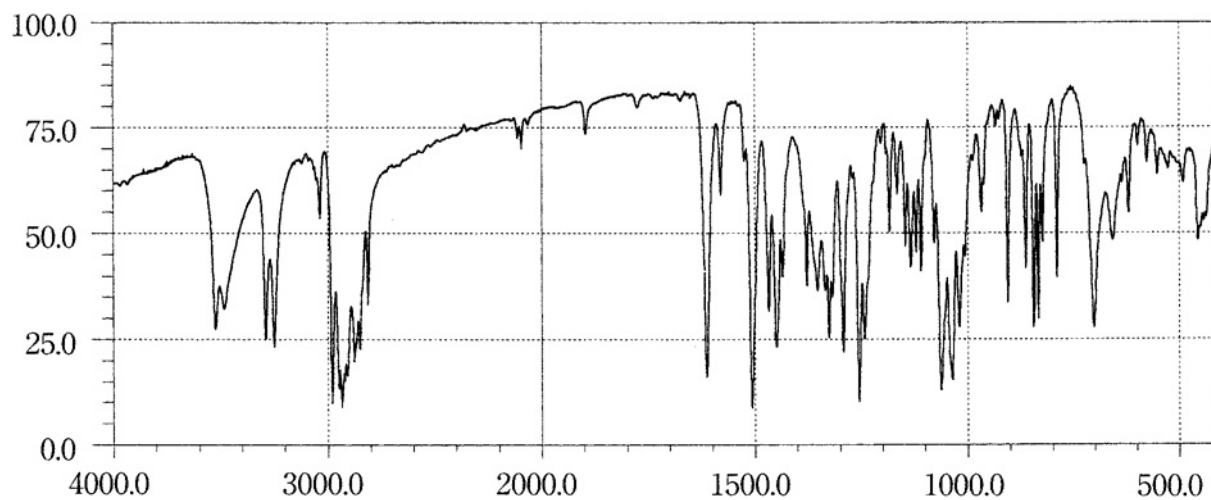


Mepivacaine Hydrochloride

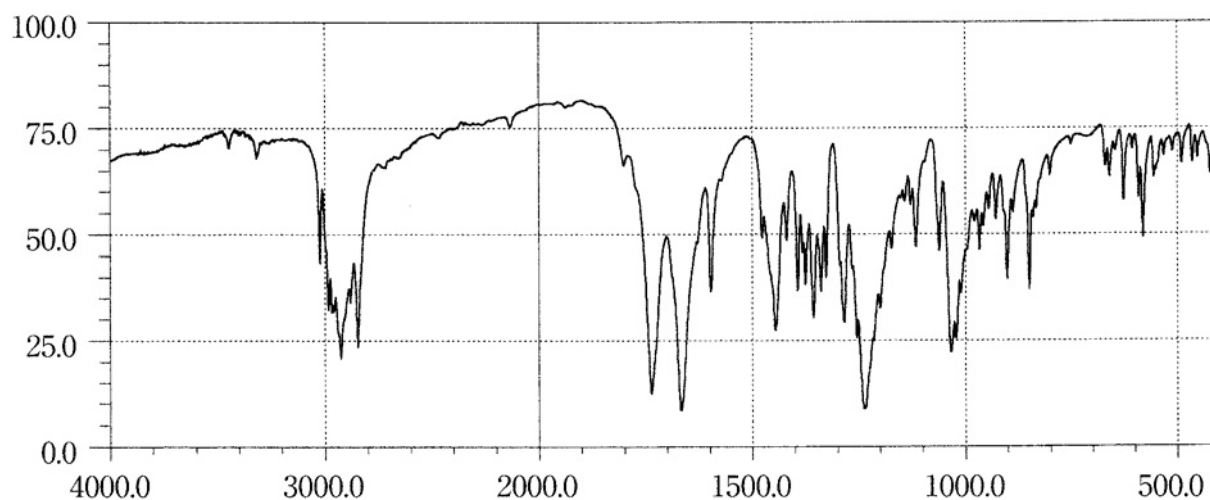
Preparation of sample: Potassium chloride disk method

Mequitazine

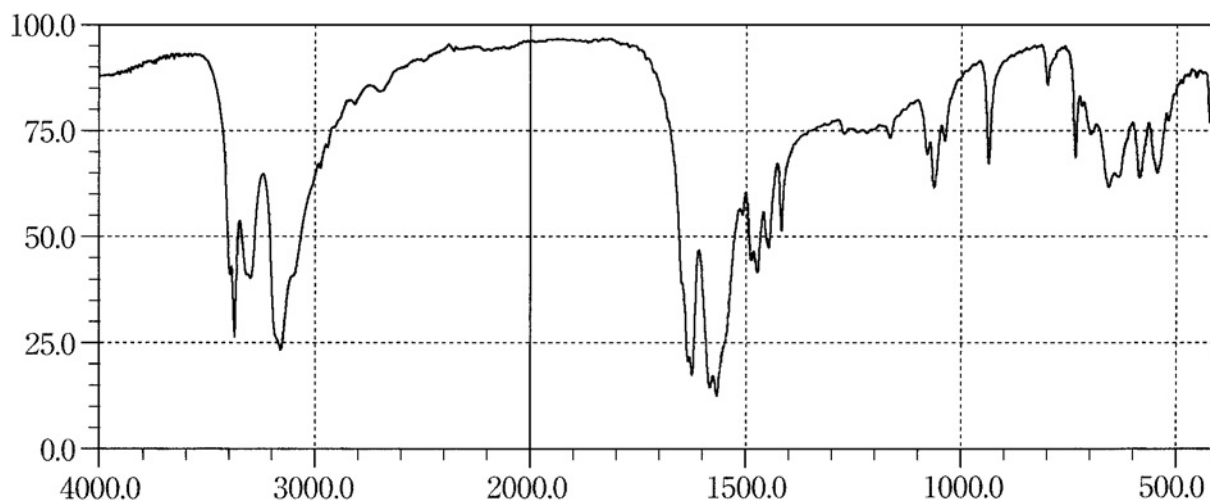
Preparation of sample: Potassium bromide disk method

Mestranol

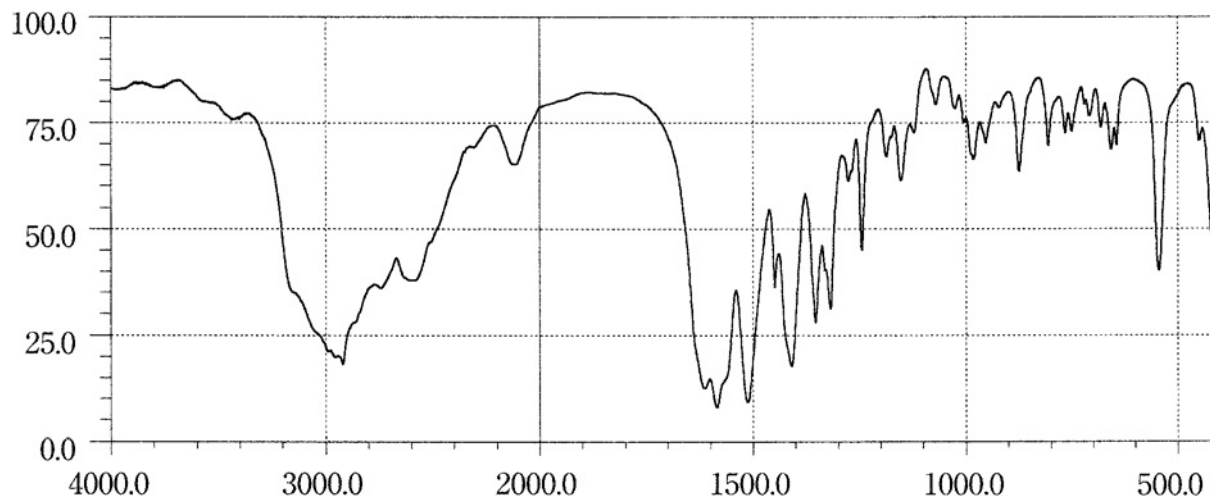
Preparation of sample: Potassium bromide disk method

Metenolone Acetate

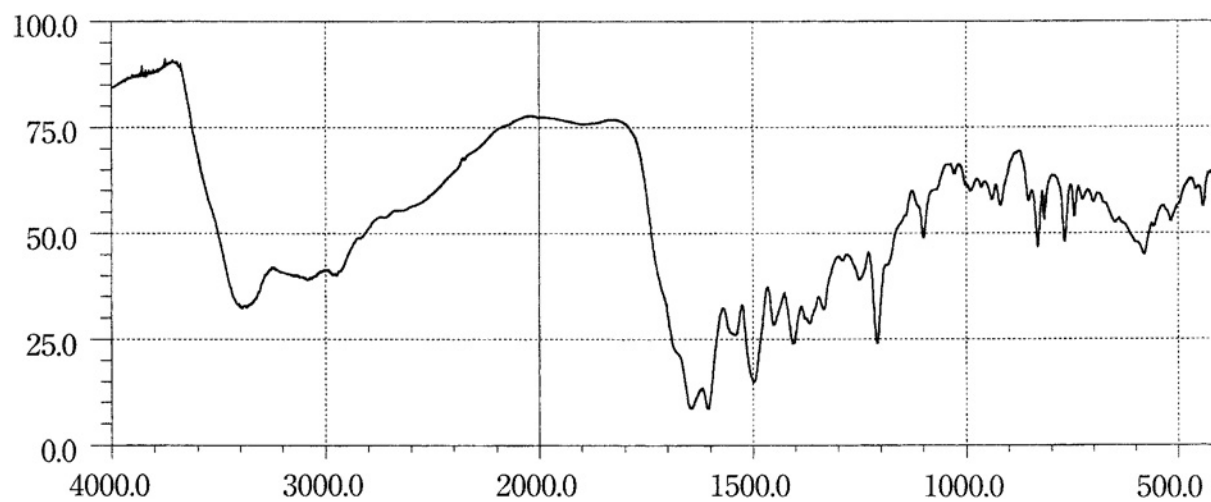
Preparation of sample: Potassium bromide disk method

Metformin Hydrochloride

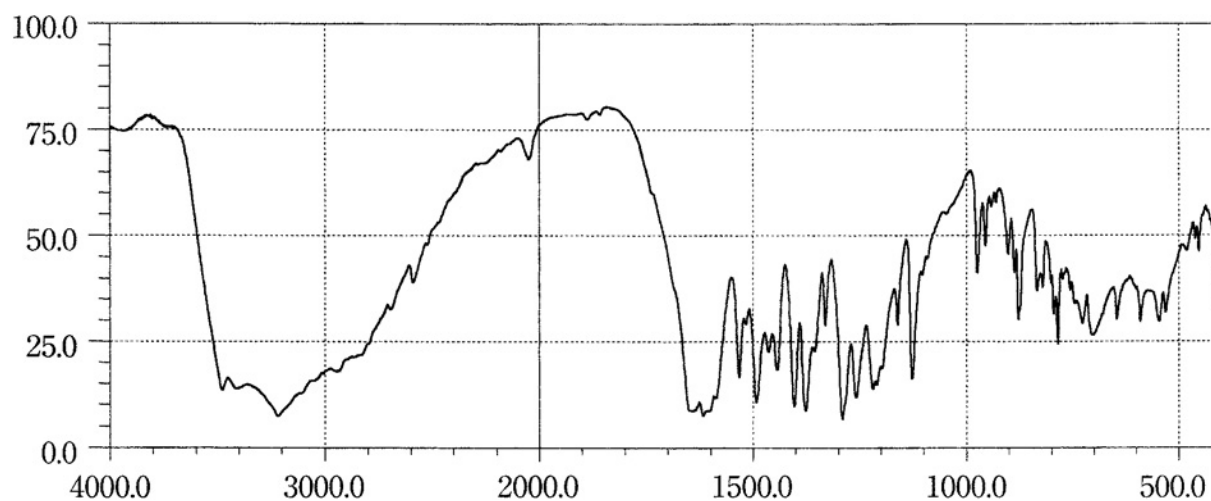
Preparation of sample: Potassium chloride disk method

L-Methionine

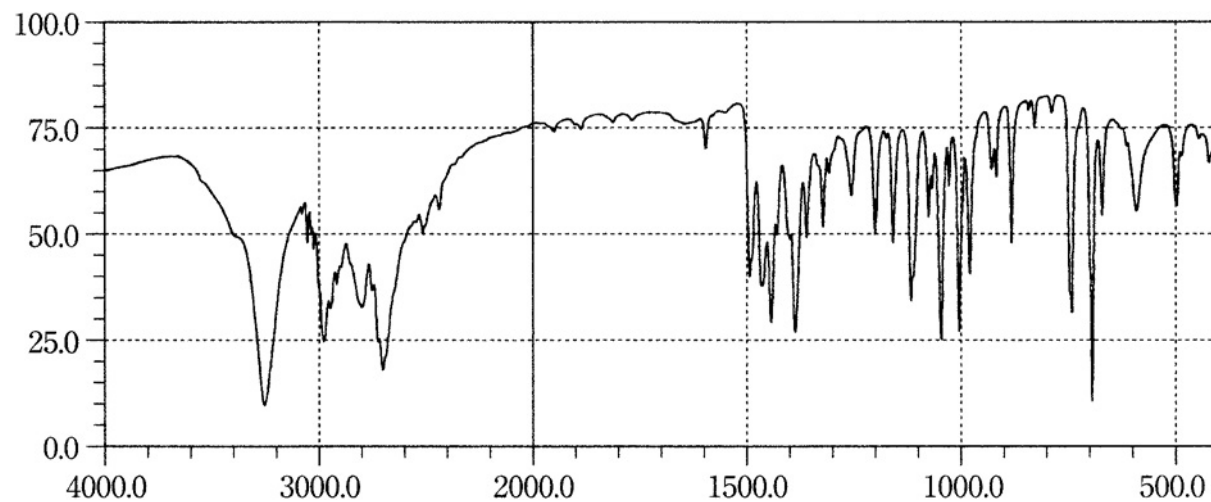
Preparation of sample: Potassium bromide disk method

Methotrexate

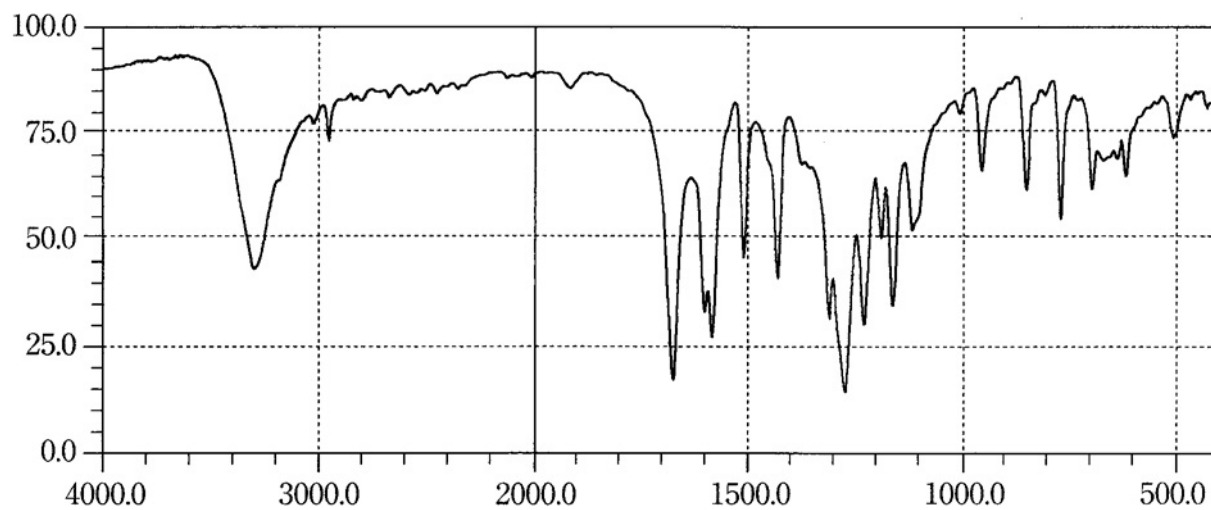
Preparation of sample: Potassium bromide disk method

Methyldopa Hydrate

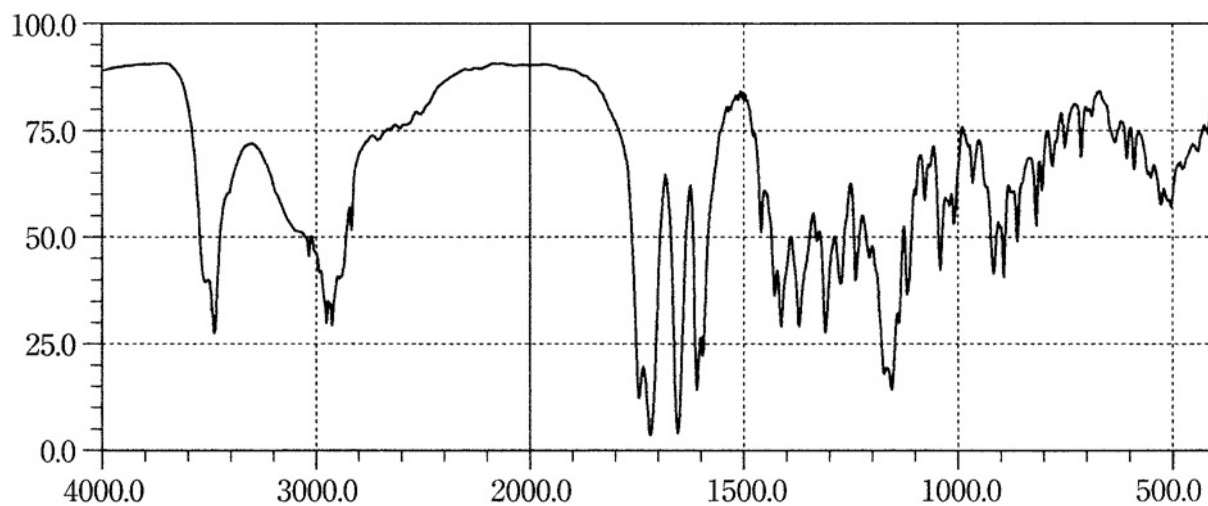
Preparation of sample: Potassium bromide disk method

dl-Methylephedrine Hydrochloride

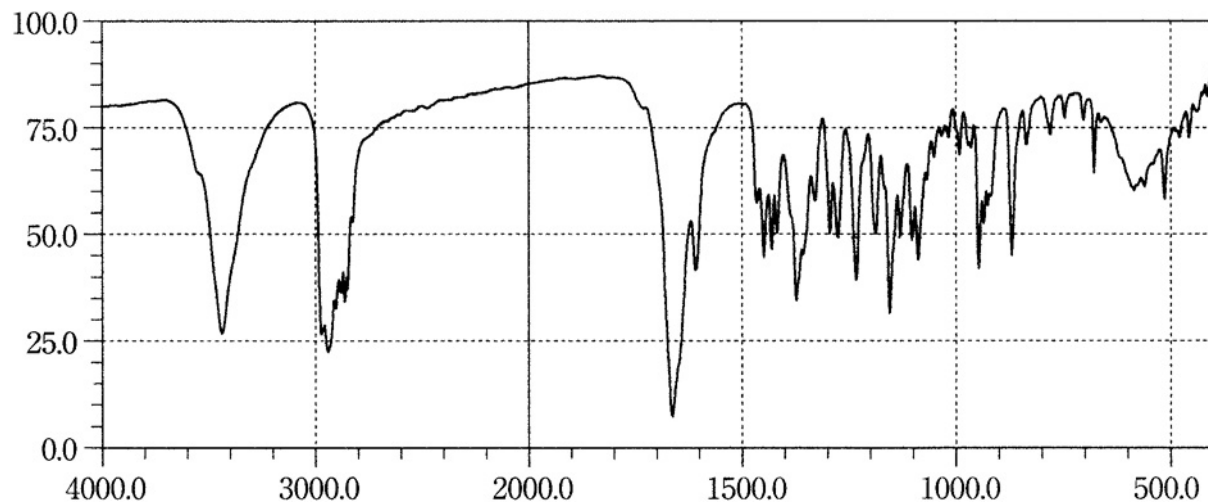
Preparation of sample: Potassium chloride disk method

Methyl Parahydroxybenzoate

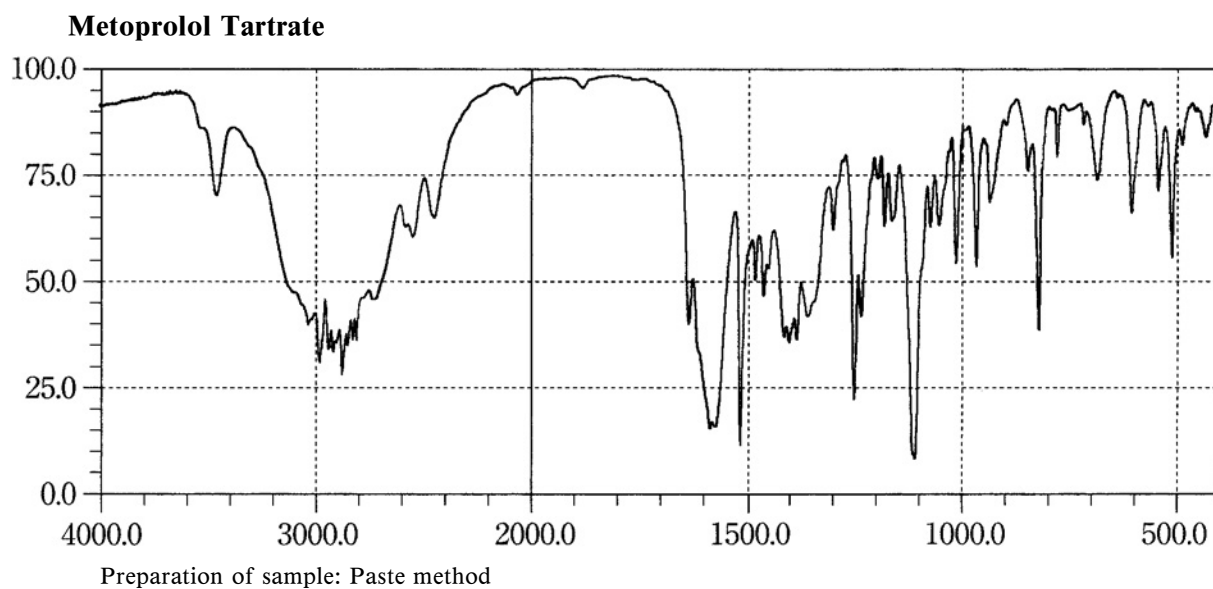
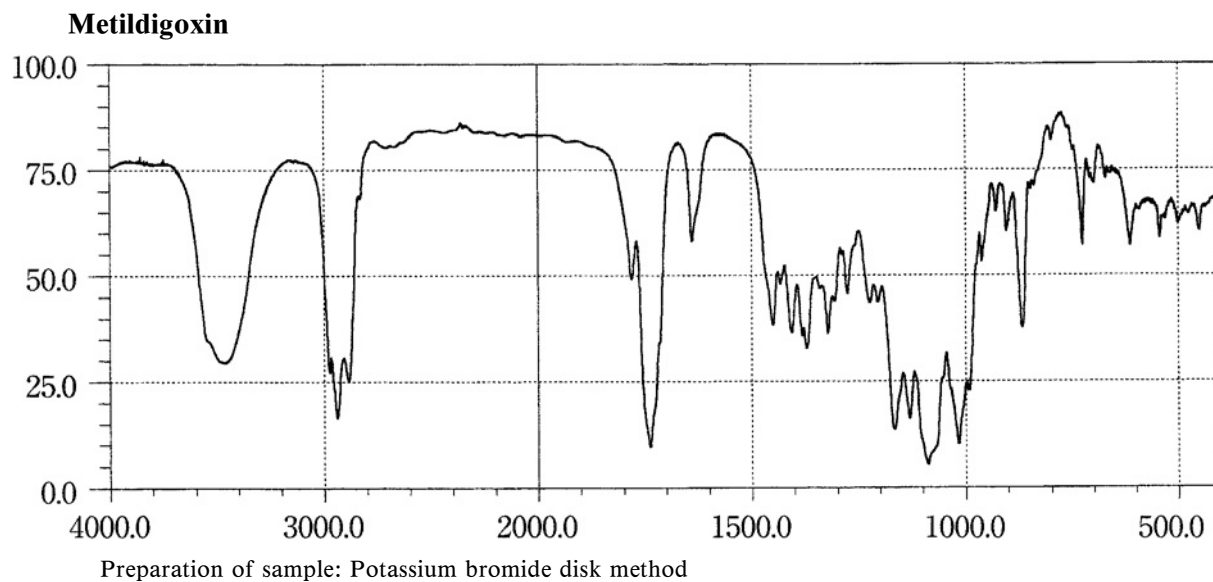
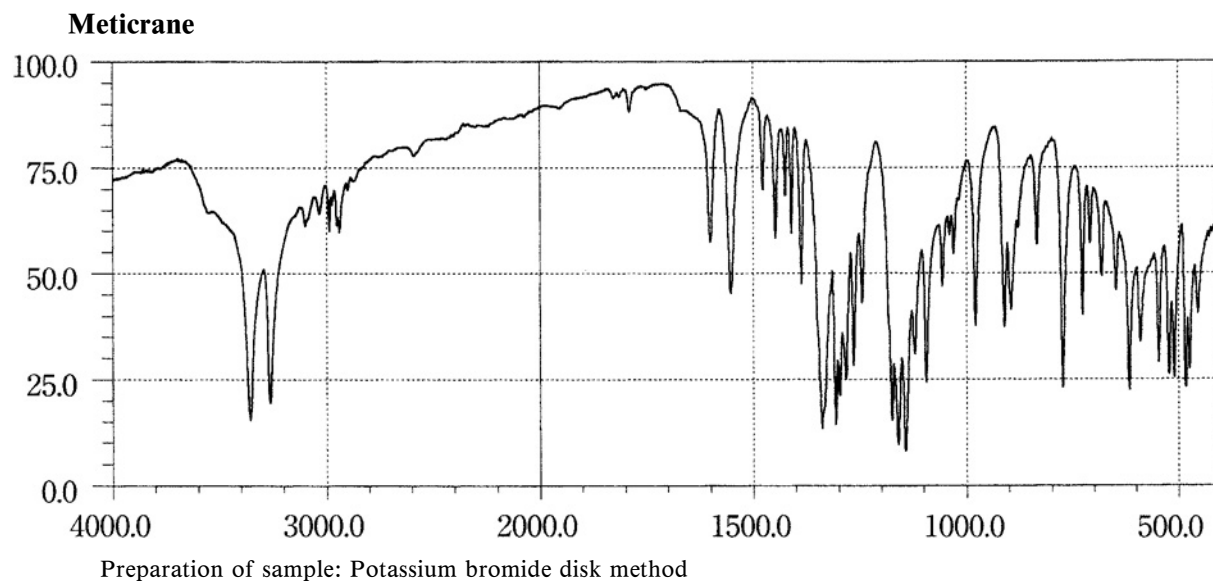
Preparation of sample: Potassium bromide disk method

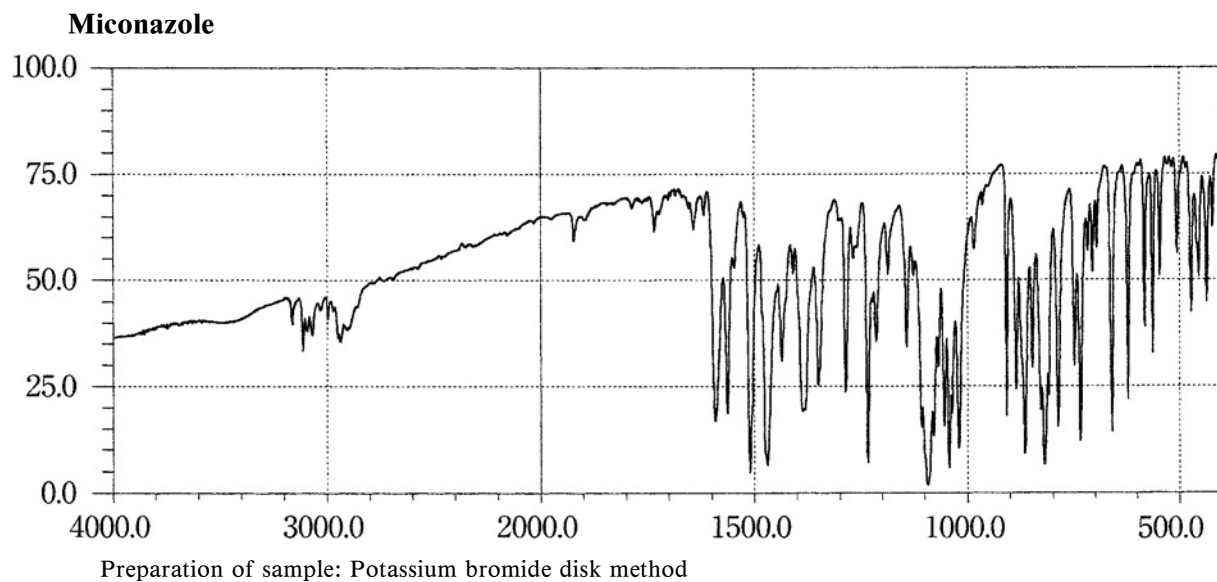
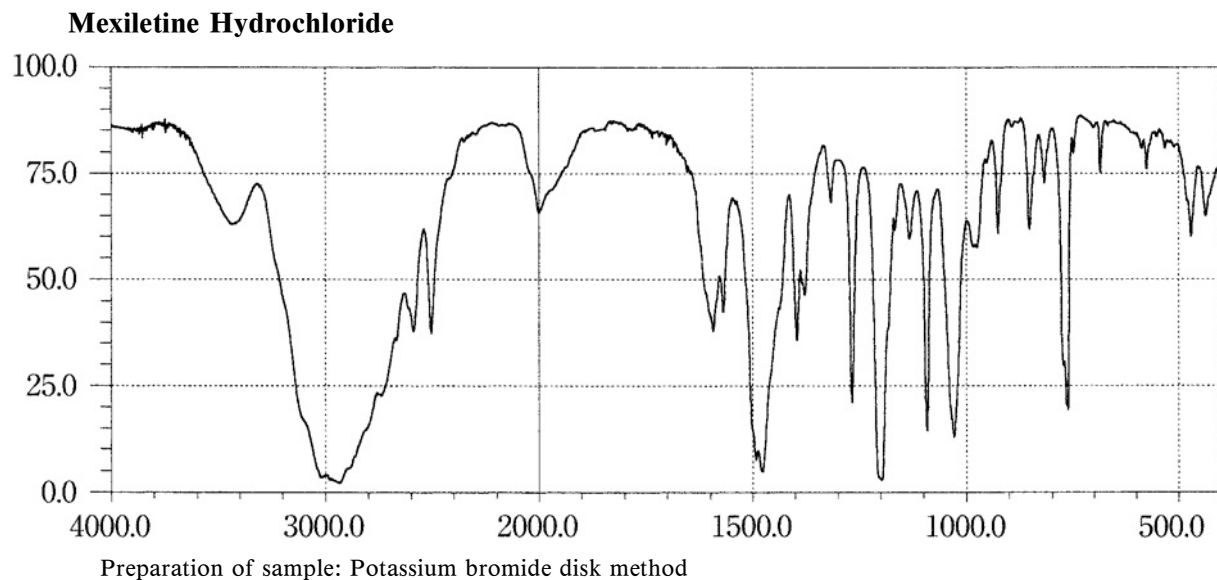
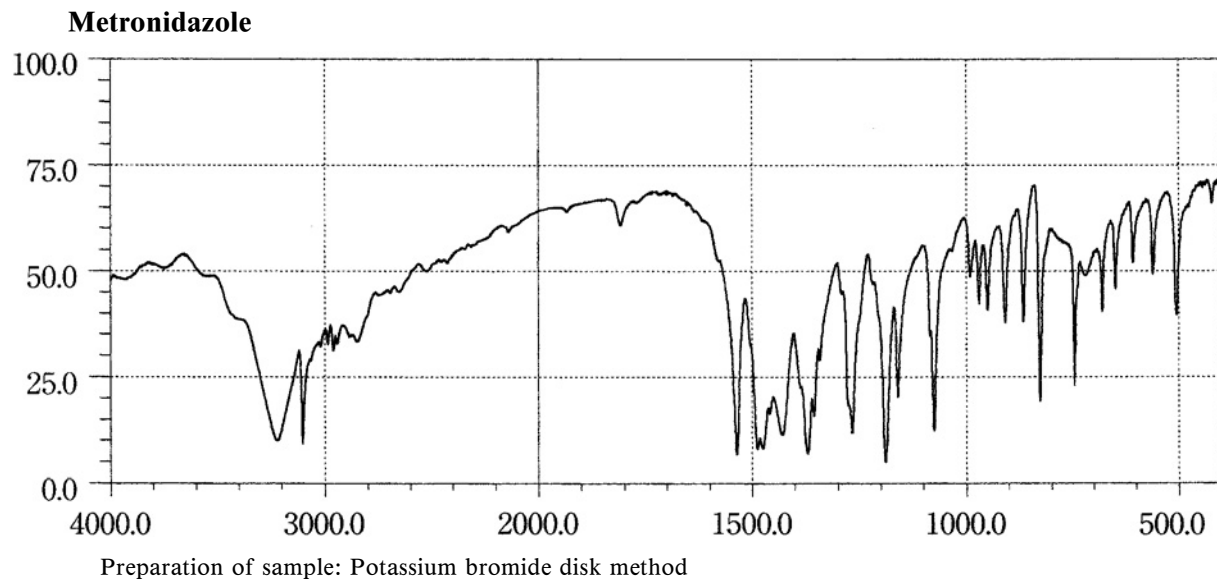
Methylprednisolone Succinate

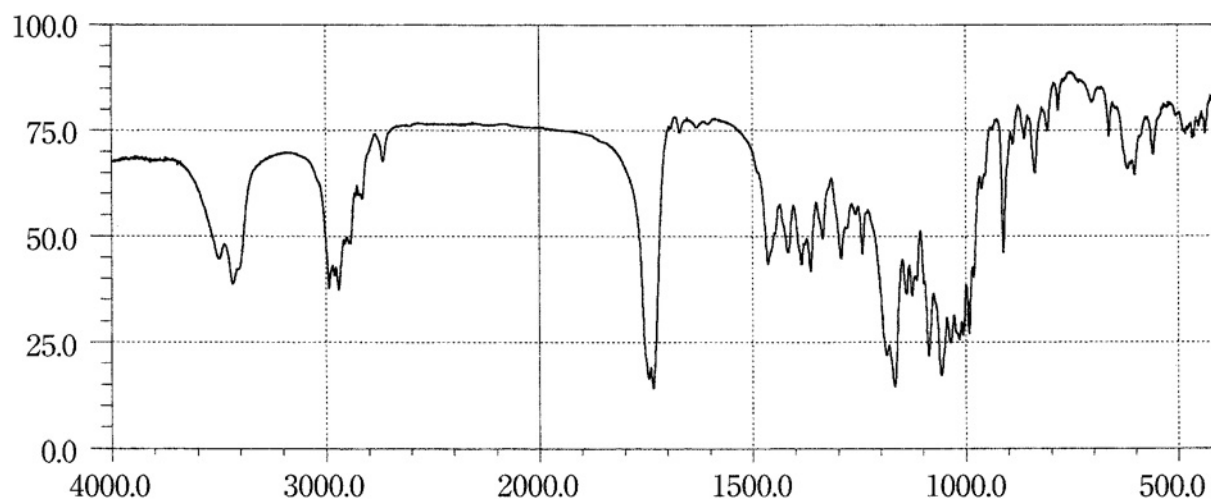
Preparation of sample: Potassium bromide disk method

Methyltestosterone

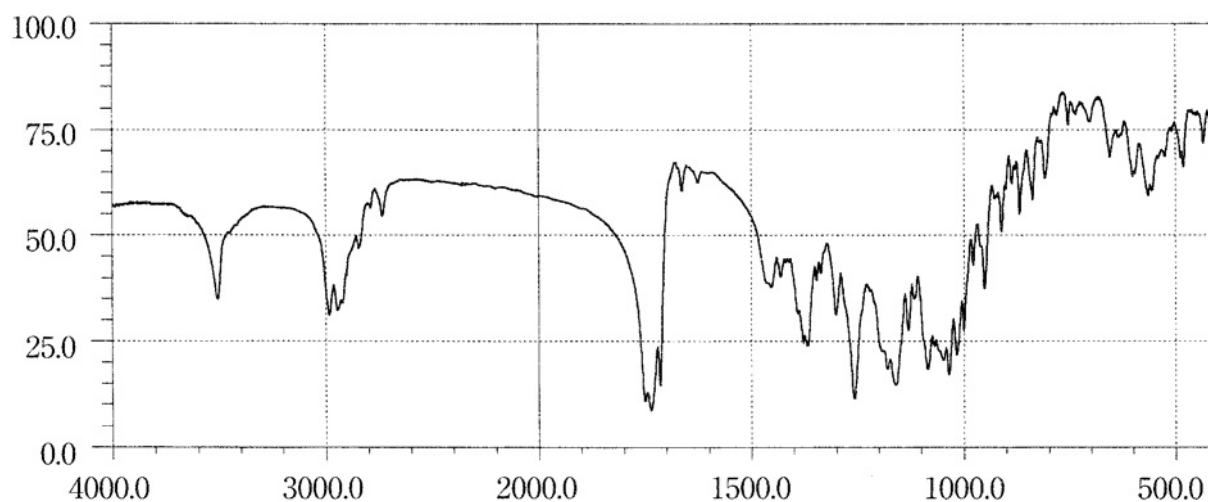
Preparation of sample: Potassium bromide disk method



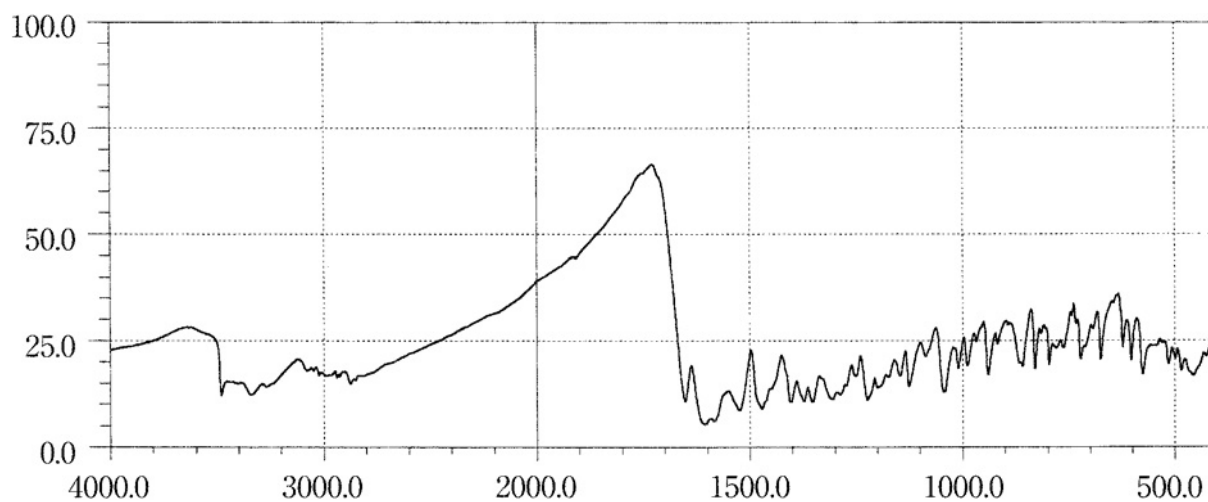


Midecamycin

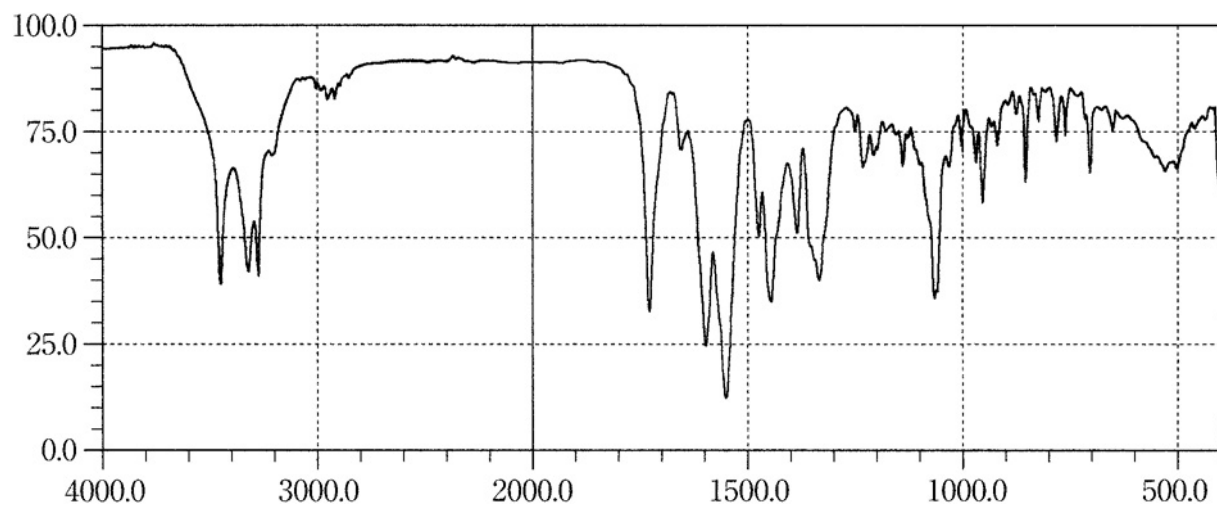
Preparation of sample: Potassium bromide disk method

Midecamycin Acetate

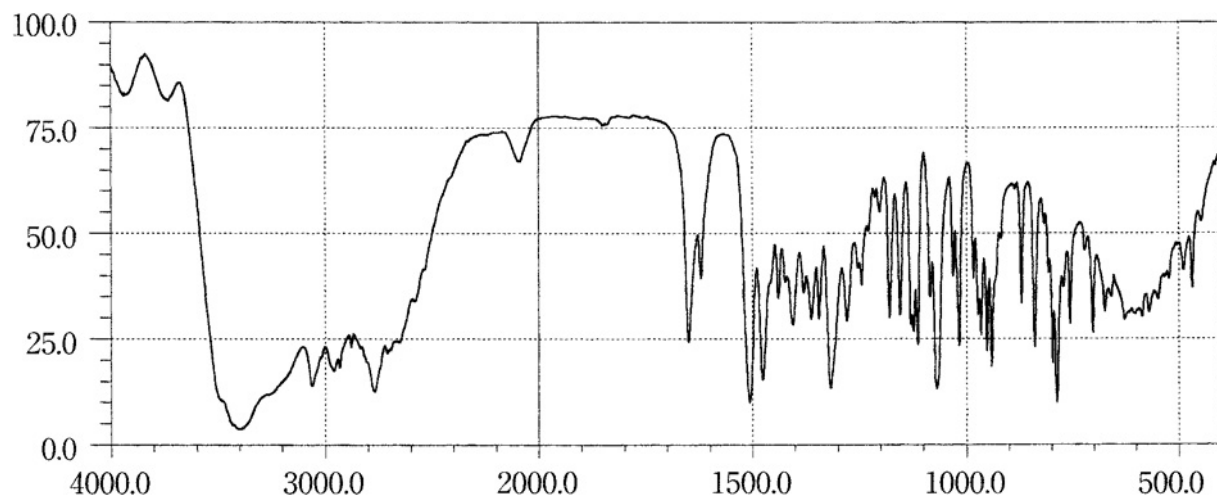
Preparation of sample: Potassium bromide disk method

Minocycline Hydrochloride

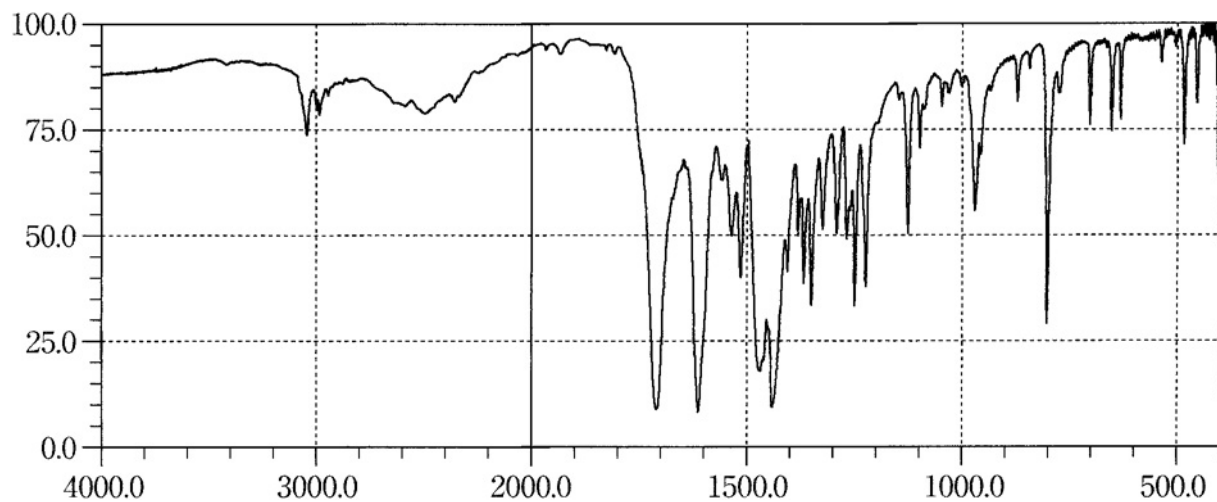
Preparation of sample: Potassium chloride disk method

Mitomycin C

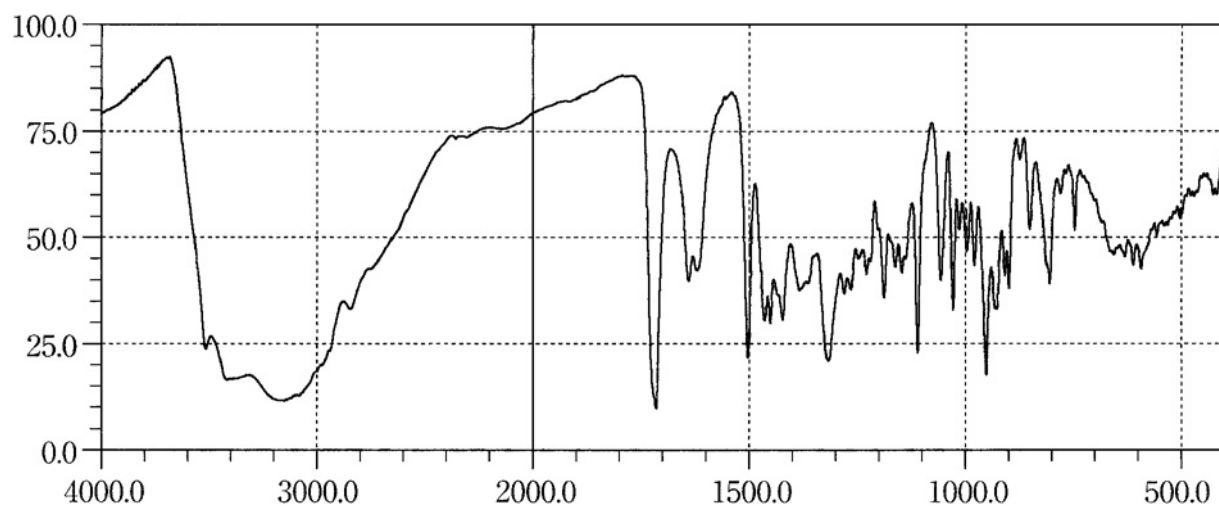
Preparation of sample: Potassium bromide disk method

Morphine Hydrochloride Hydrate

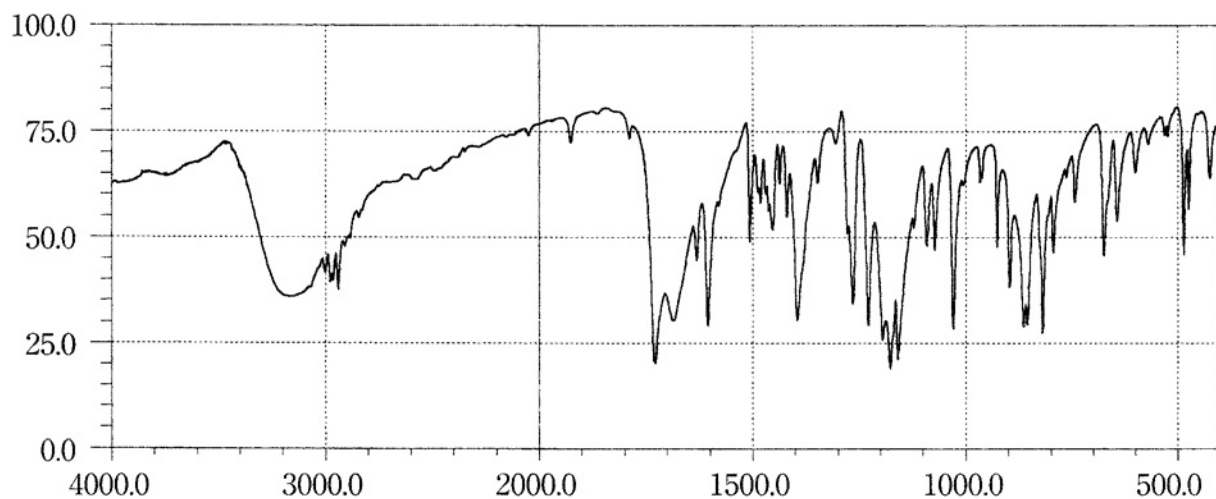
Preparation of sample: Potassium bromide disk method

Nalidixic Acid

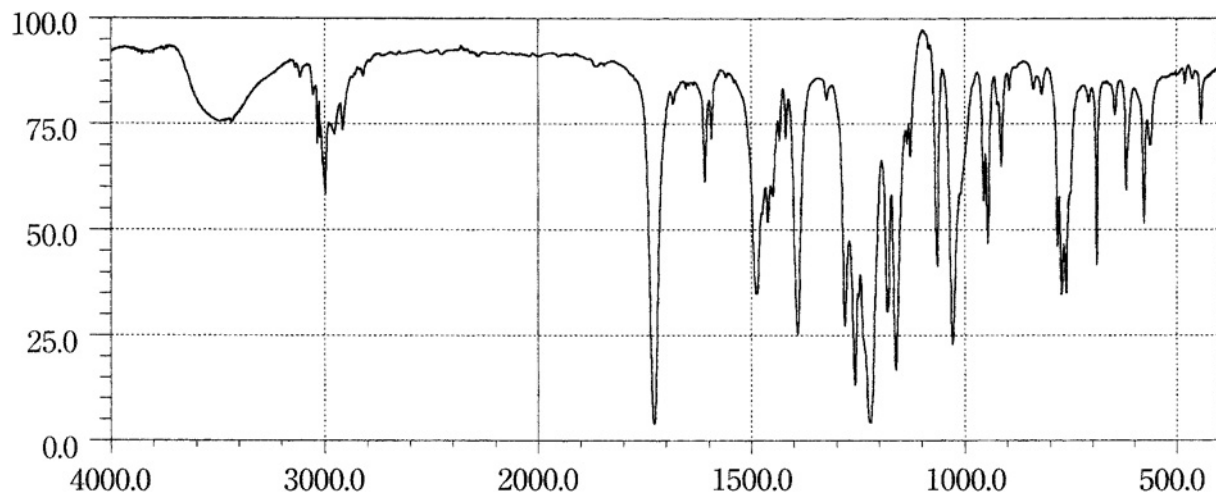
Preparation of sample: Potassium bromide disk method

Naloxone Hydrochloride

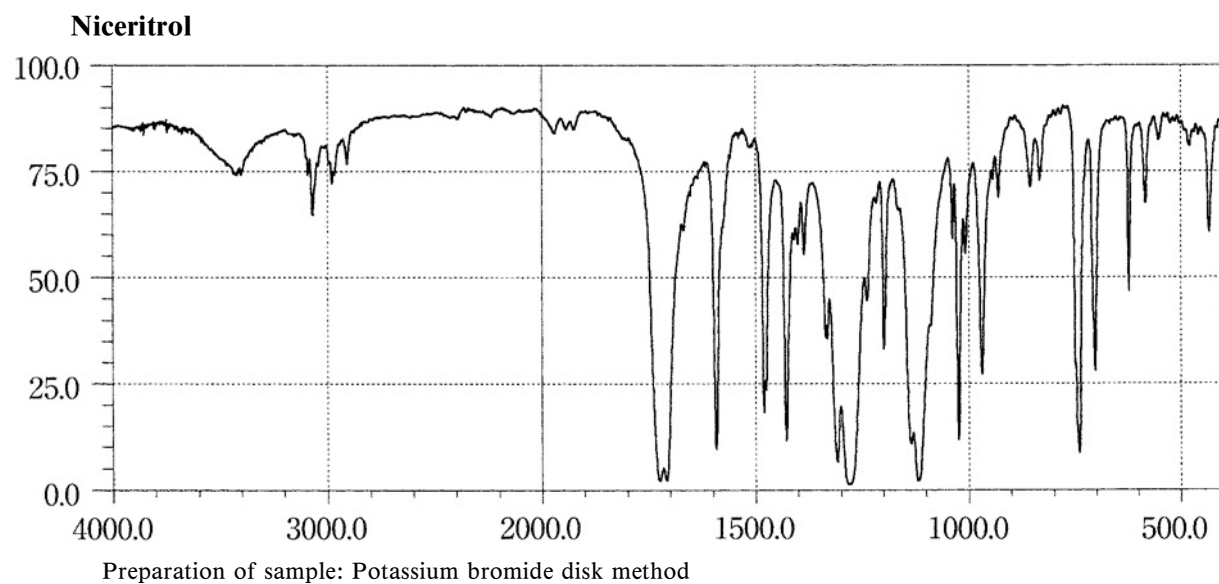
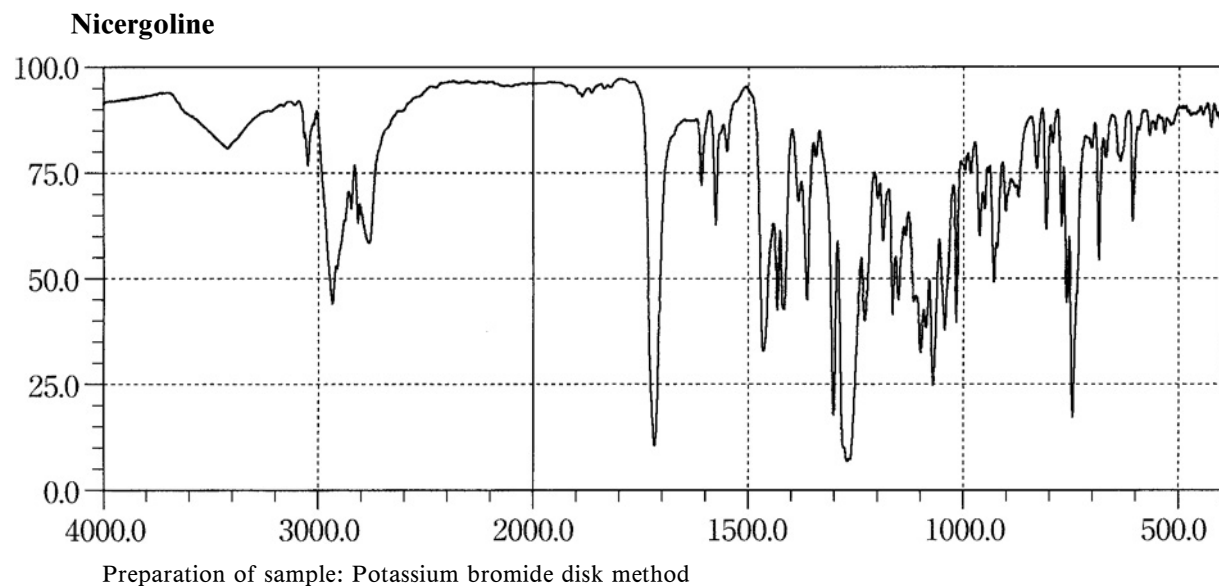
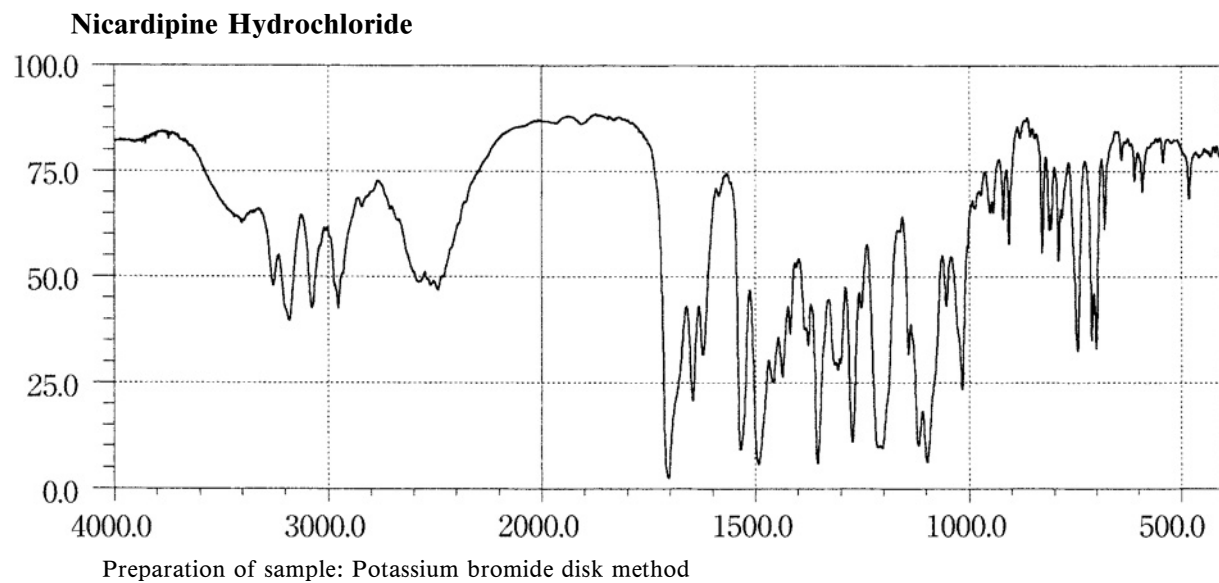
Preparation of sample: Potassium chloride disk method

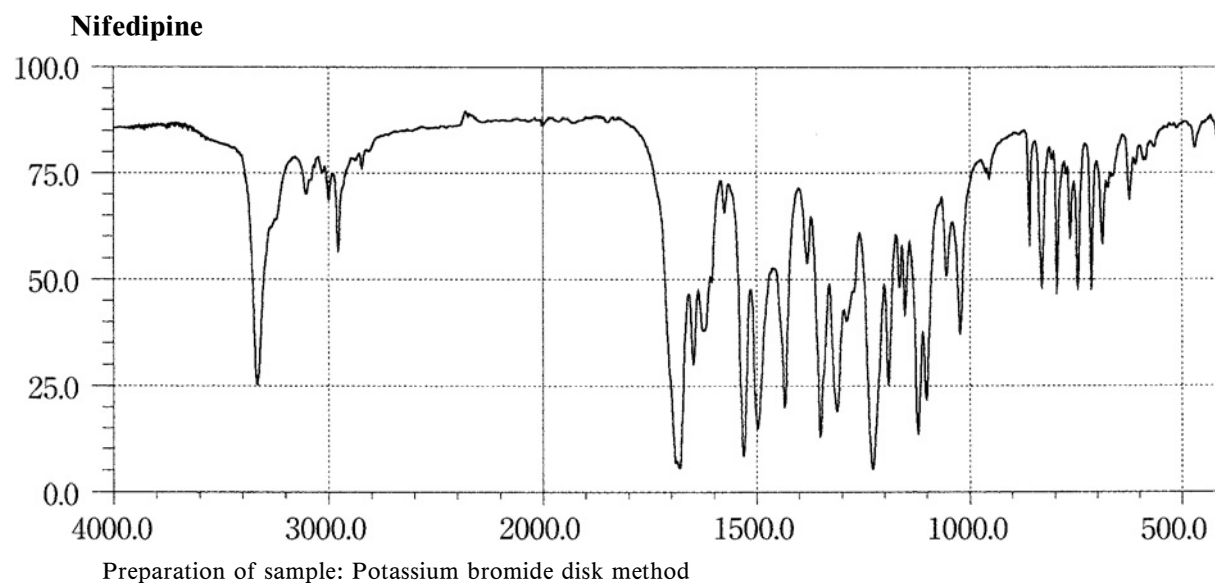
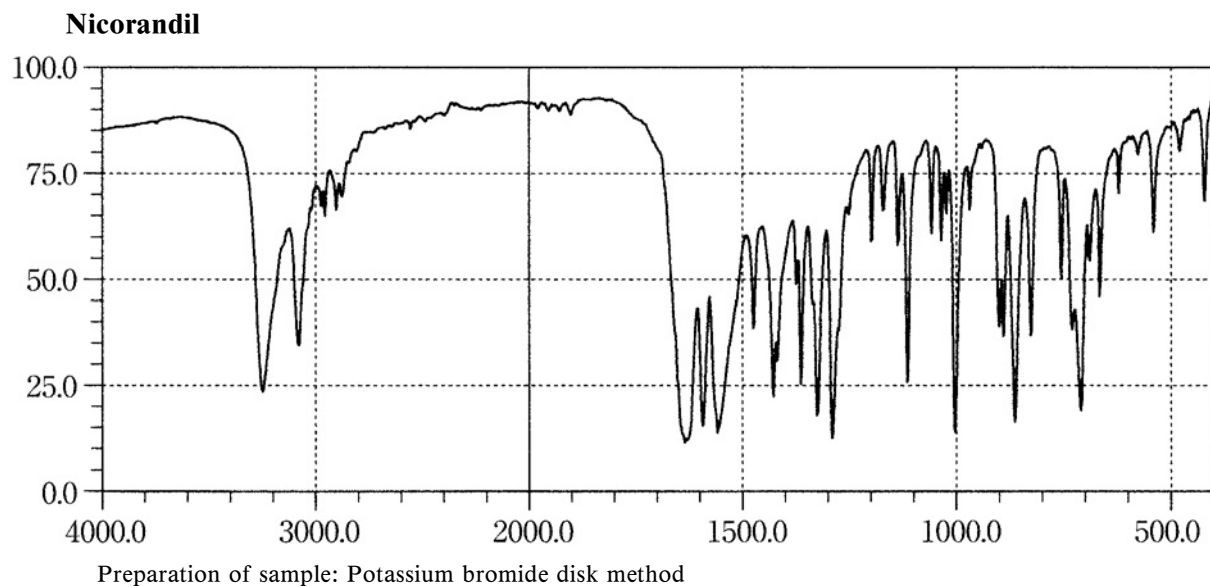
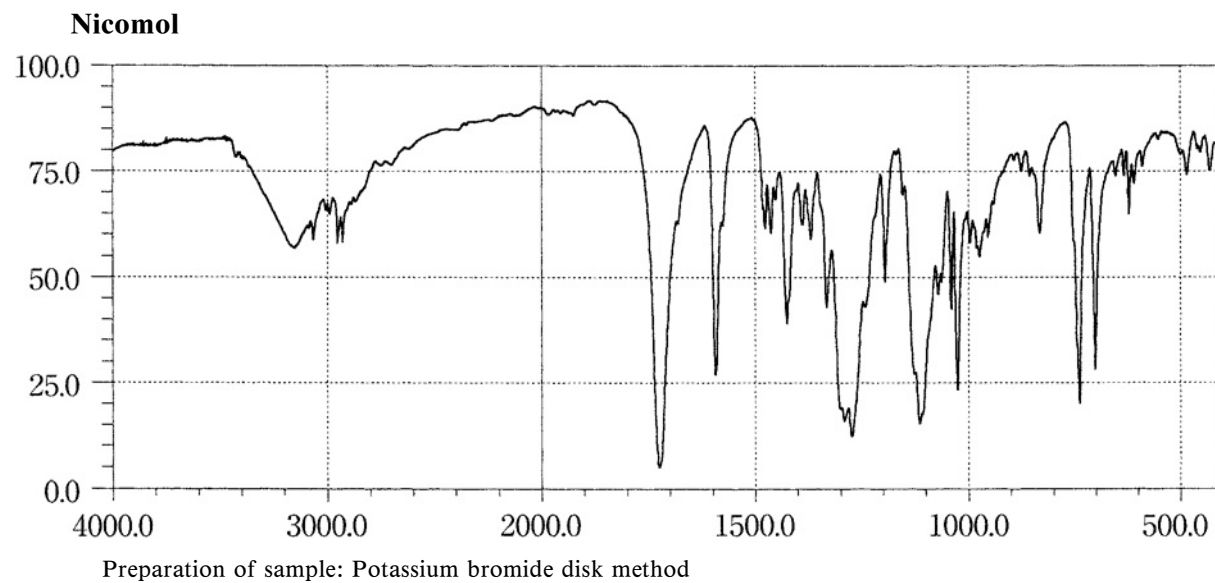
Naproxen

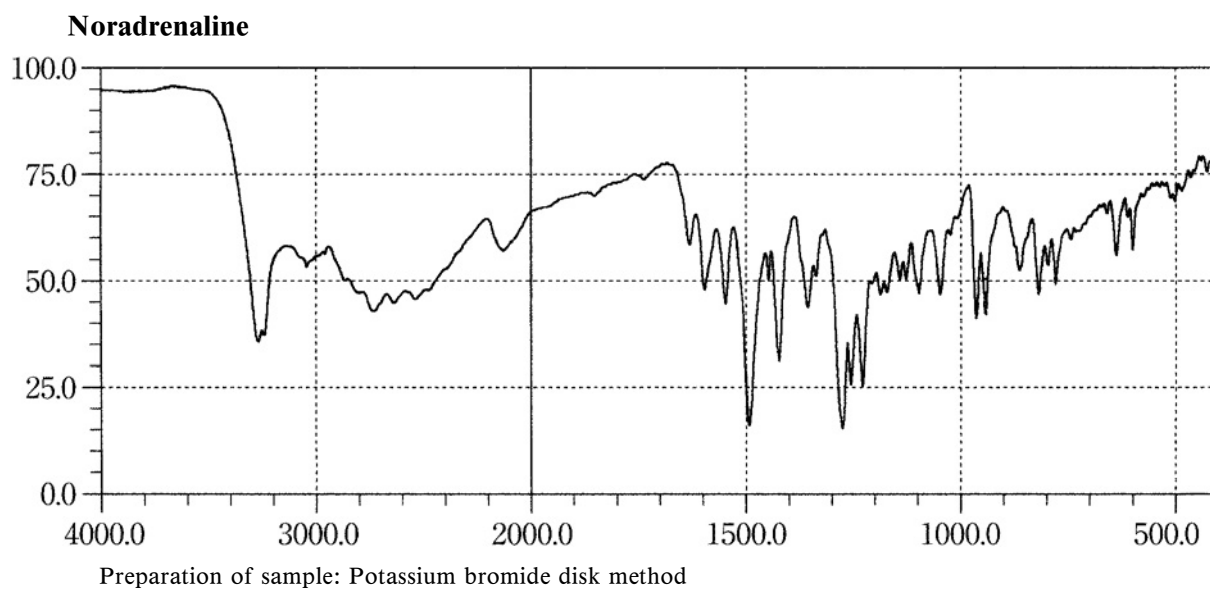
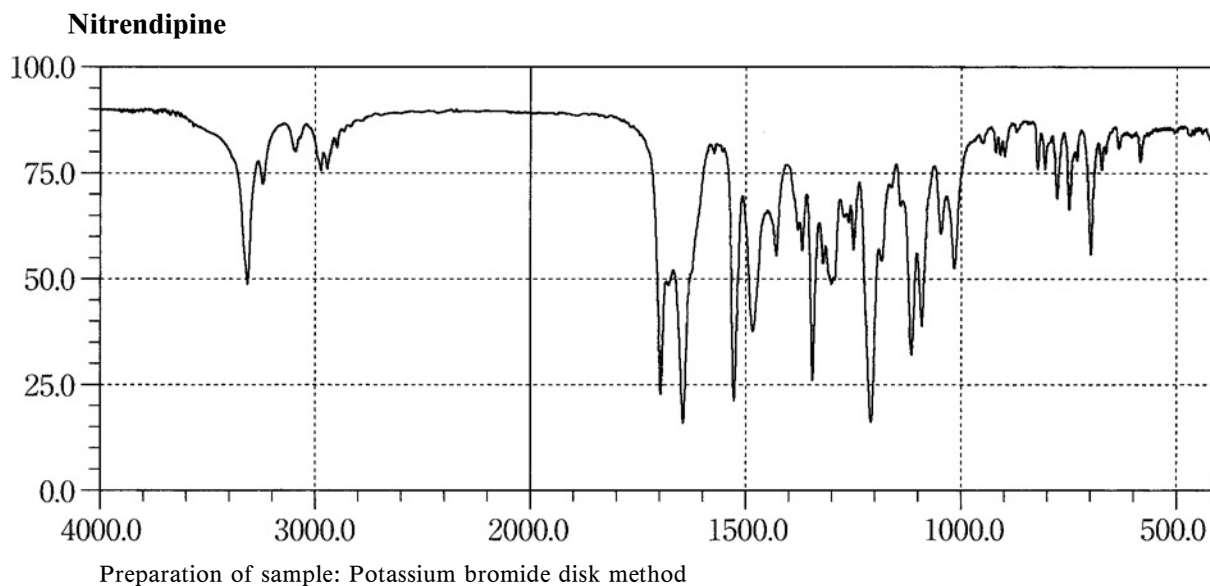
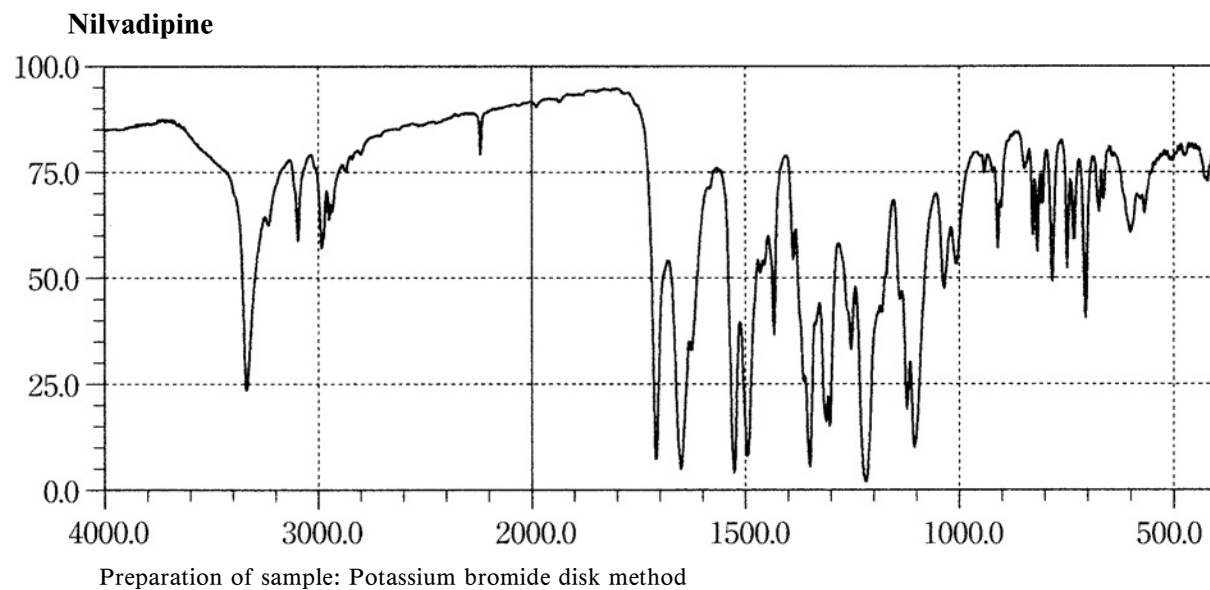
Preparation of sample: Potassium bromide disk method

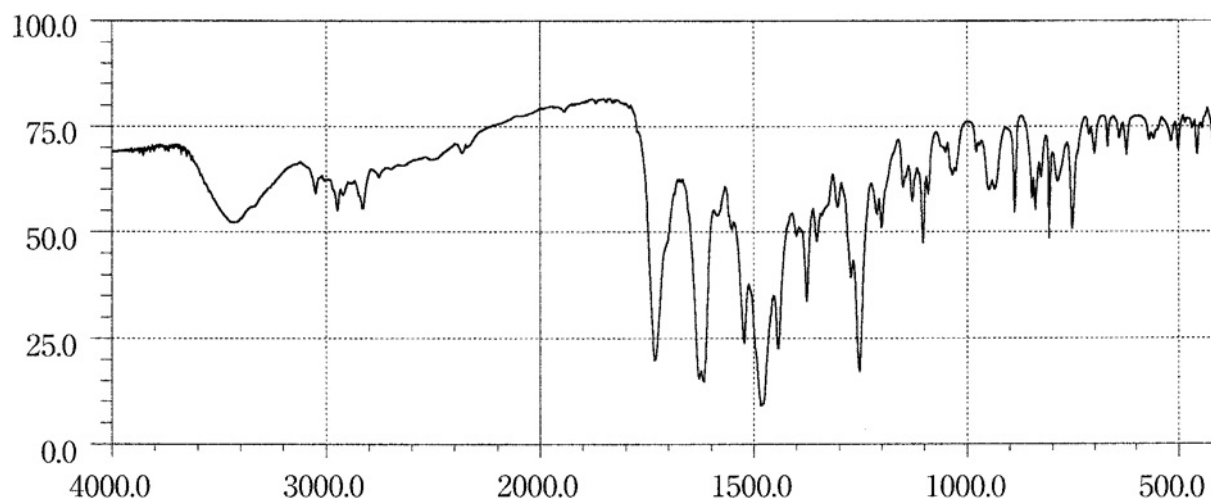
Neostigmine Methylsulfate

Preparation of sample: Potassium bromide disk method

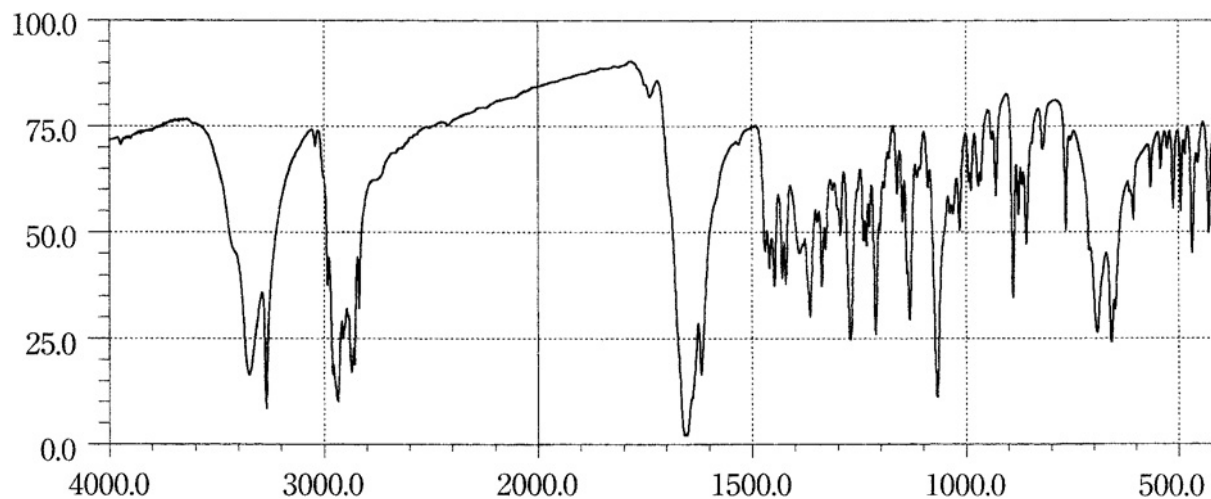




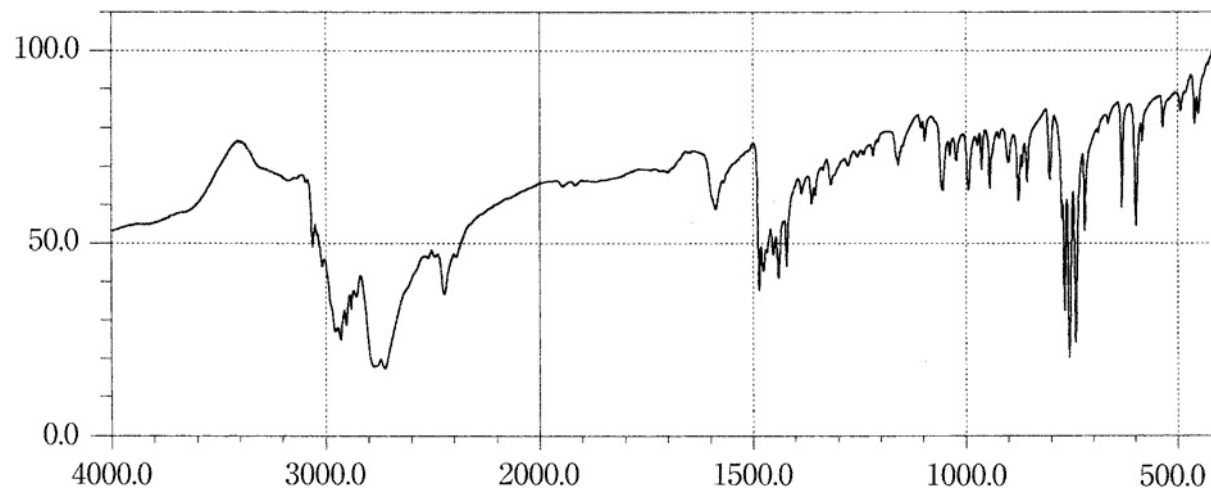


Norfloxacine

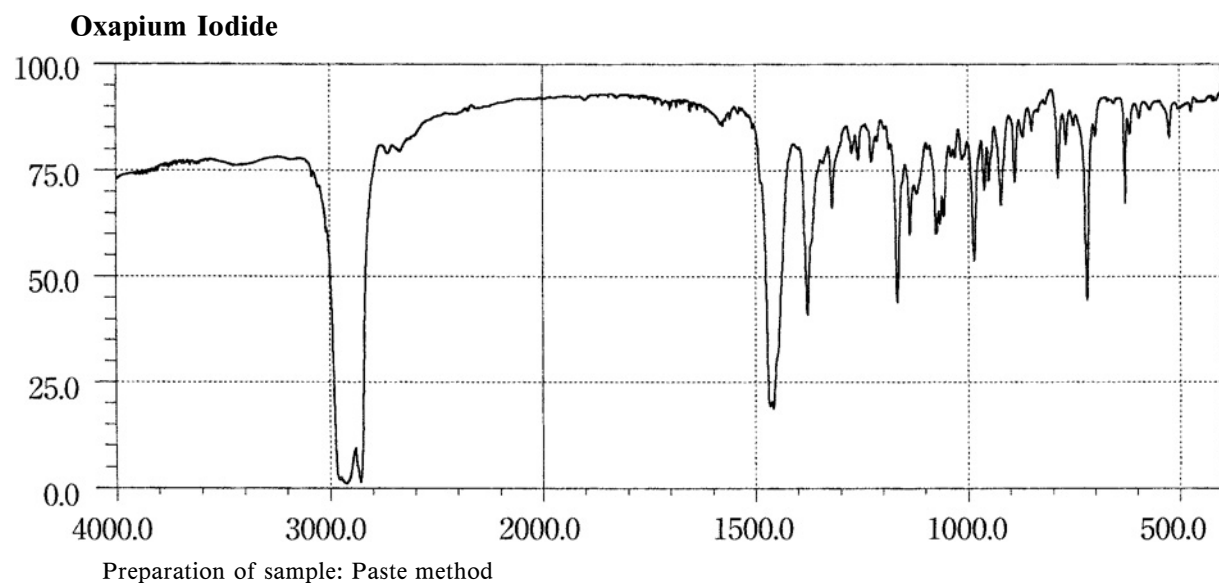
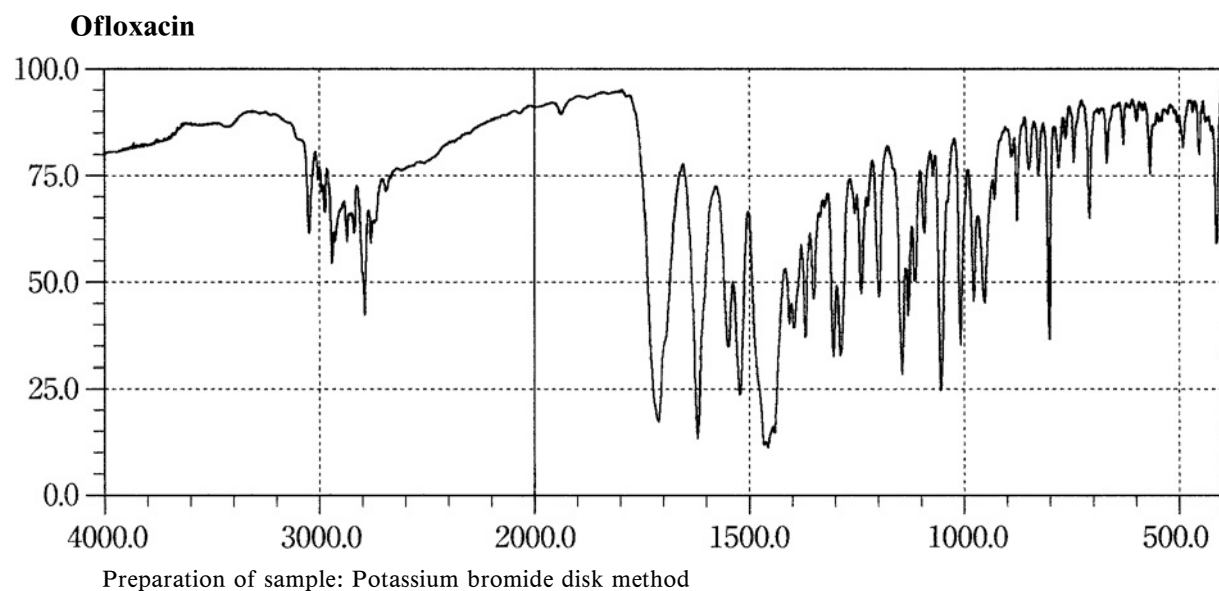
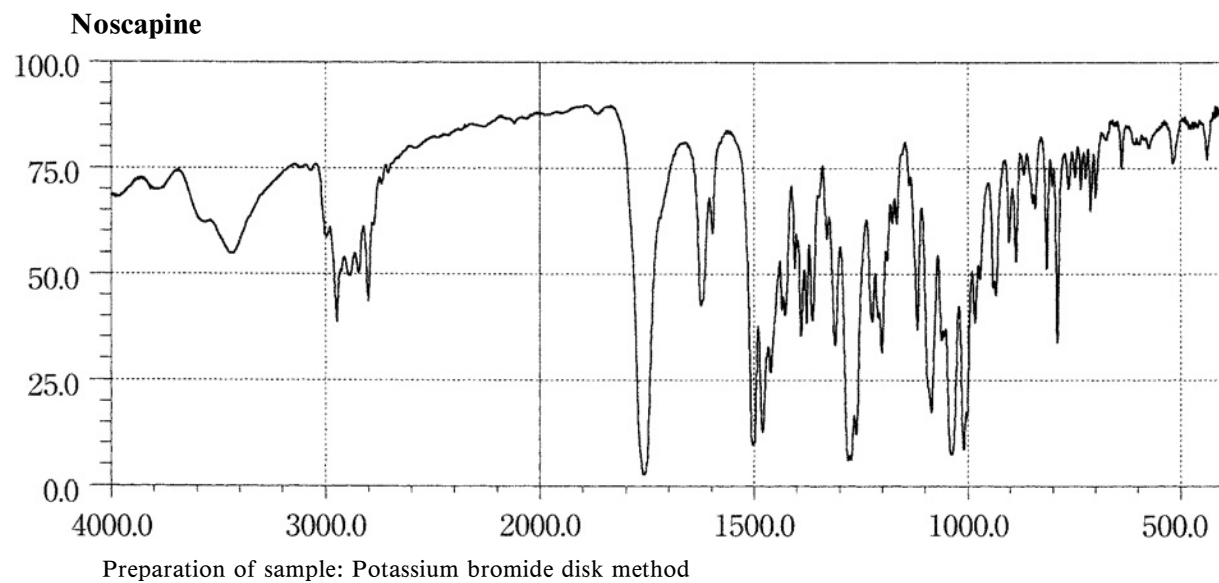
Preparation of sample: Potassium bromide disk method

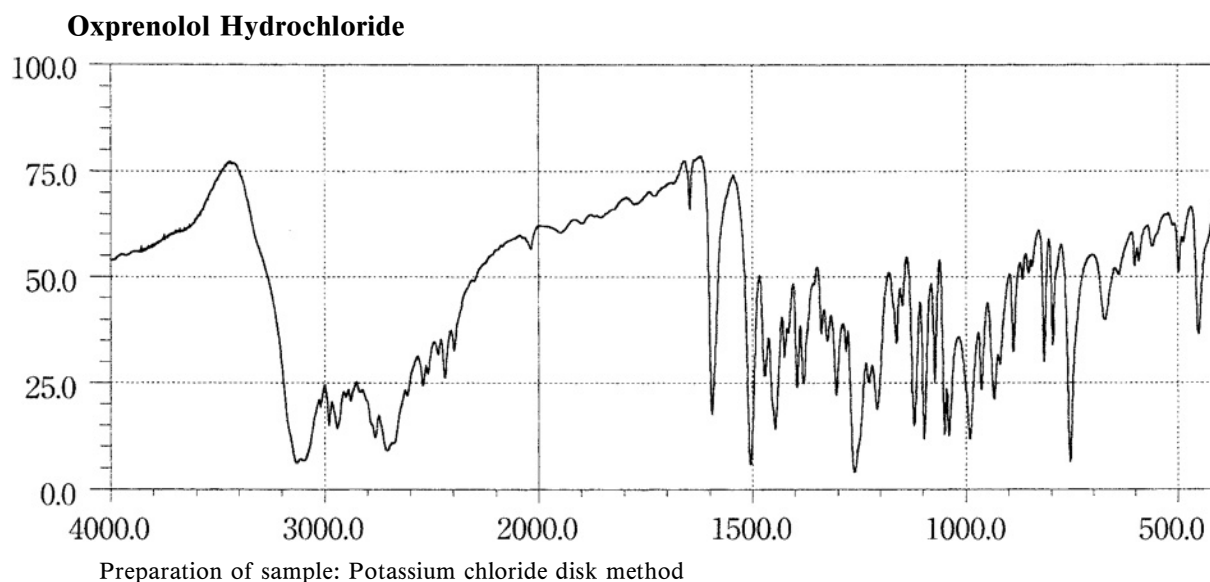
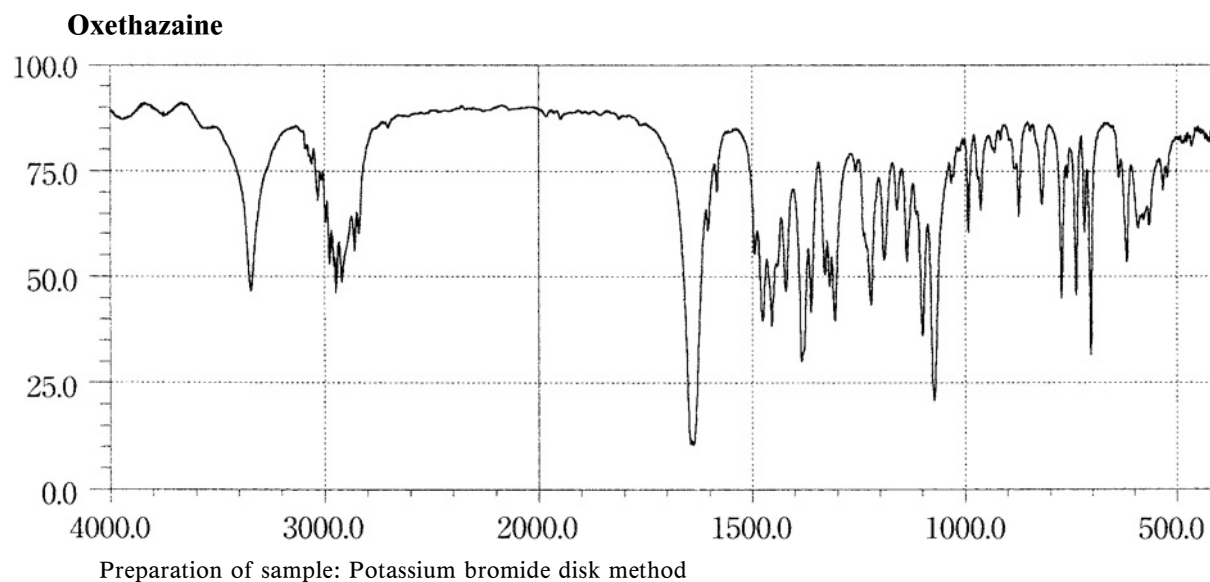
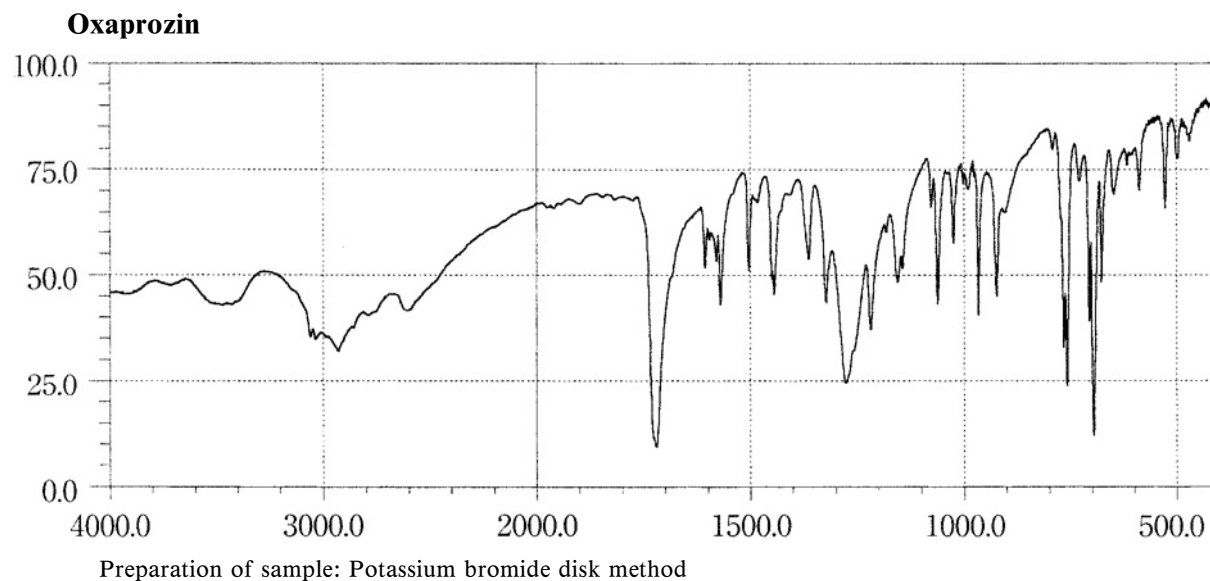
Norgestrel

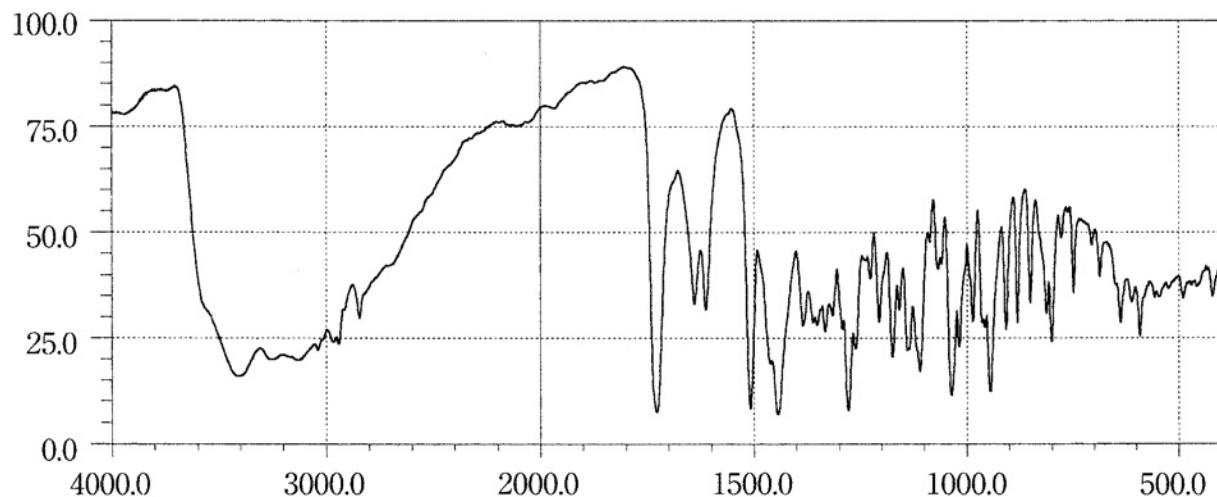
Preparation of sample: Potassium bromide disk method

Nortriptyline Hydrochloride

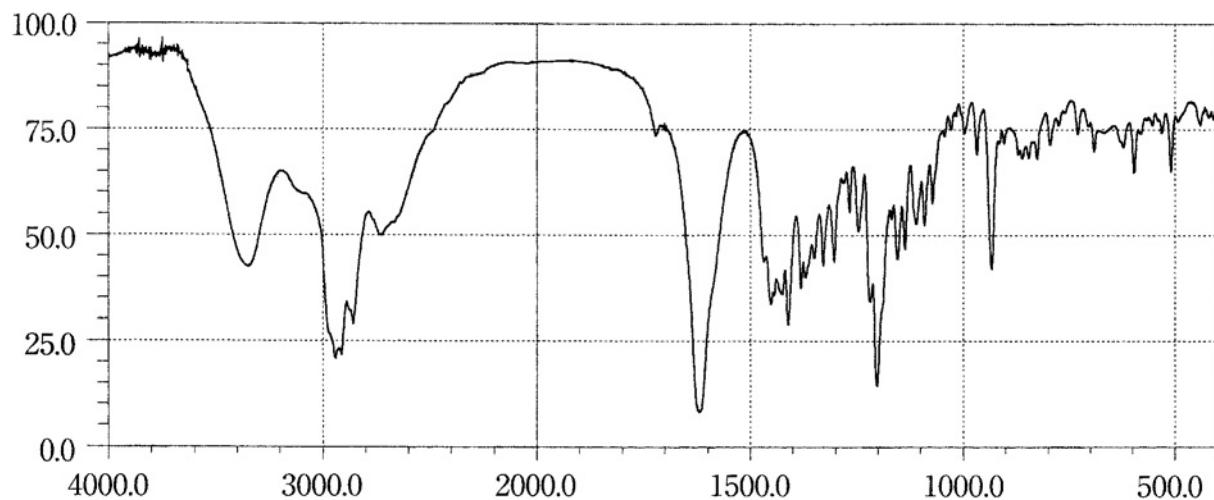
Preparation of sample: Potassium chloride disk method



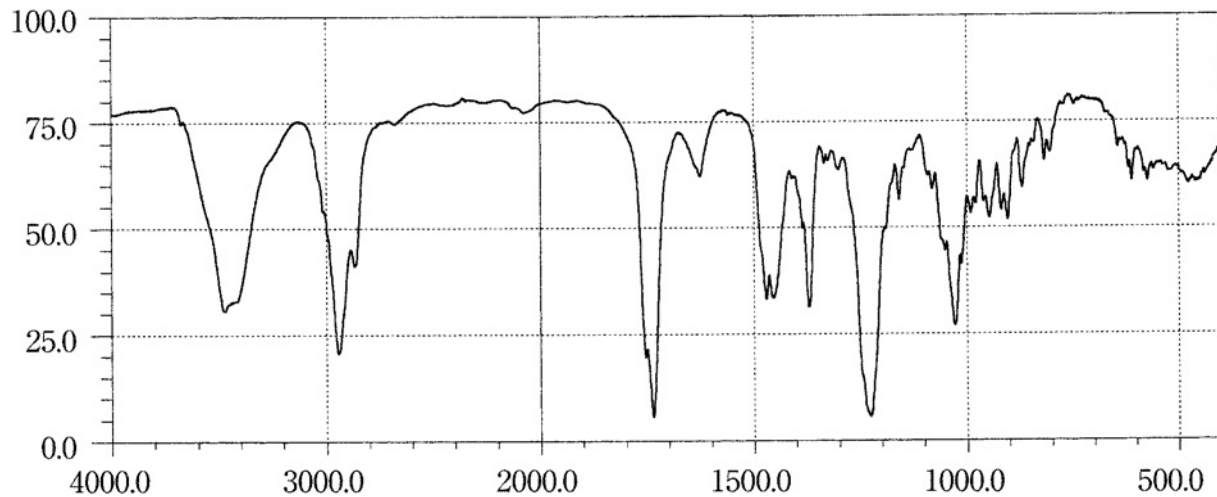


Oxycodone Hydrochloride Hydrate

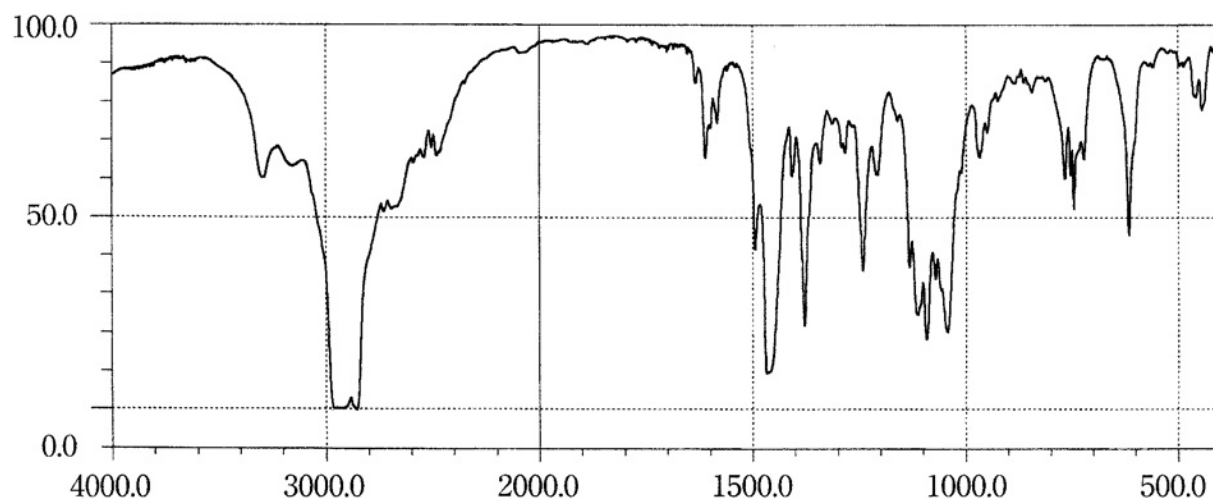
Preparation of sample: Potassium bromide disk method

Oxymetholone

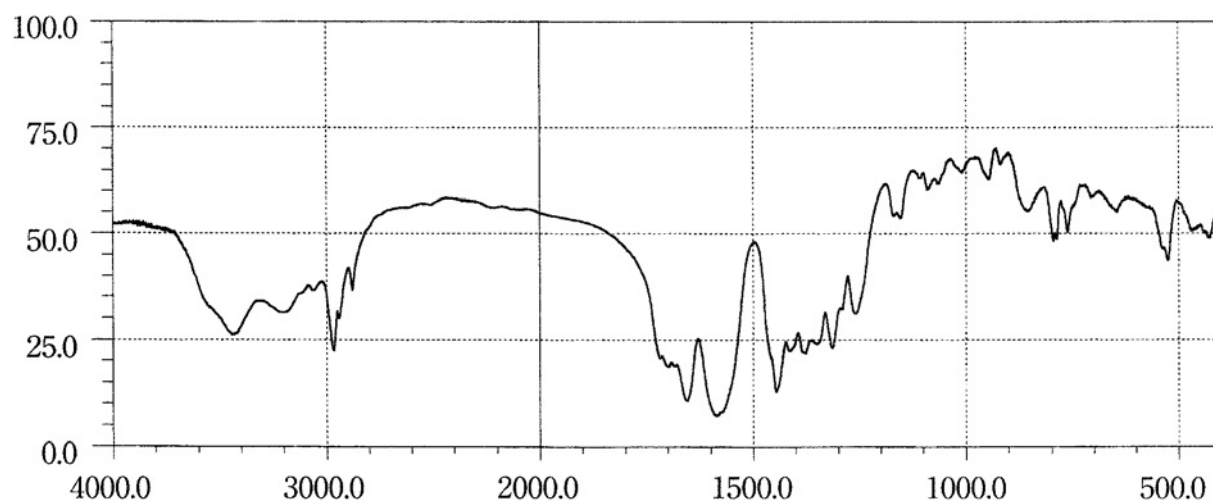
Preparation of sample: Potassium bromide disk method

Pancuronium Bromide

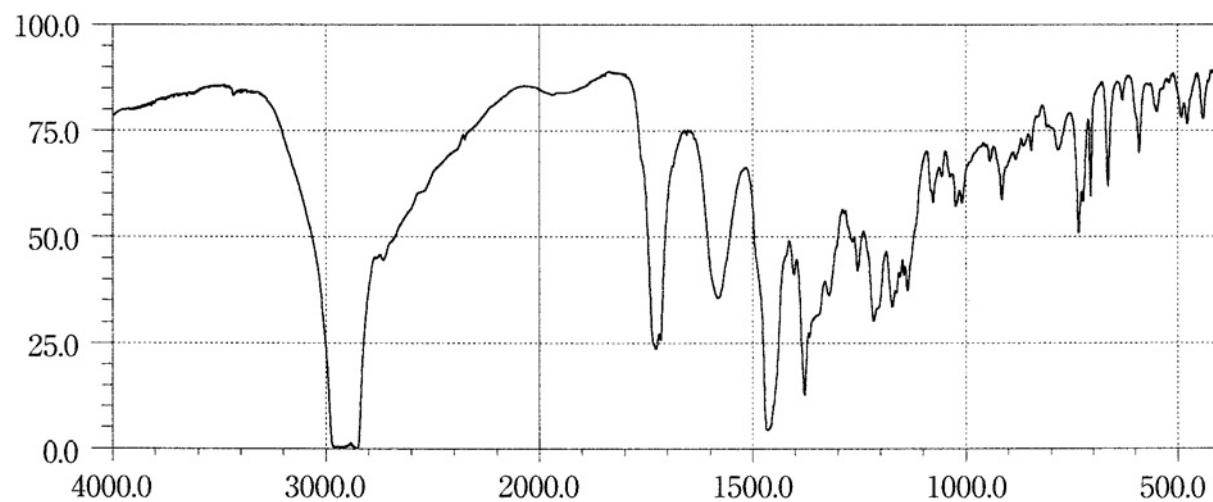
Preparation of sample: Potassium bromide disk method

Penbutolol Sulfate

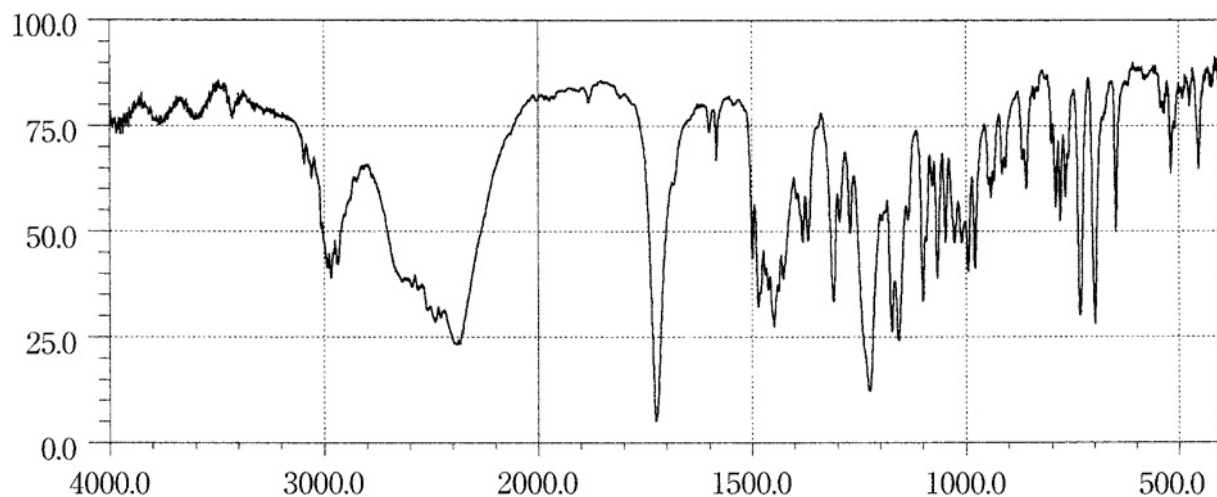
Preparation of sample: Paste method

Pentobarbital Calcium

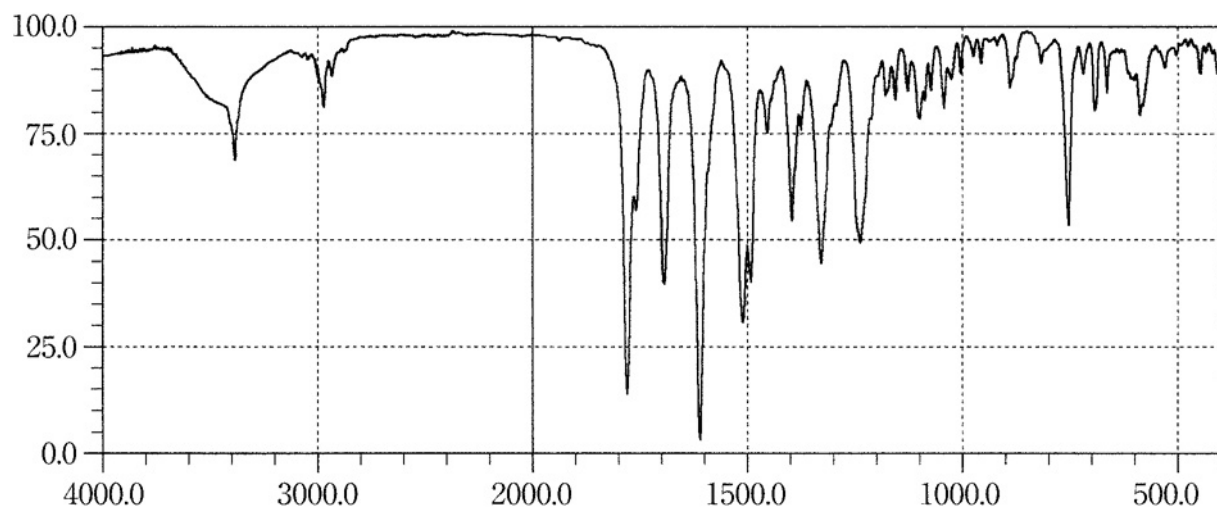
Preparation of sample: Potassium bromide disk method

Pentoxyverine Citrate

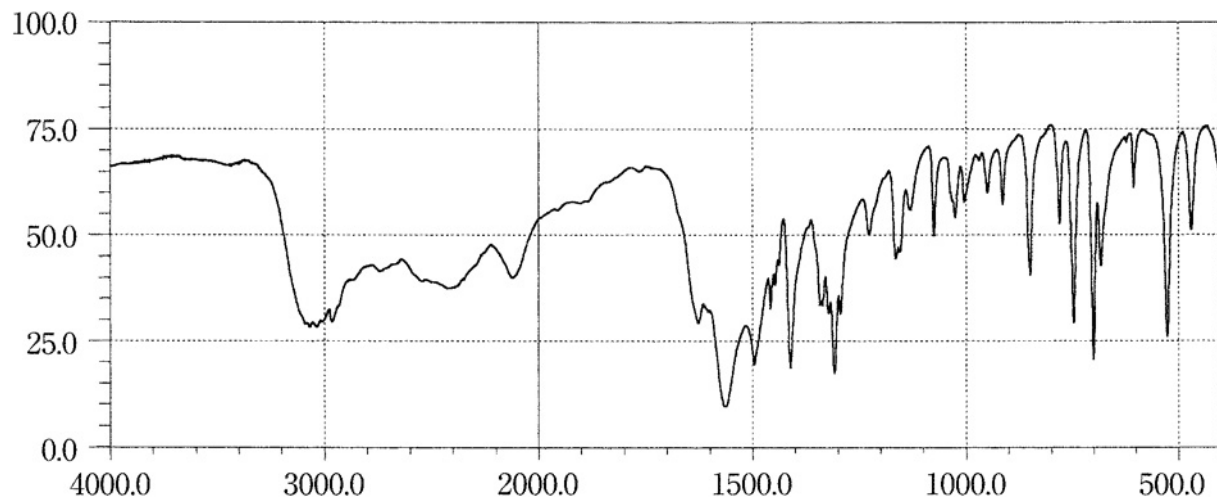
Preparation of sample: Paste method

Pethidine Hydrochloride

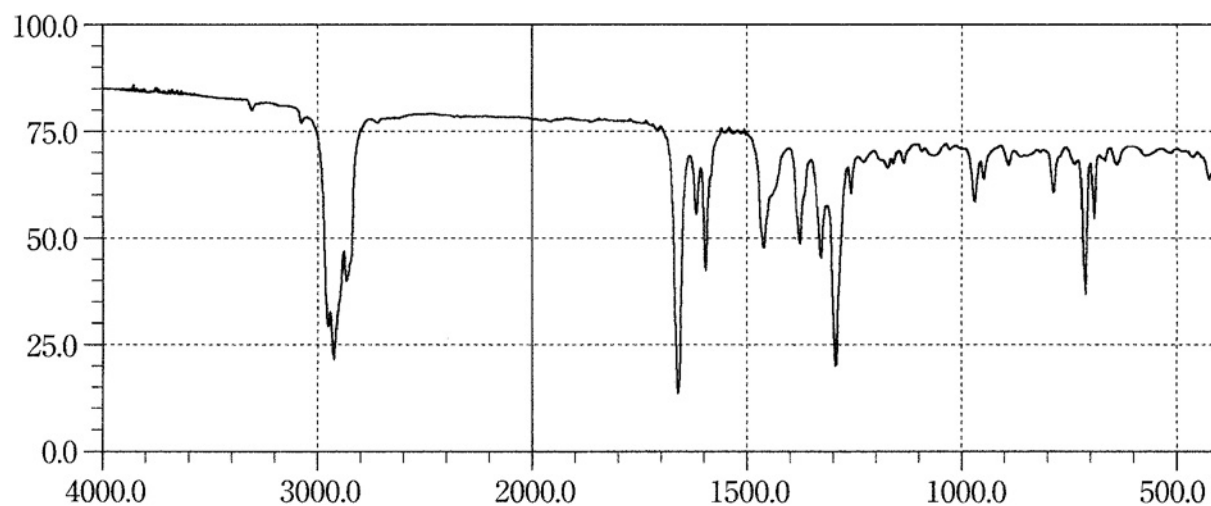
Preparation of sample: Potassium bromide disk method

Phenethicillin Potassium

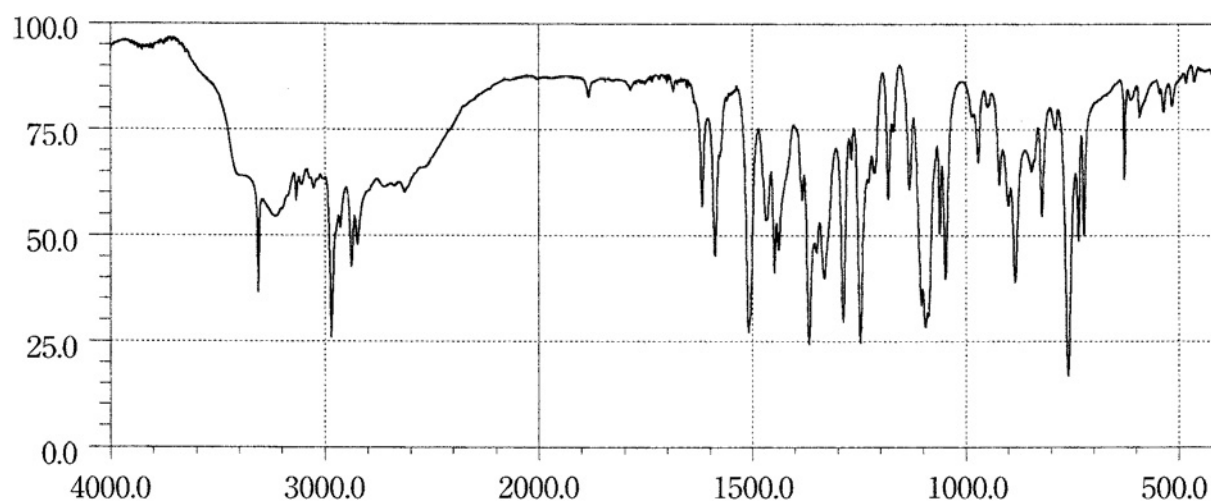
Preparation of sample: Potassium bromide disk method

L-Phenylalanine

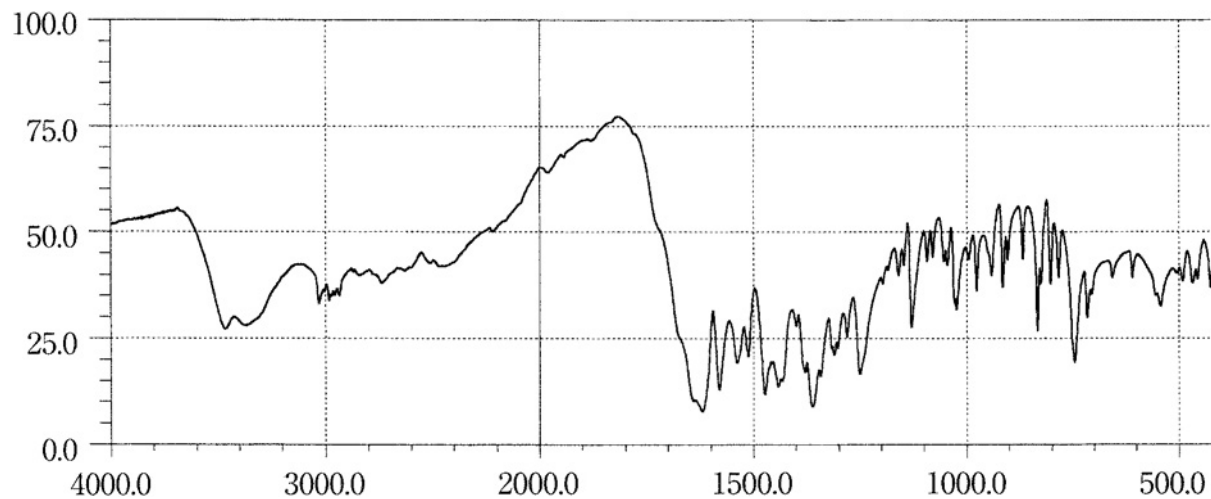
Preparation of sample: Potassium bromide disk method

Phytonadione

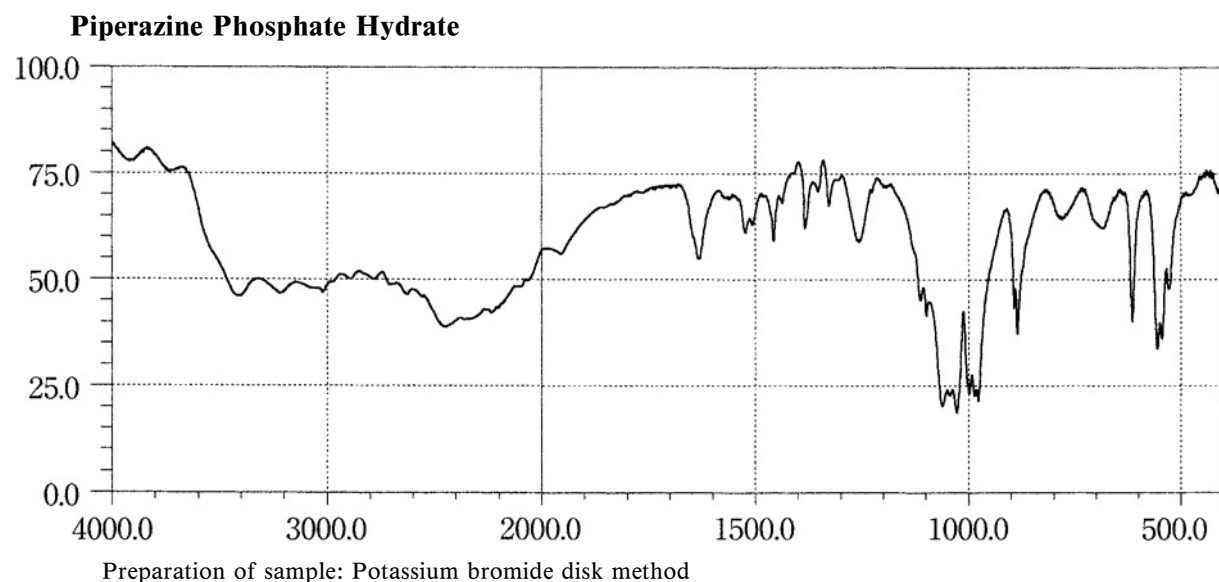
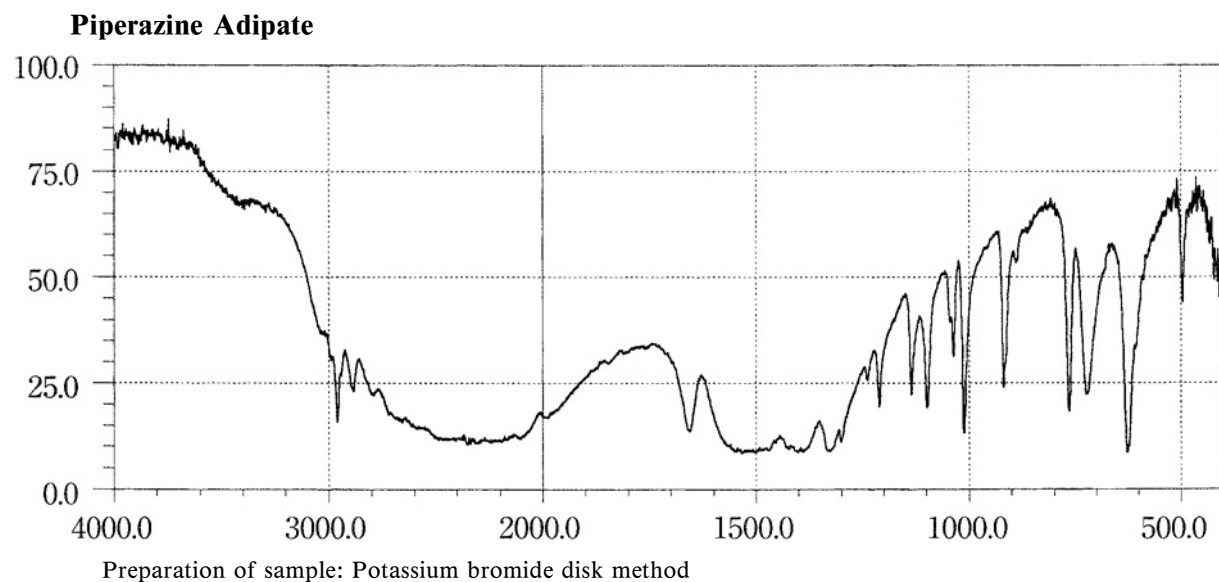
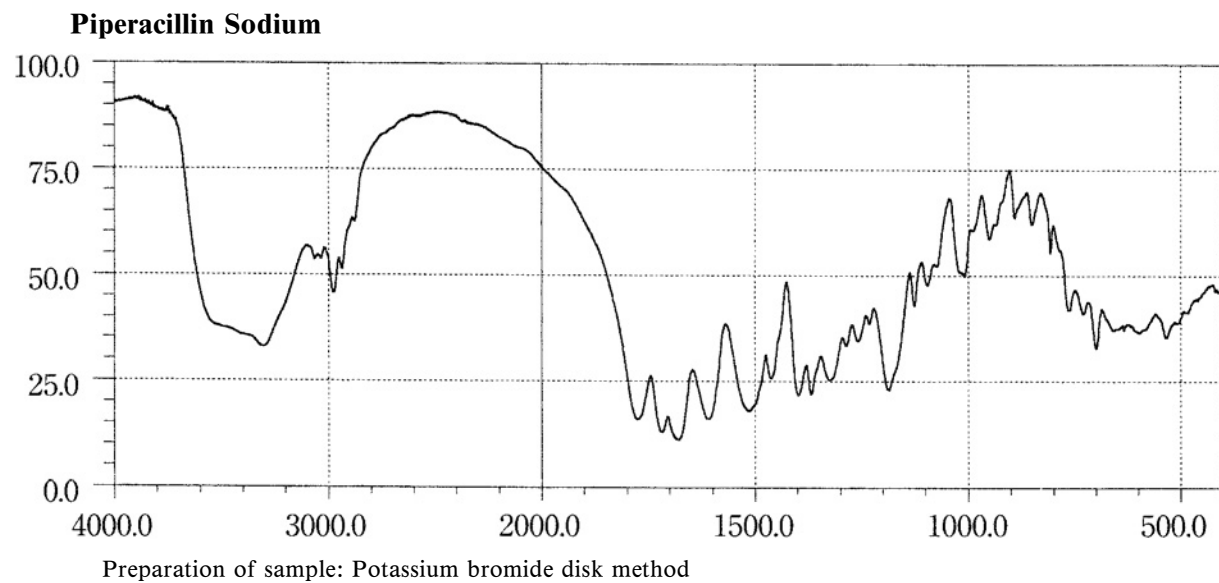
Preparation of sample: Liquid film method

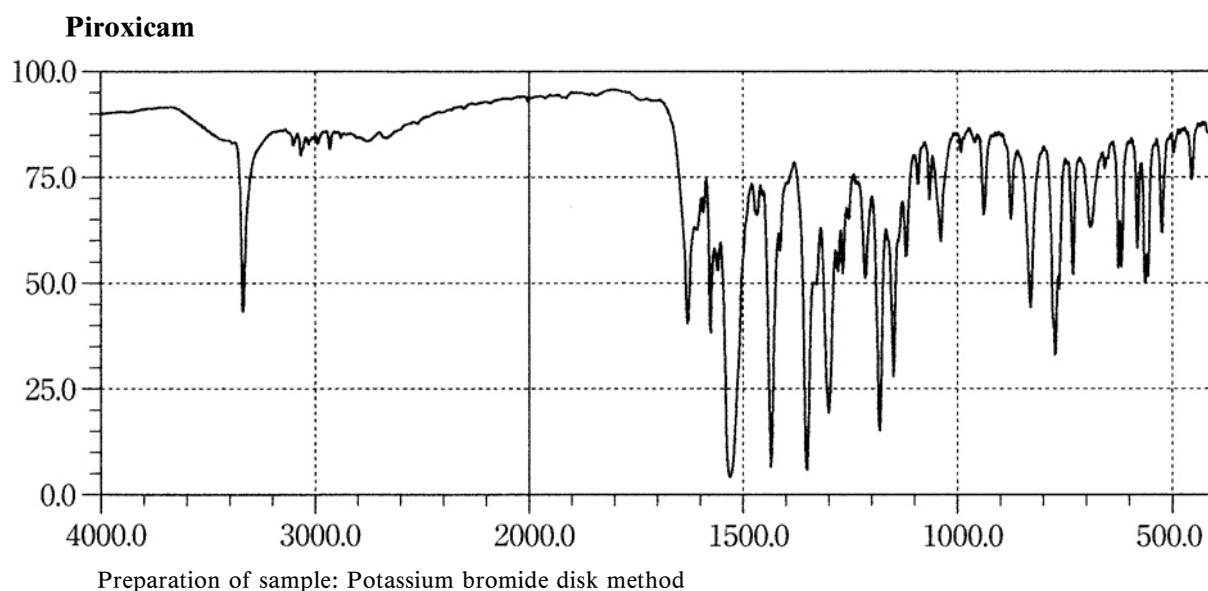
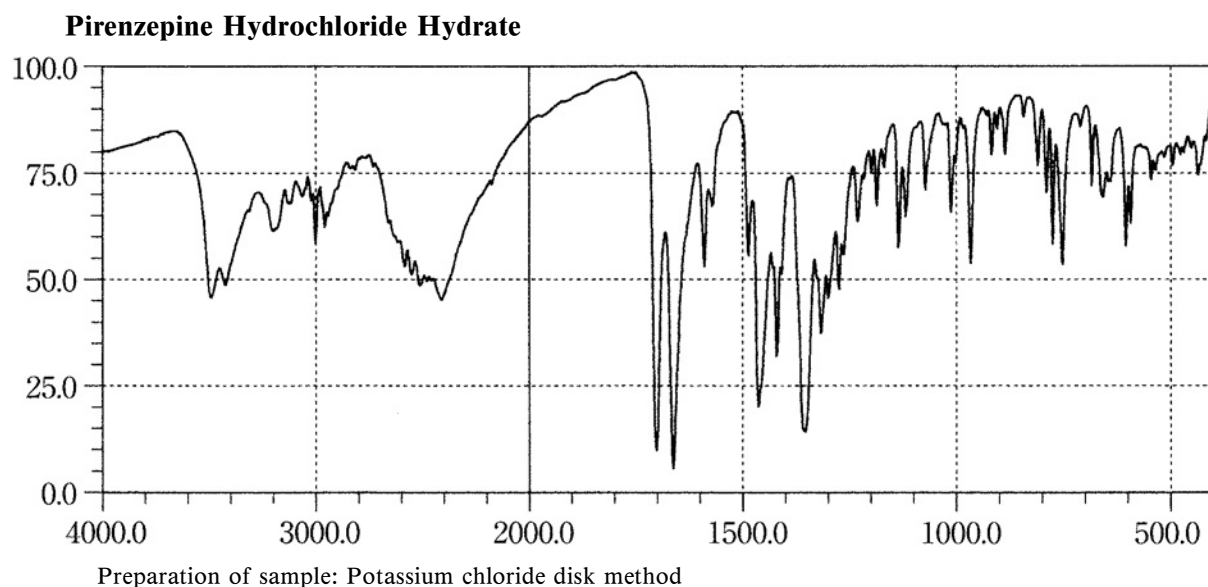
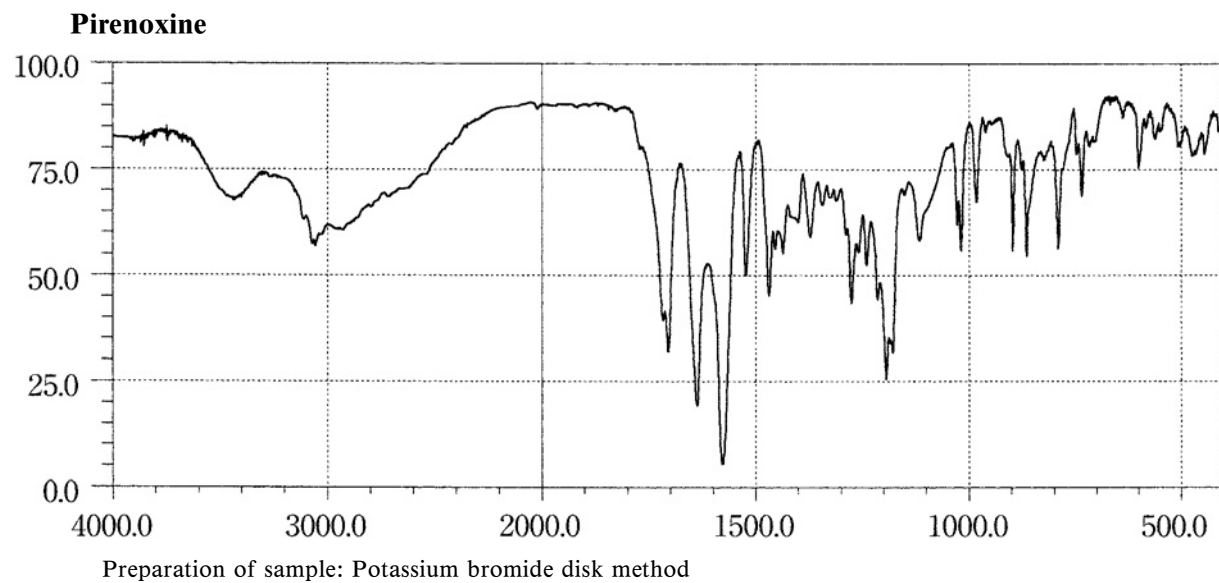
Pindolol

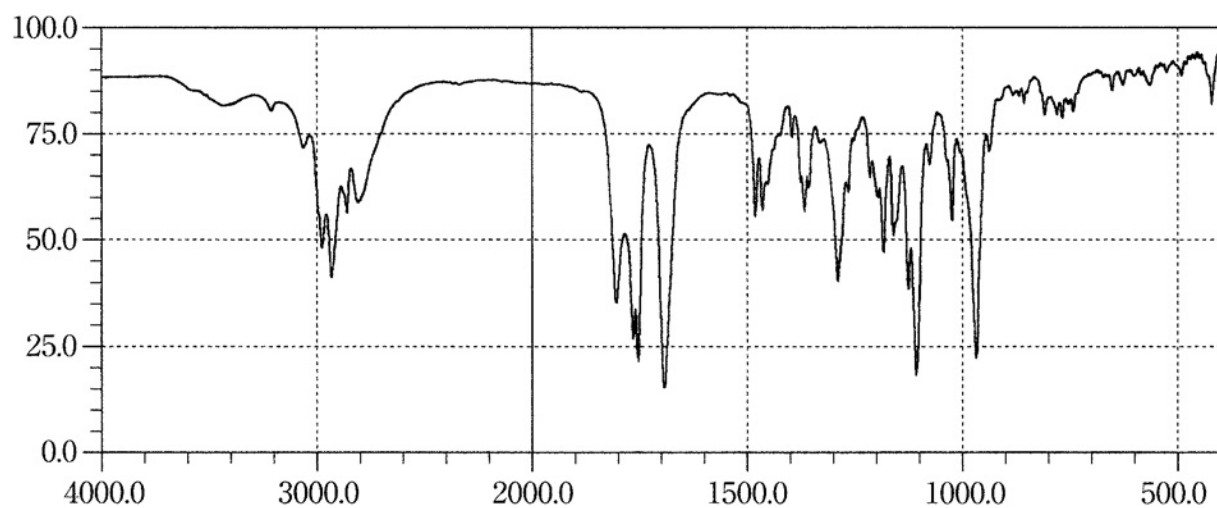
Preparation of sample: Potassium bromide disk method

Pipemidic Acid Hydrate

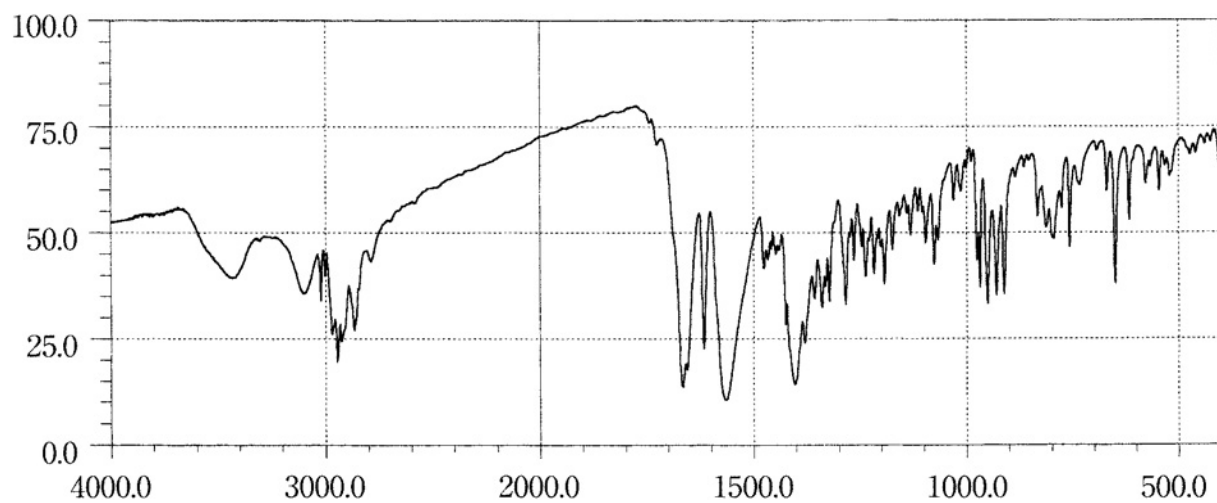
Preparation of sample: Potassium bromide disk method



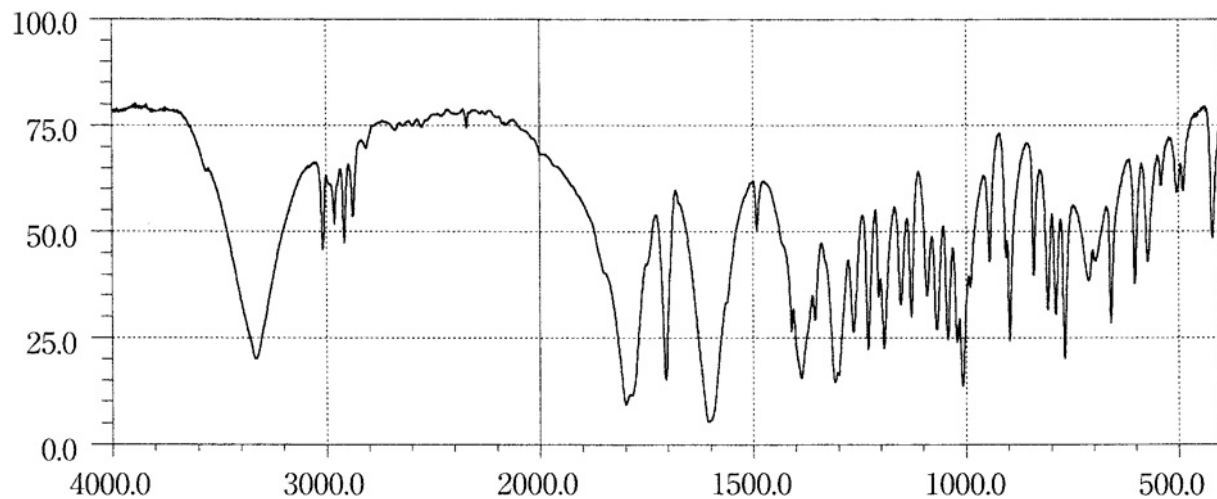


Pivmecillinam Hydrochloride

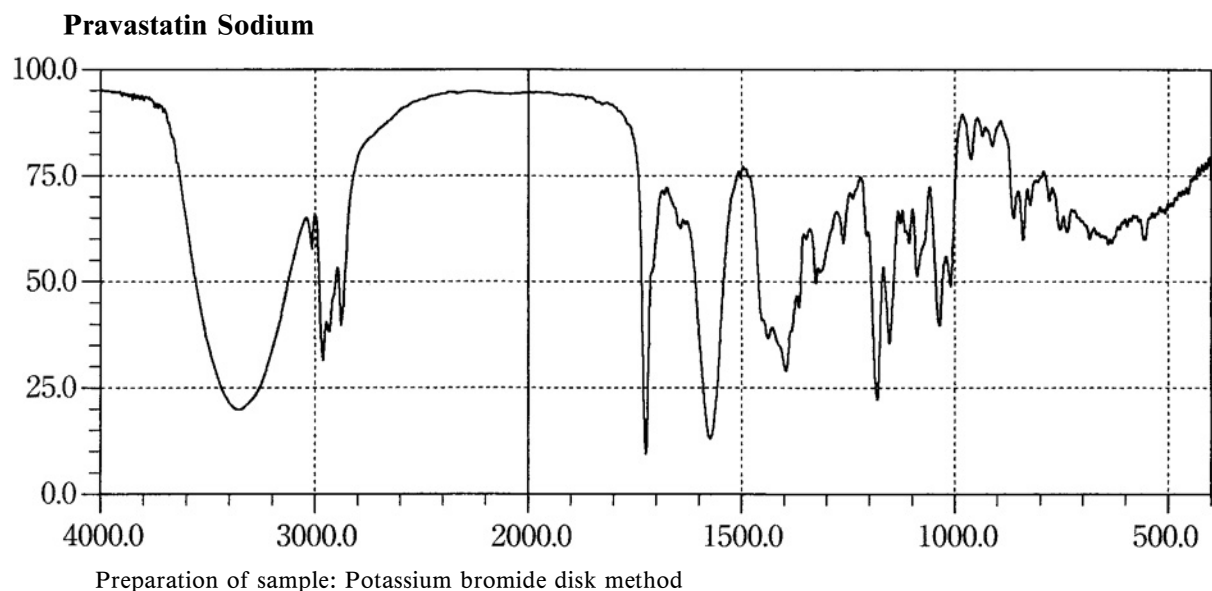
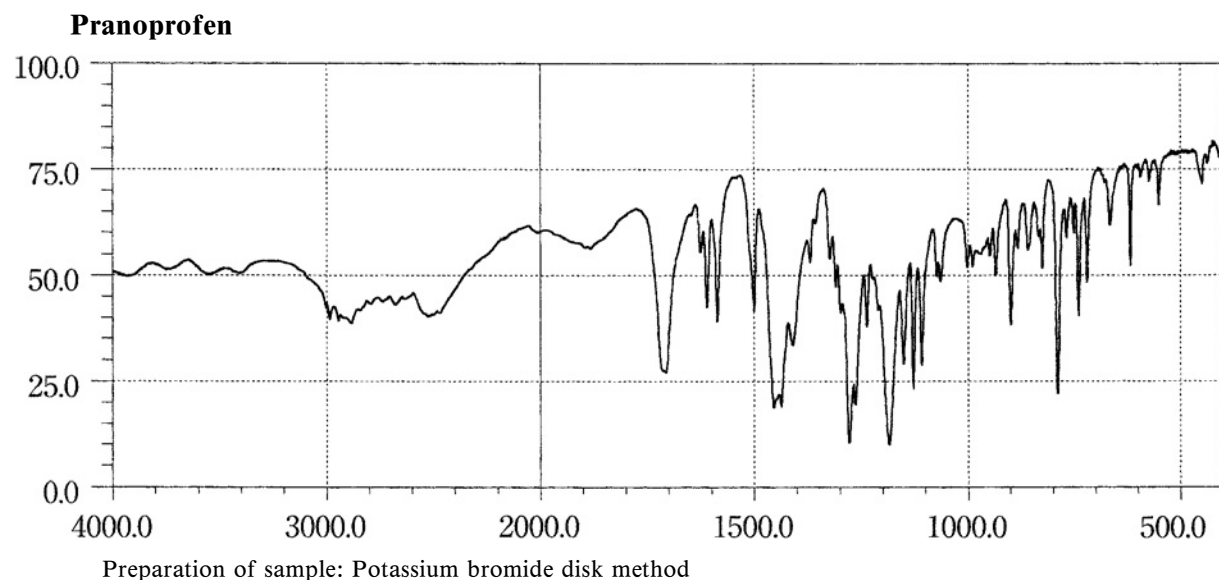
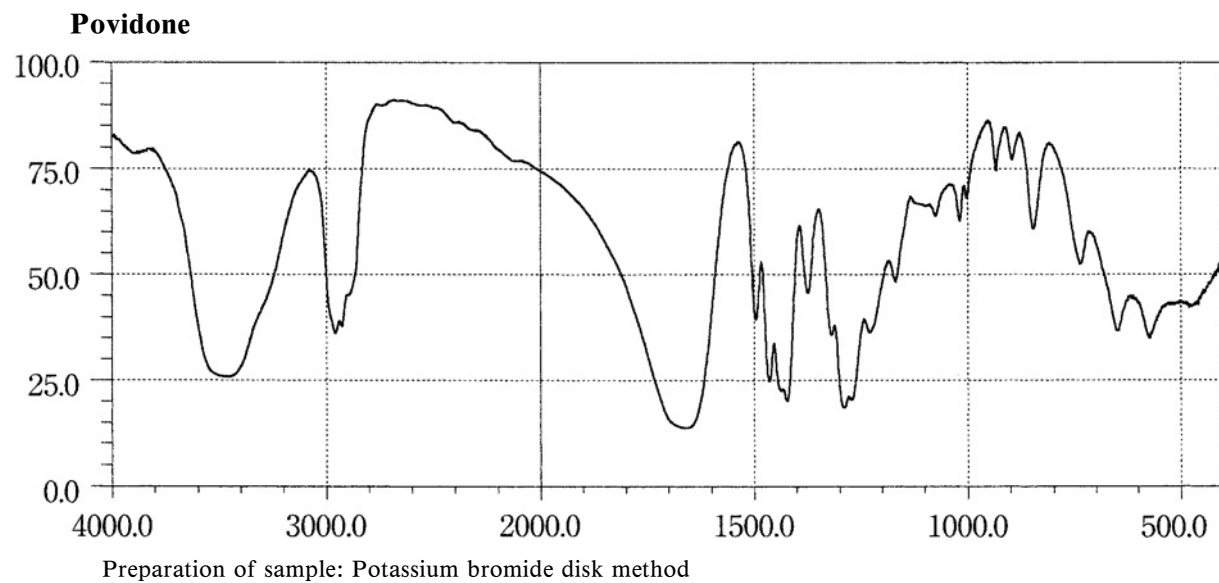
Preparation of sample: Potassium bromide disk method

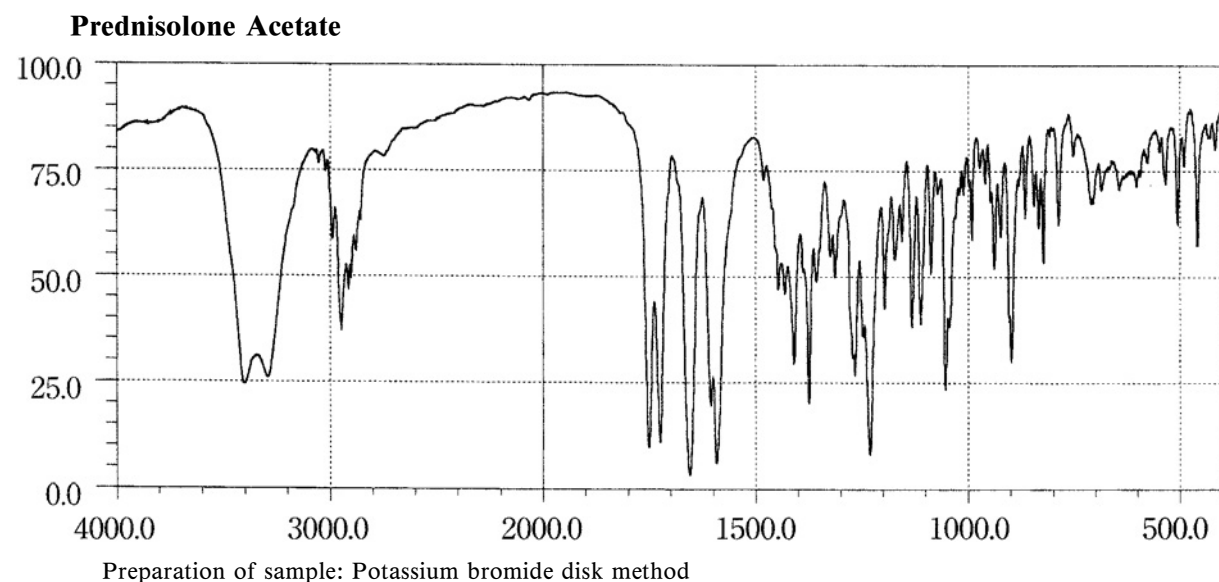
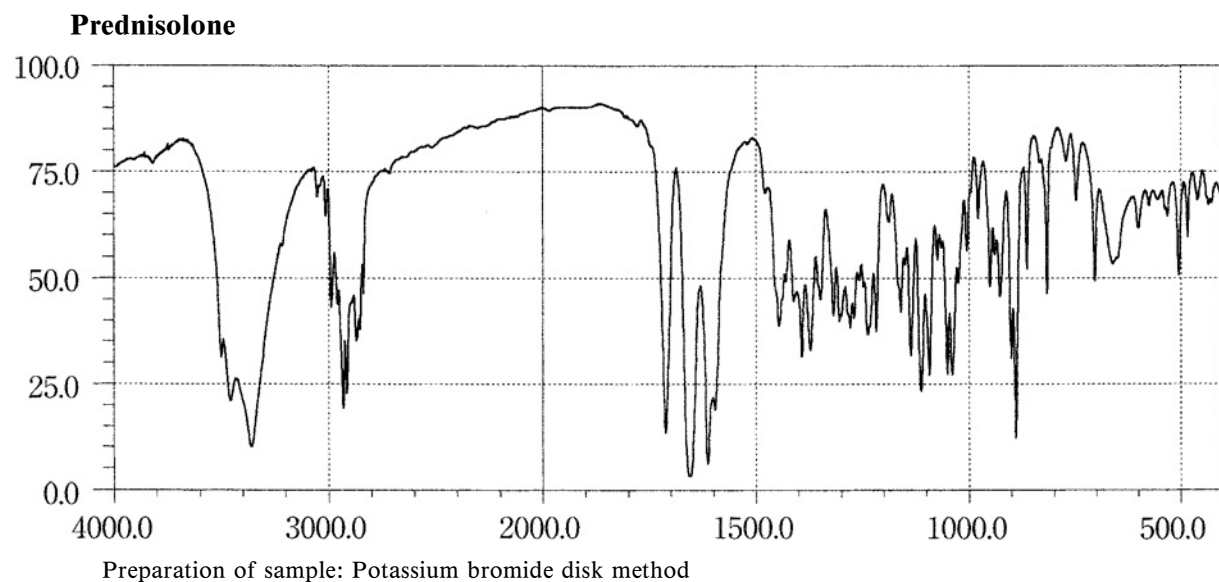
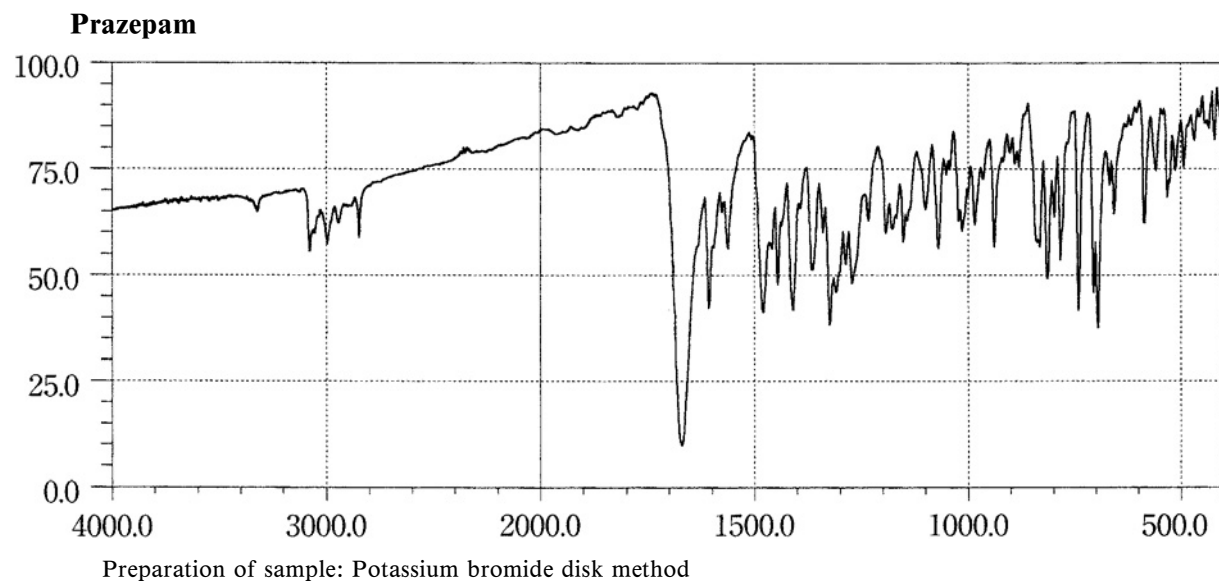
Potassium Canrenoate

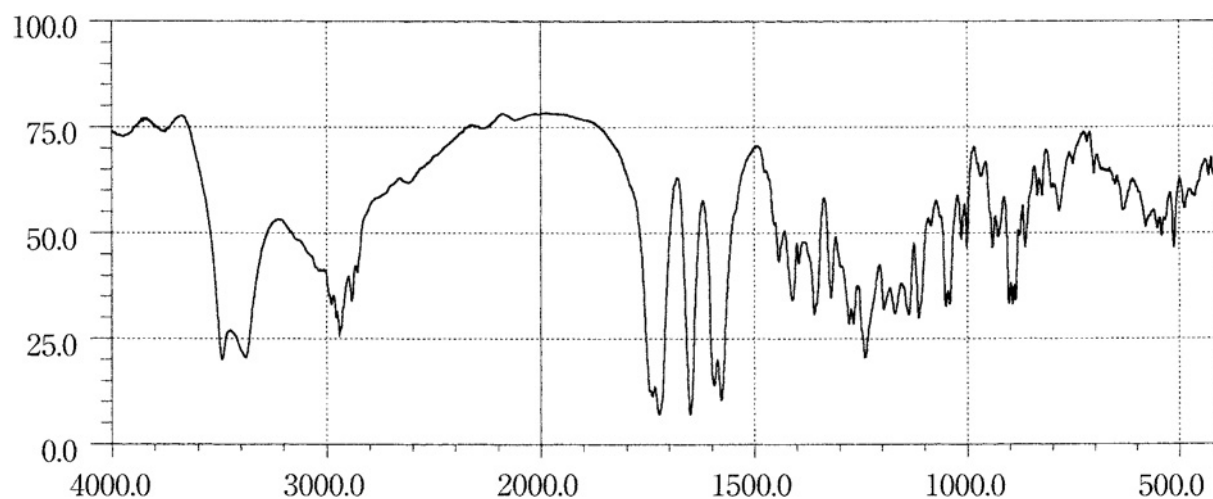
Preparation of sample: Potassium bromide disk method

Potassium Clavulanate

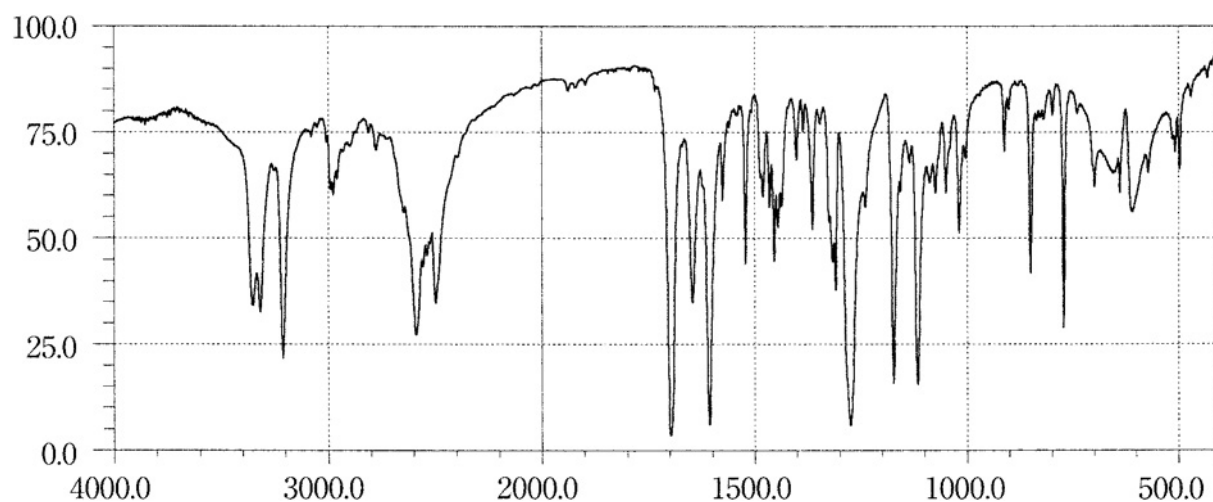
Preparation of sample: Potassium bromide disk method



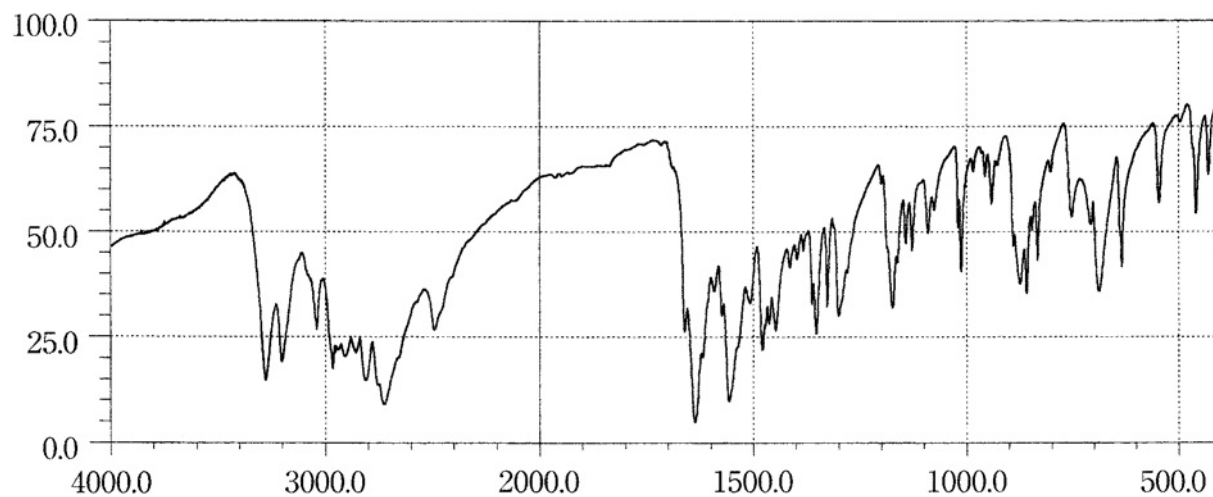


Prednisolone Succinate

Preparation of sample: Potassium bromide disk method

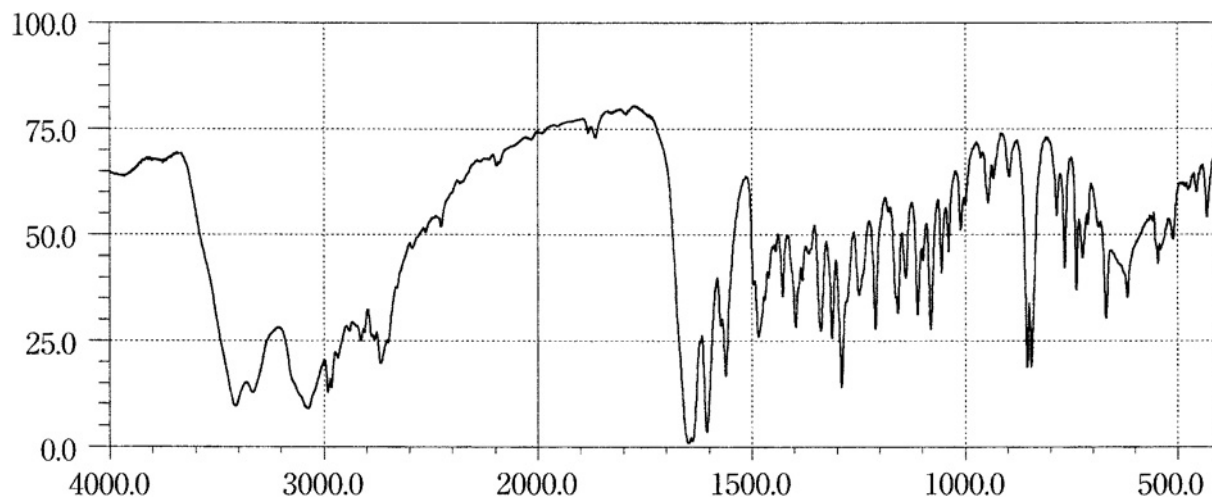
Procaine Hydrochloride

Preparation of sample: Potassium chloride disk method

Procarbazine Hydrochloride

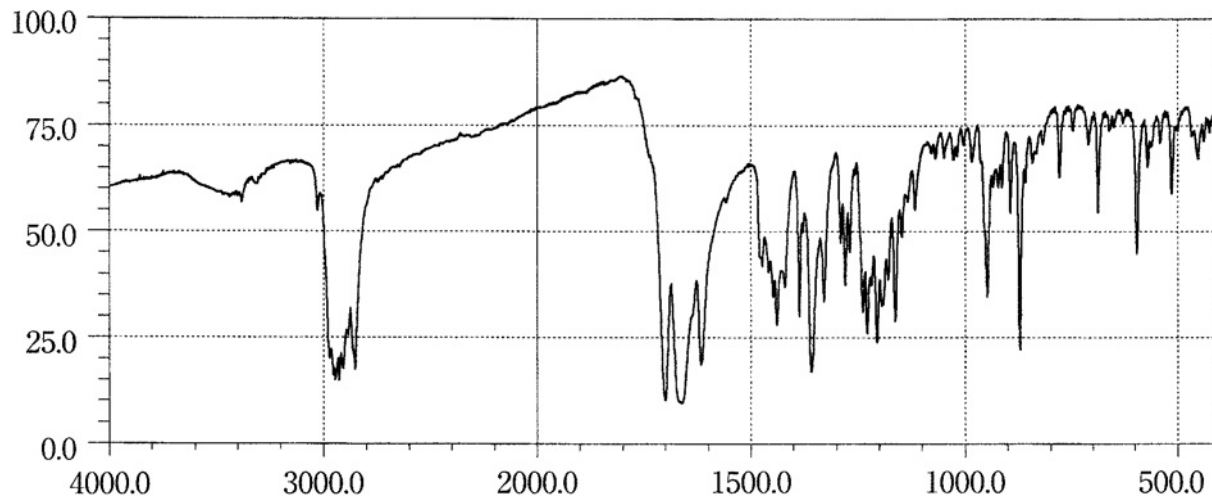
Preparation of sample: Potassium chloride disk method

Procaterol Hydrochloride Hydrate



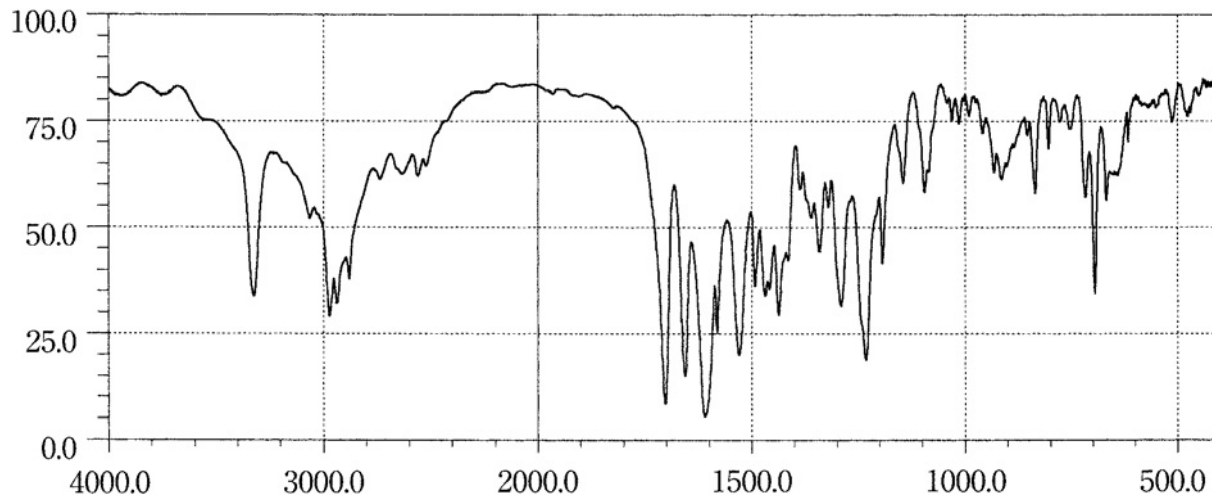
Preparation of sample: Potassium bromide disk method

Progesterone

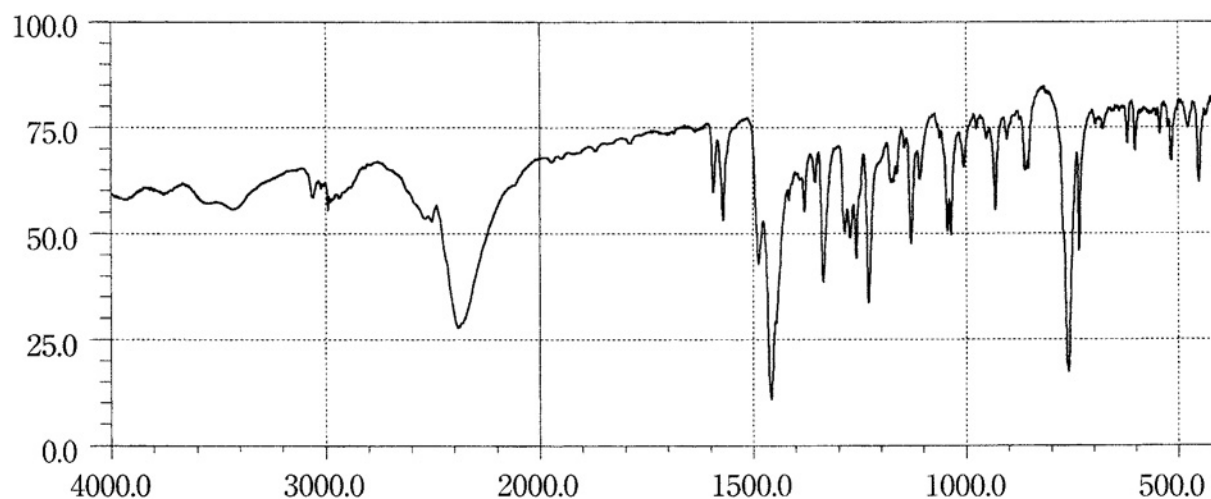


Preparation of sample: Potassium bromide disk method

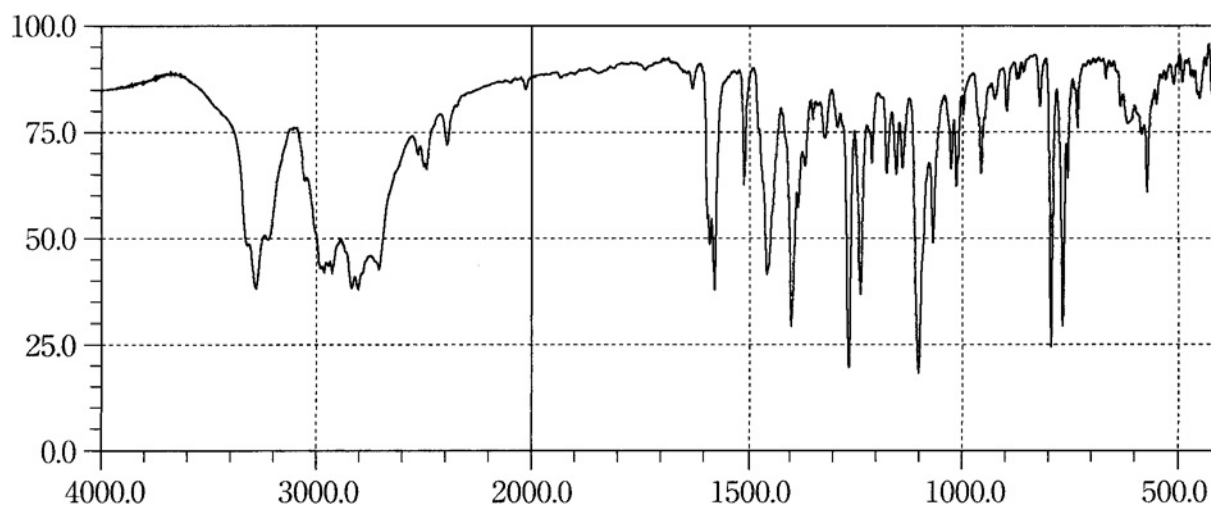
Proglumide



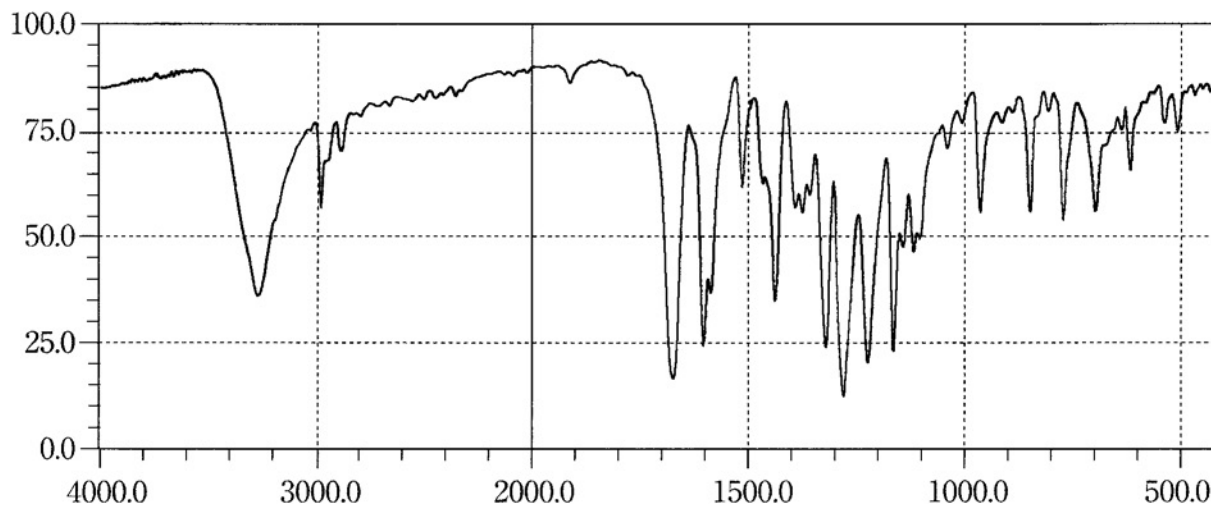
Preparation of sample: Potassium bromide disk method

Promethazine Hydrochloride

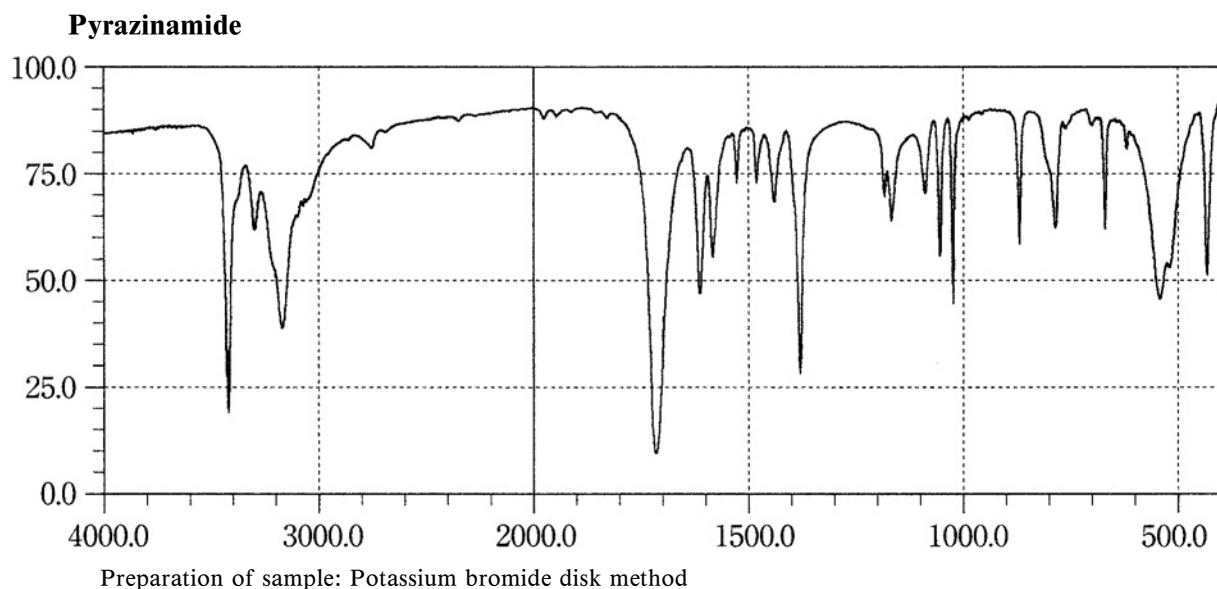
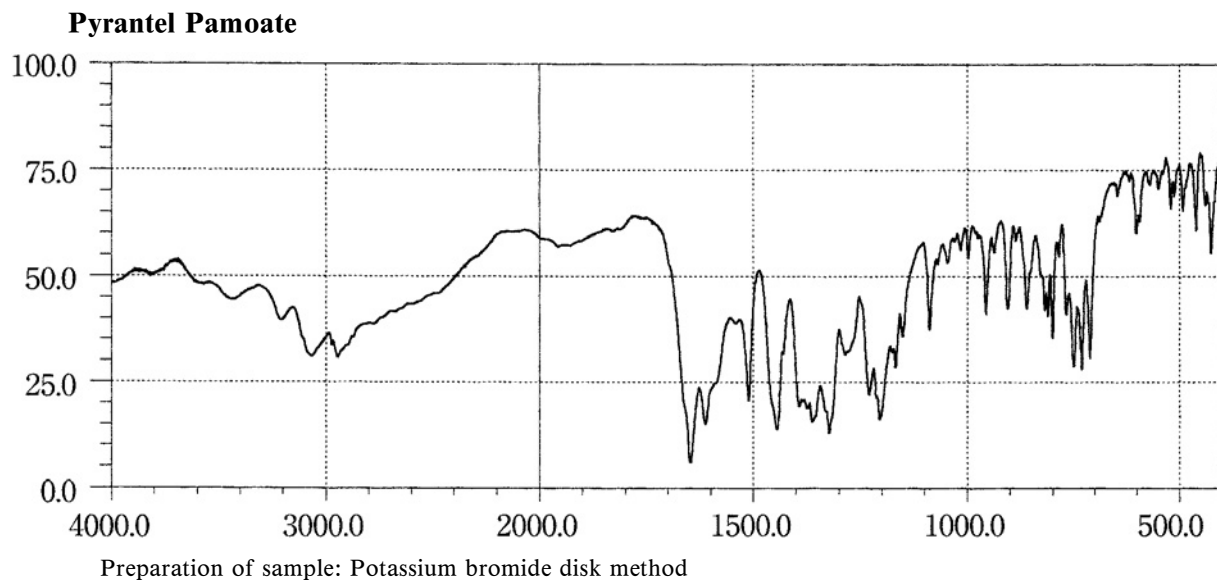
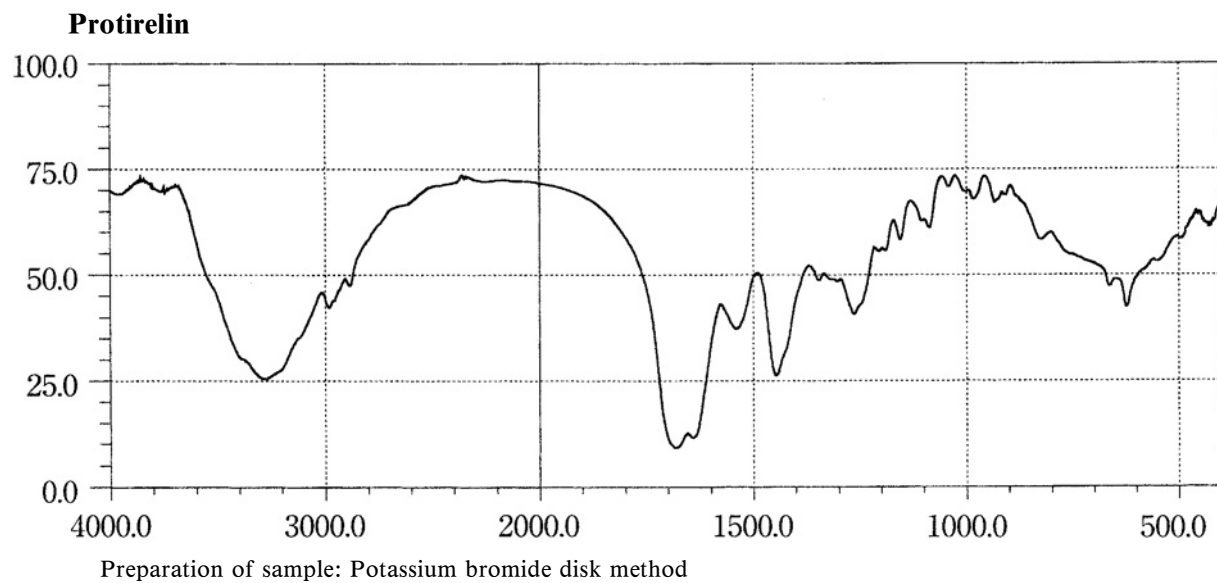
Preparation of sample: Potassium bromide disk method

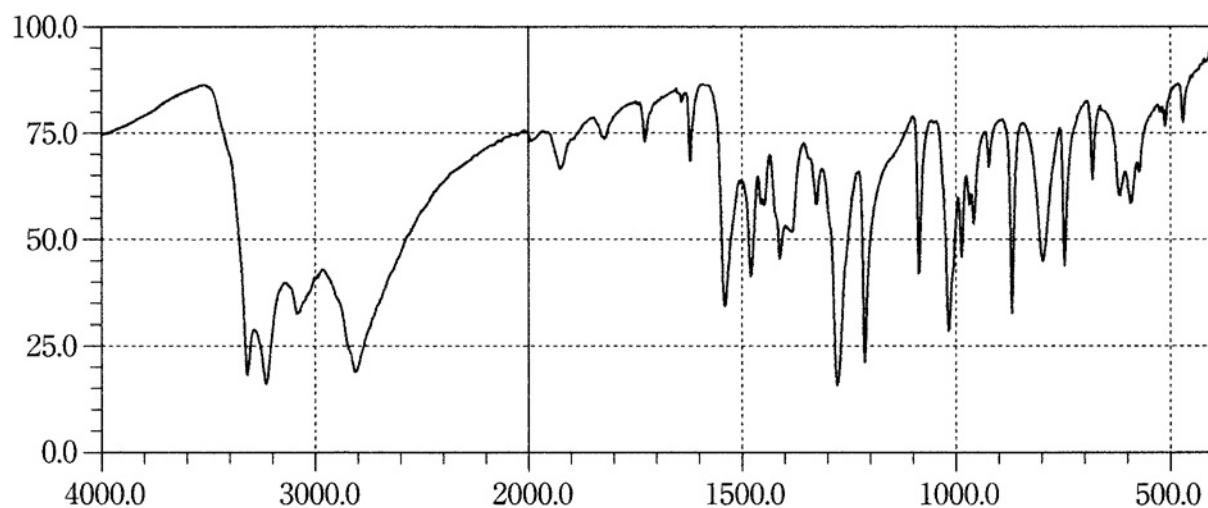
Propranolol Hydrochloride

Preparation of sample: Potassium chloride disk method

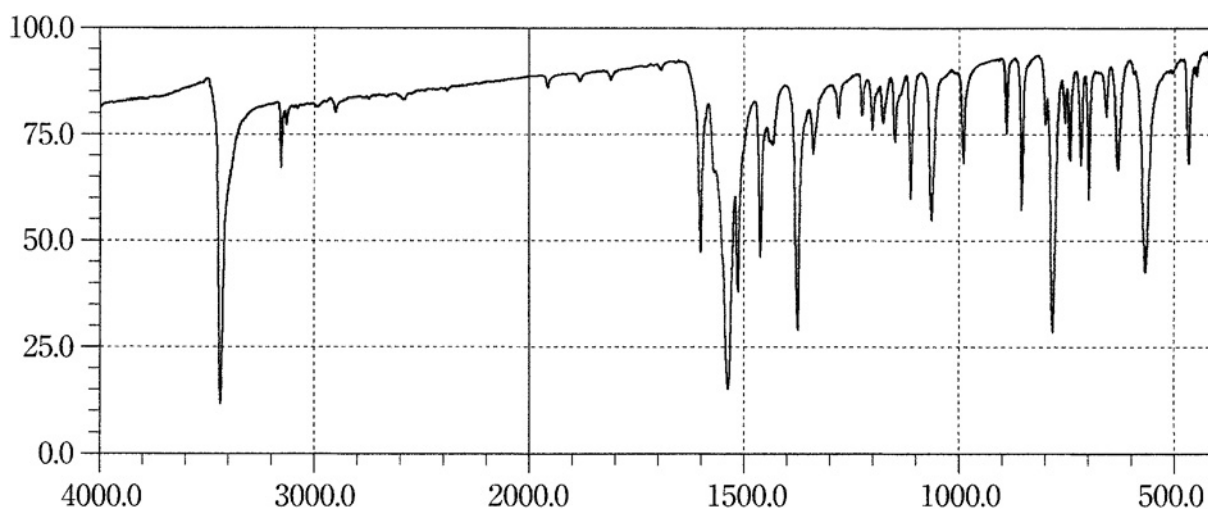
Propyl Parahydroxybenzoate

Preparation of sample: Potassium bromide disk method

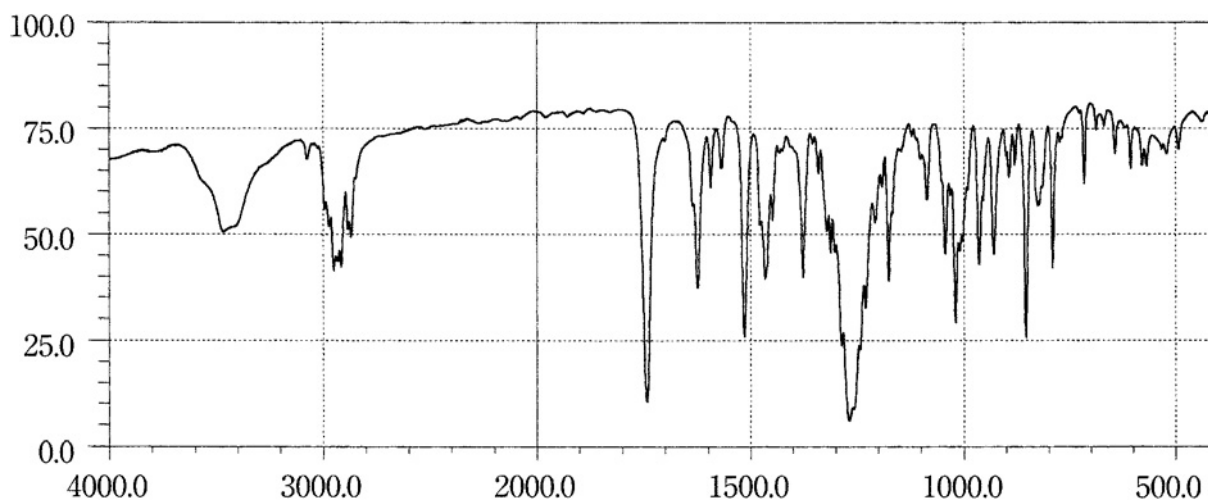


Pyridoxine Hydrochloride

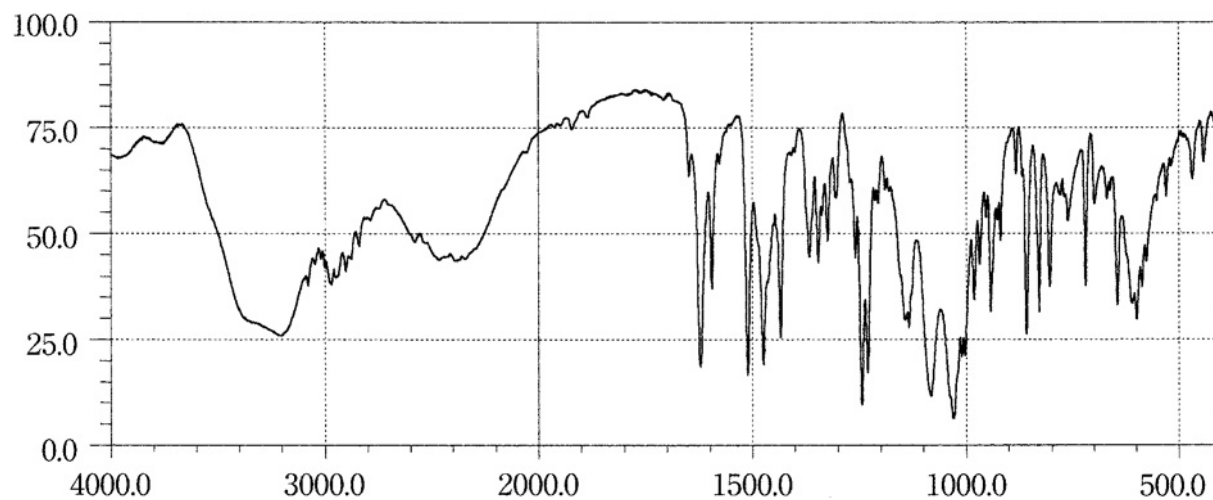
Preparation of sample: Potassium chloride disk method

Pyrrrolnitrin

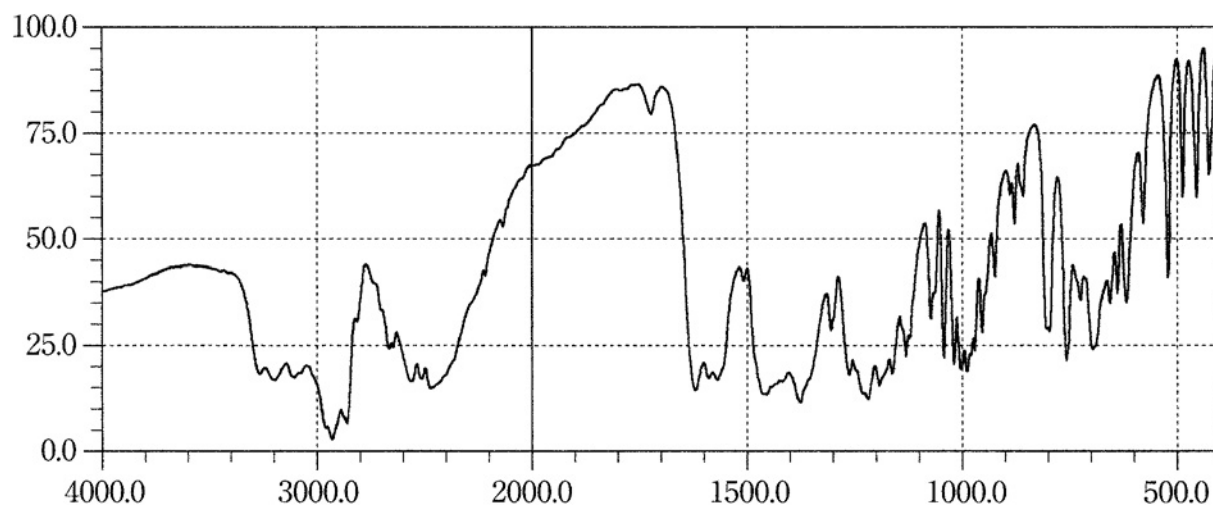
Preparation of sample: Potassium bromide disk method

Quinine Ethyl Carbonate

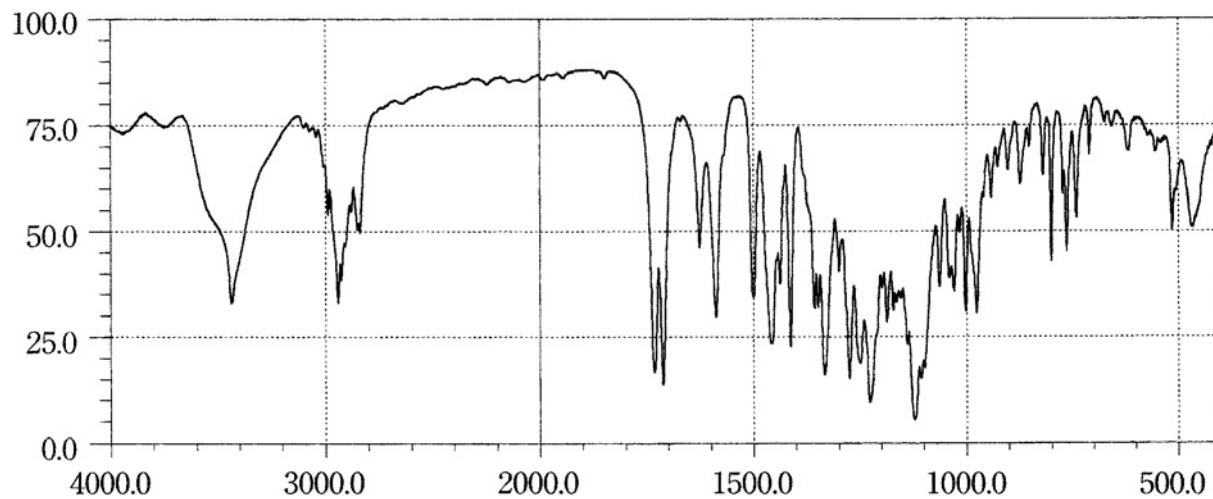
Preparation of sample: Potassium bromide disk method

Quinine Sulfate Hydrate

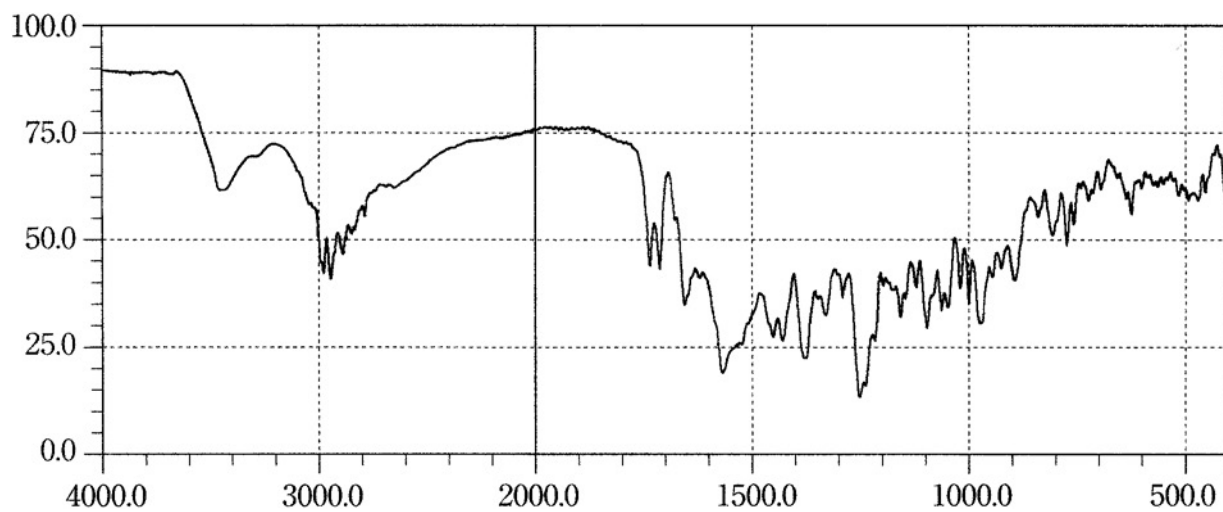
Preparation of sample: Potassium bromide disk method

Ranitidine Hydrochloride

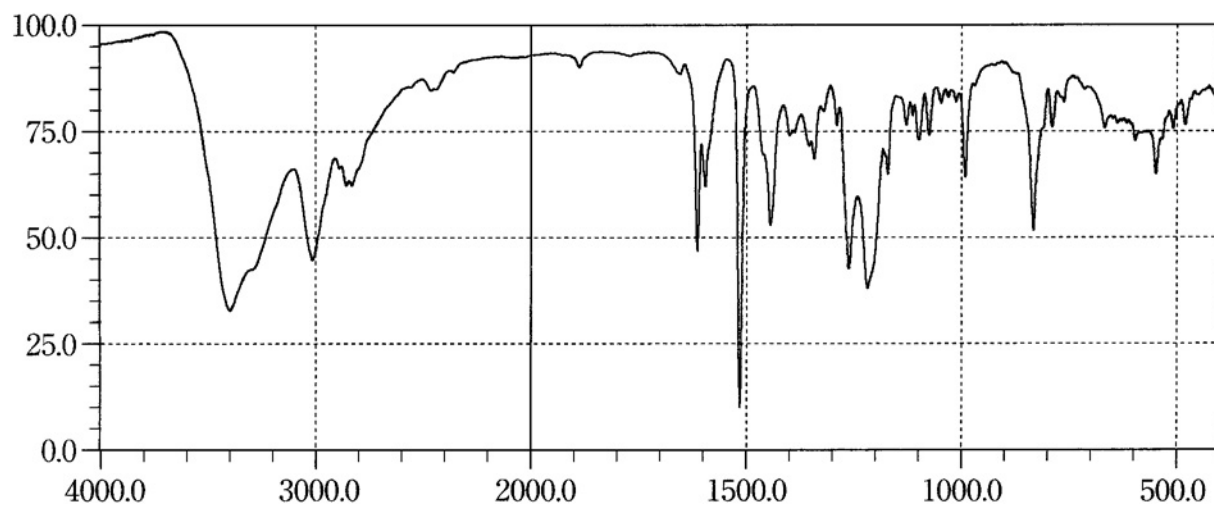
Preparation of sample: Paste method

Reserpine

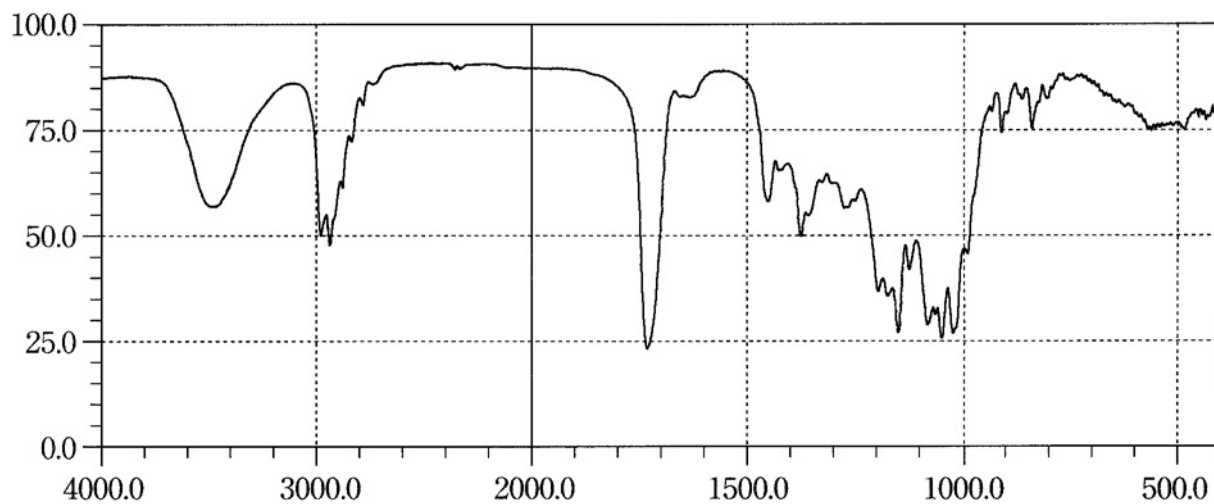
Preparation of sample: Potassium bromide disk method

Rifampicin

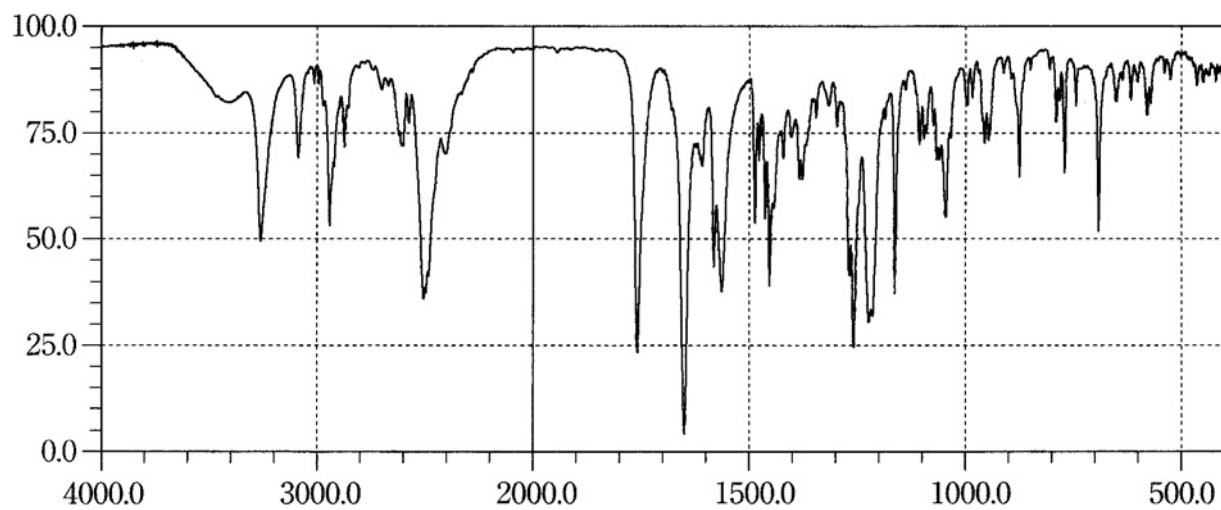
Preparation of sample: Potassium bromide disk method

Ritodrine Hydrochloride

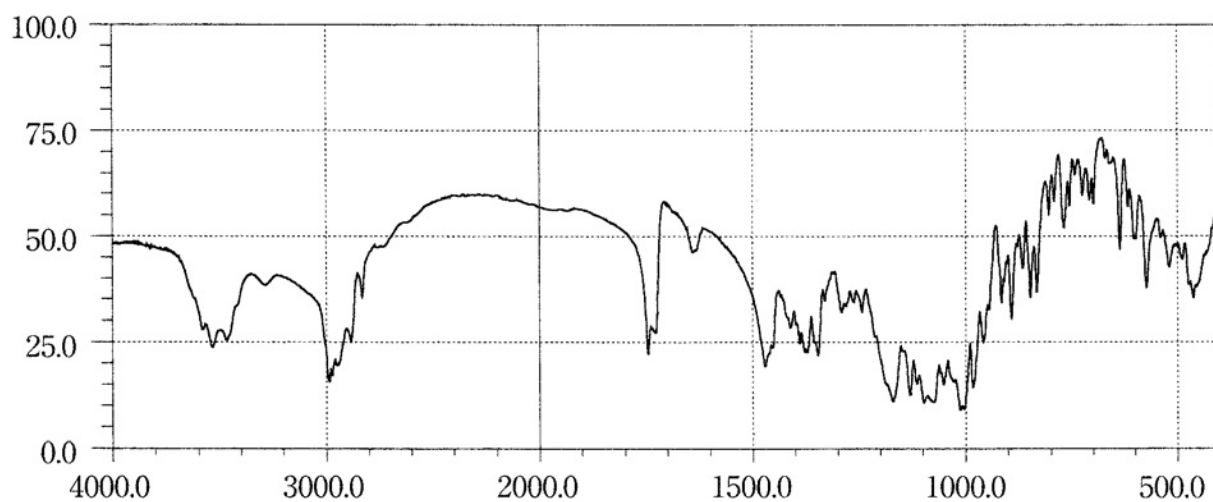
Preparation of sample: Potassium chloride disk method

Rokitamycin

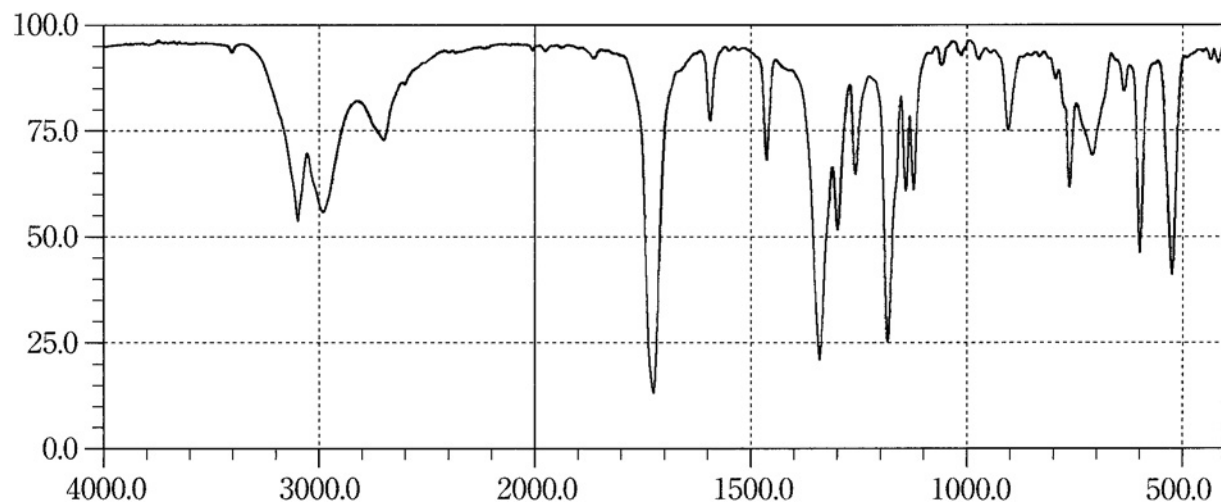
Preparation of sample: Potassium bromide disk method

Roxatidine Acetate Hydrochloride

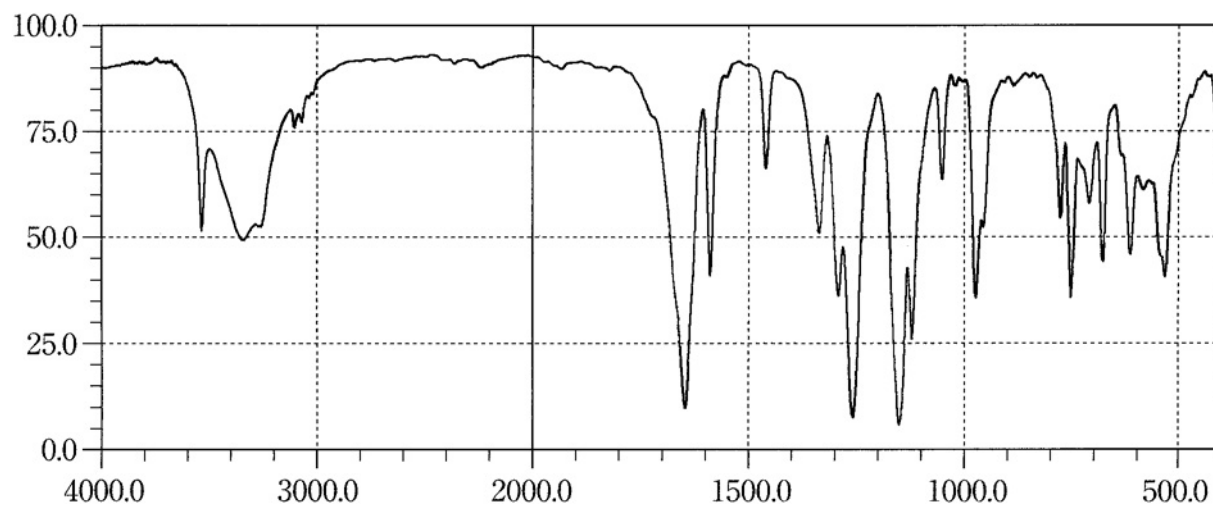
Preparation of sample: Potassium chloride disk method

Roxithromycin

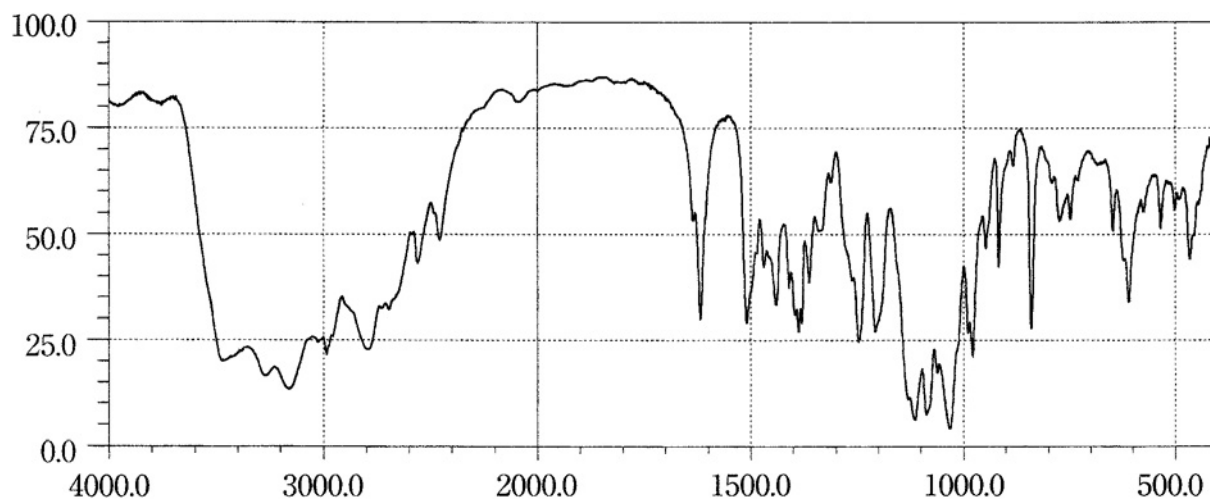
Preparation of sample: Potassium bromide disk method

Saccharin

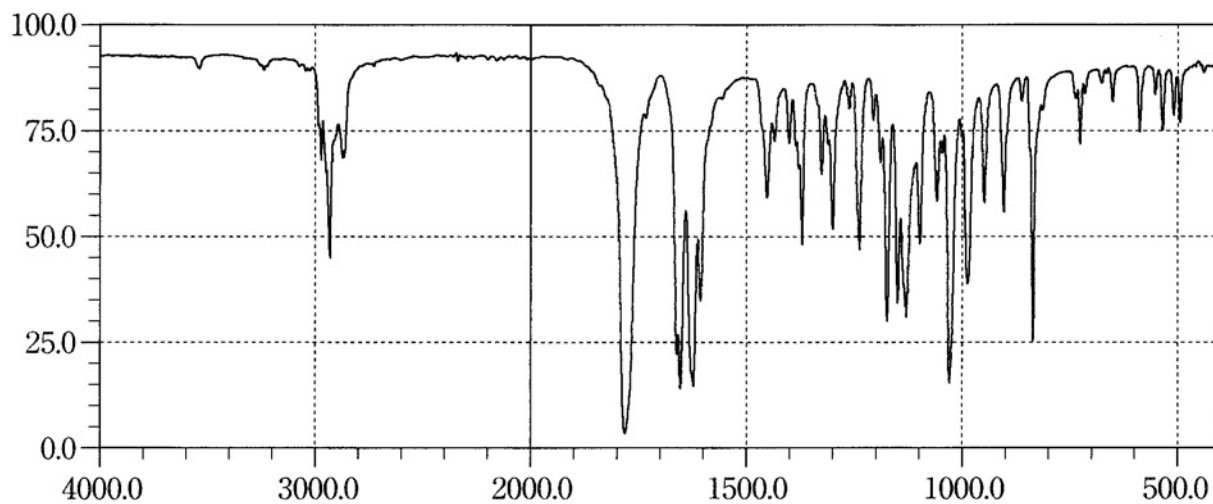
Preparation of sample: Potassium bromide disk method

Saccharin Sodium Hydrate

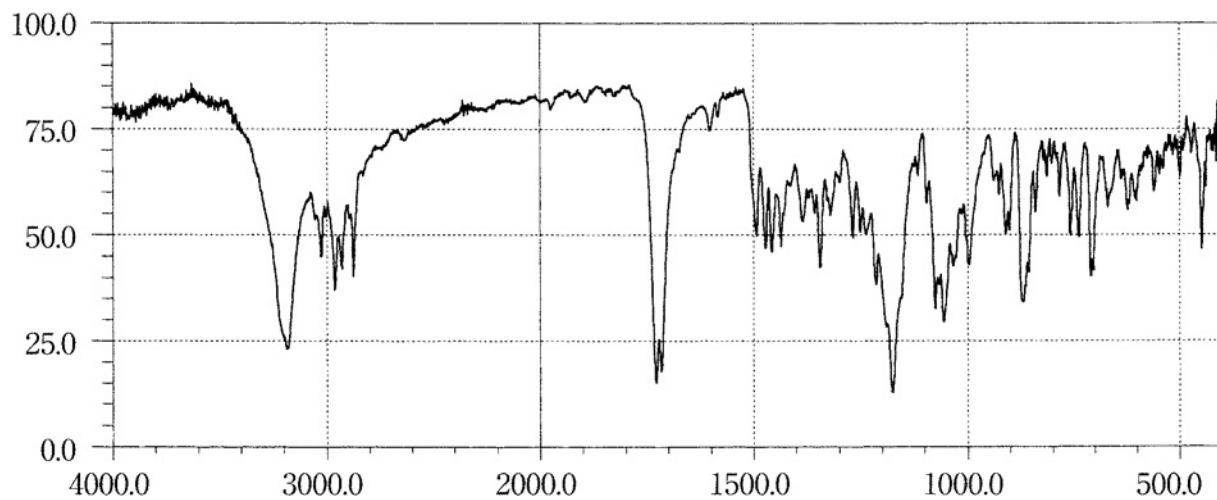
Preparation of sample: Potassium bromide disk method

Salbutamol Sulfate

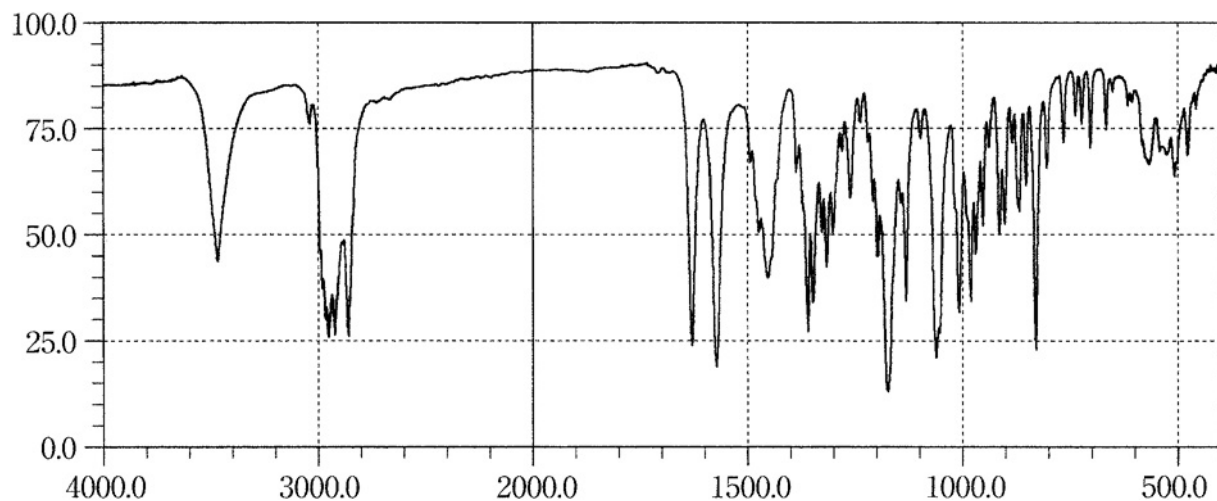
Preparation of sample: Potassium bromide disk method

Santonin

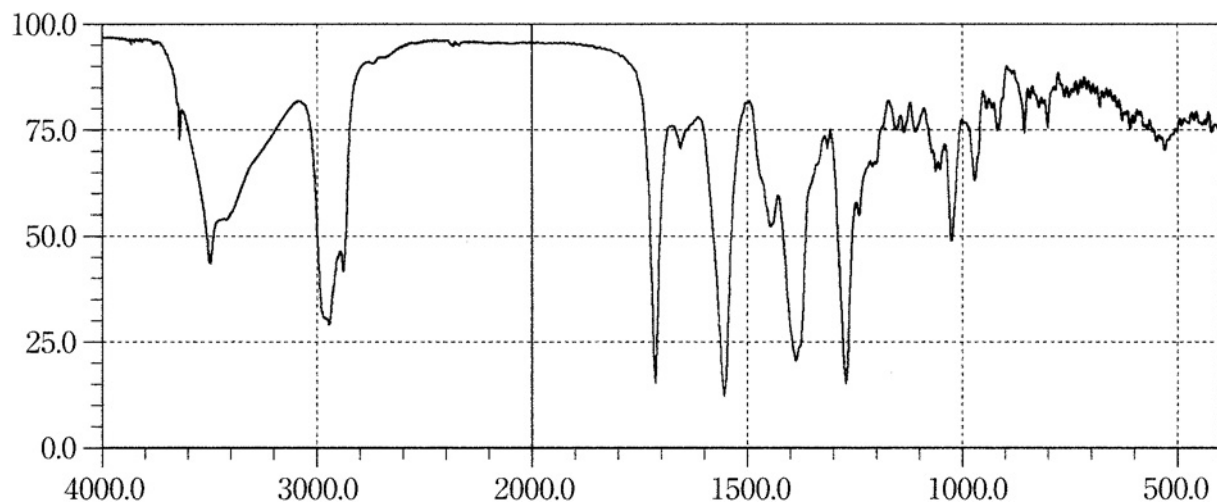
Preparation of sample: Potassium bromide disk method

Scopolamine Butylbromide

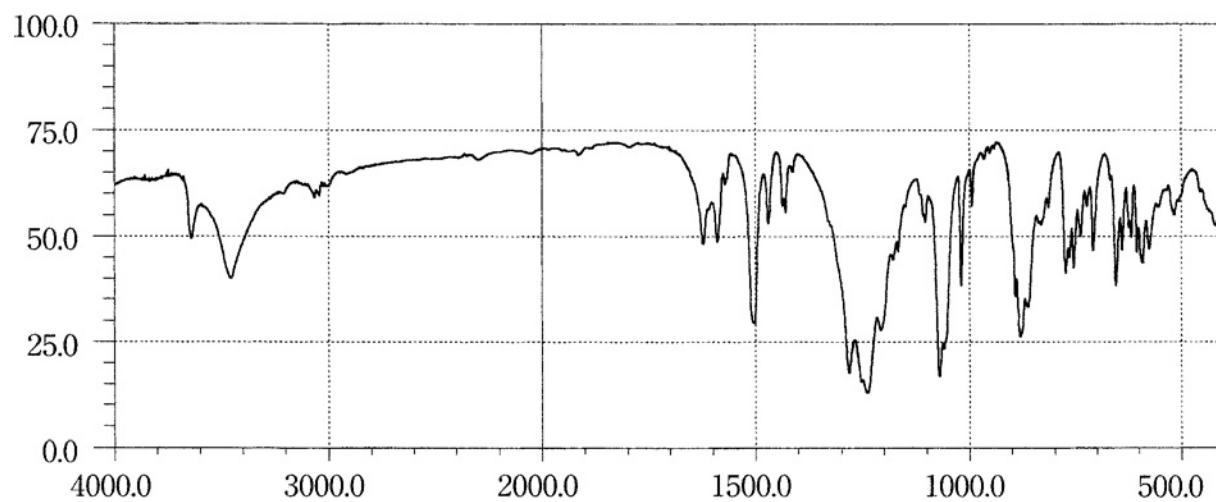
Preparation of sample: Potassium bromide disk method

Siccanin

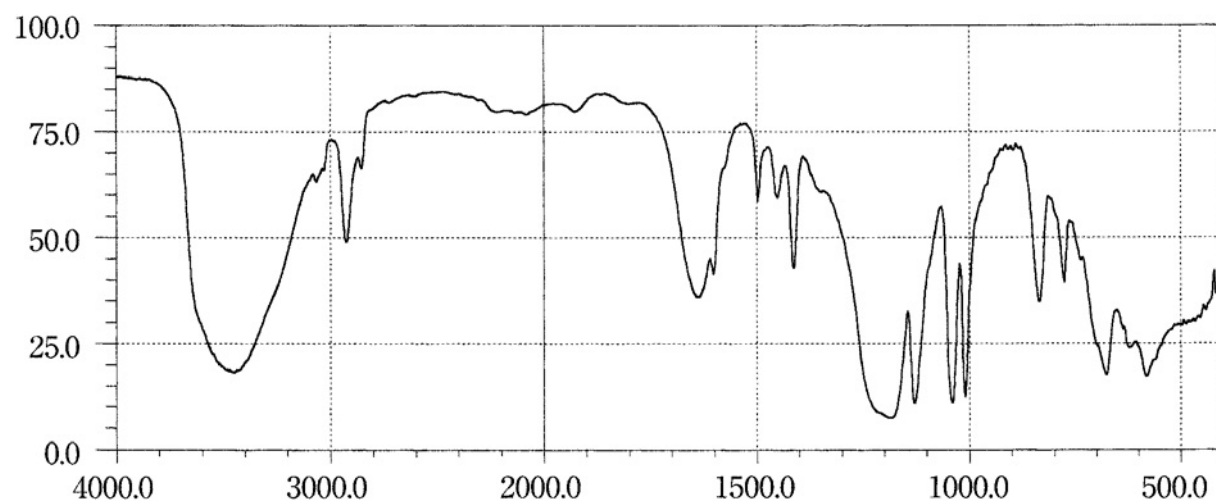
Preparation of sample: Potassium bromide disk method

Sodium Fusidate

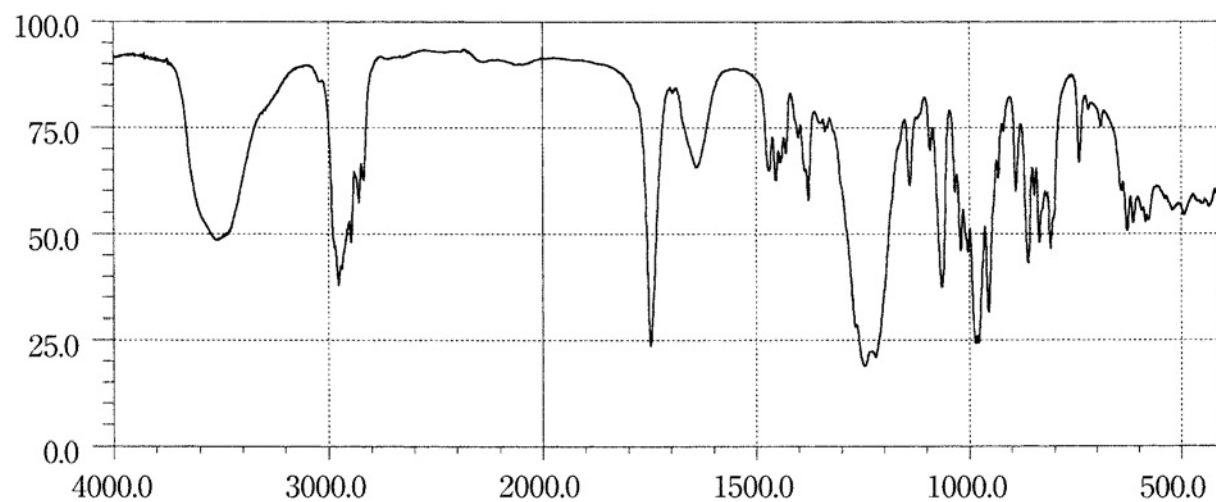
Preparation of sample: Potassium bromide disk method

Sodium Picosulfate Hydrate

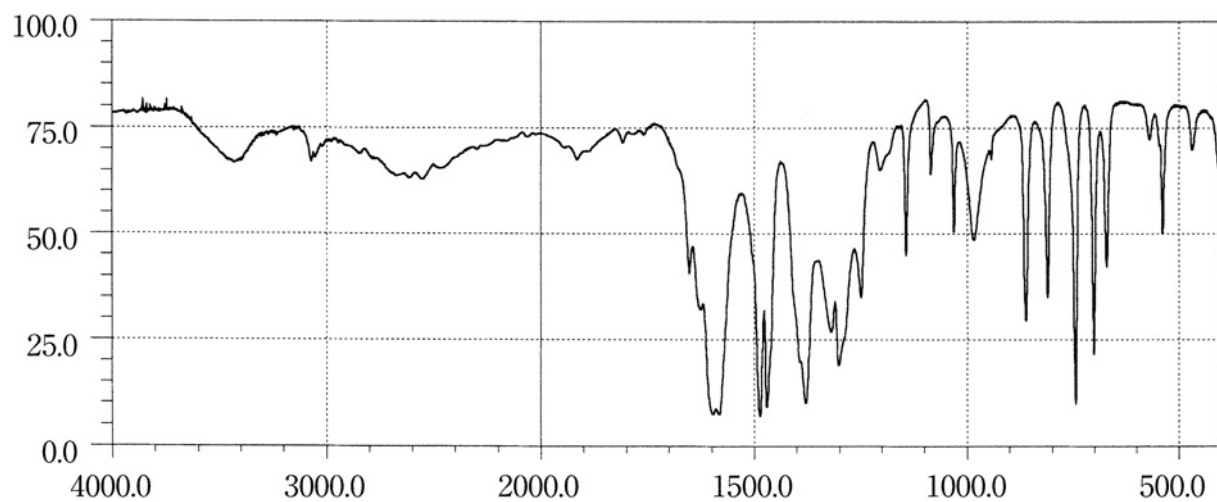
Preparation of sample: Potassium bromide disk method

Sodium Polystyrene Sulfonate

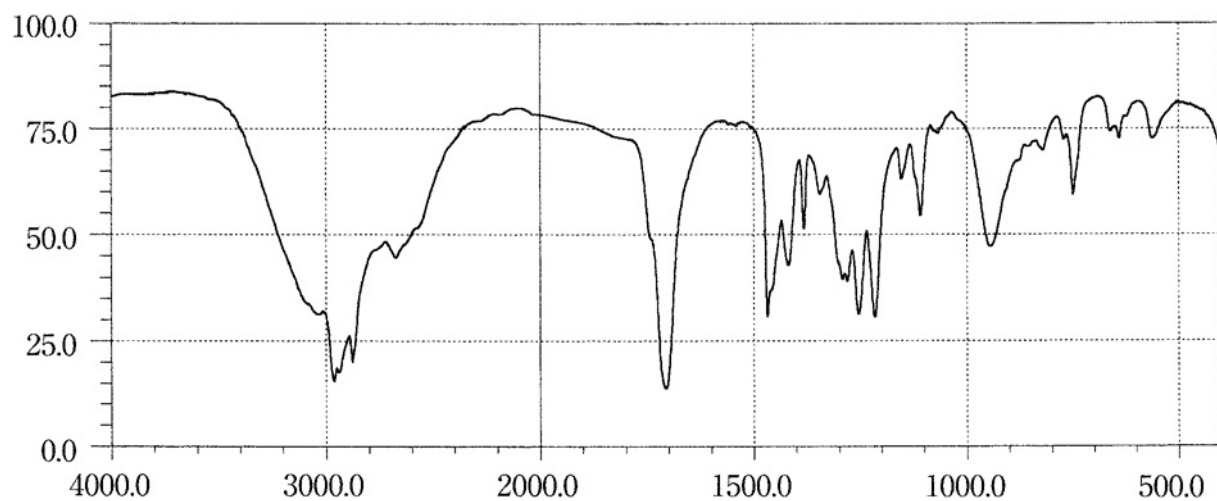
Preparation of sample: Potassium bromide disk method

Sodium Prasterone Sulfate Hydrate

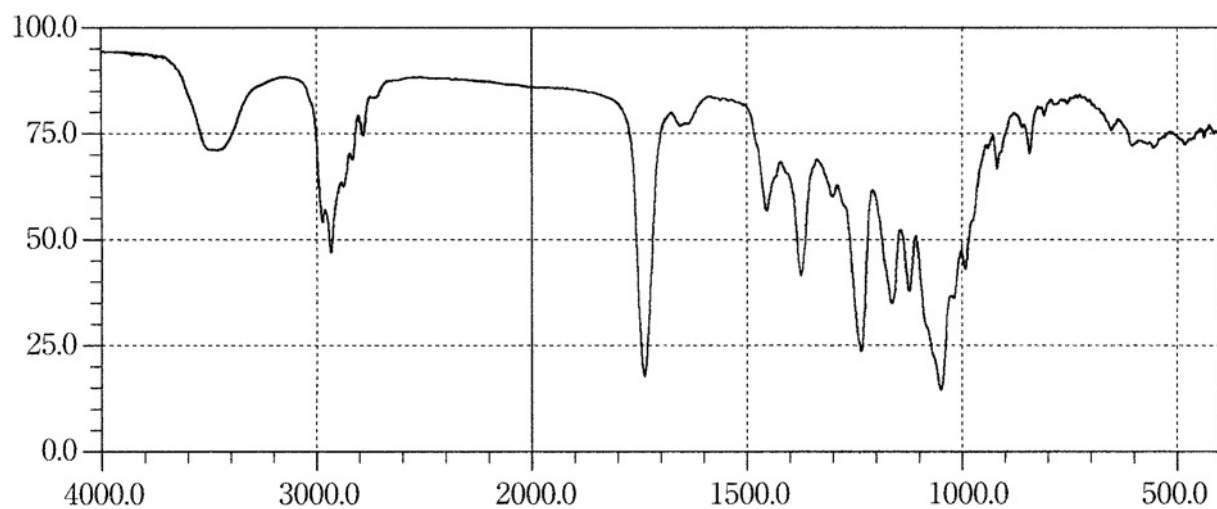
Preparation of sample: Potassium bromide disk method

Sodium Salicylate

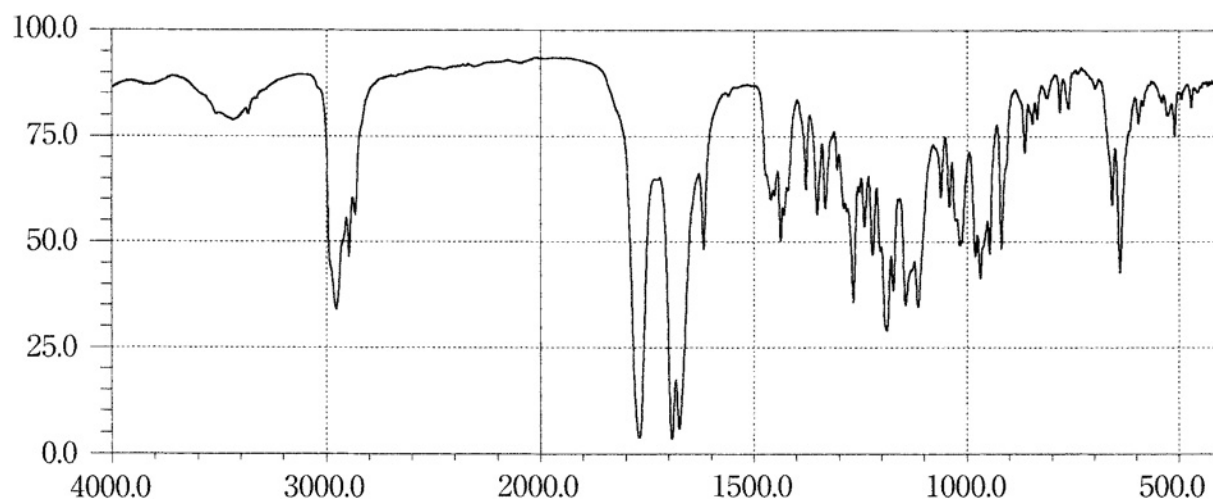
Preparation of sample: Potassium bromide disk method

Sodium Valproate

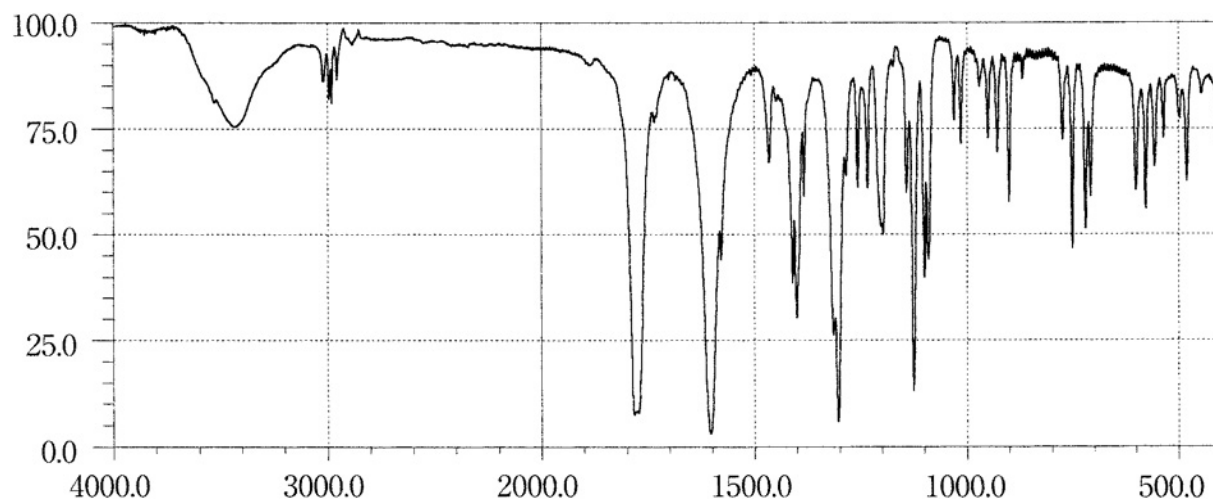
Preparation of sample: Liquid film method

Spiramycin Acetate

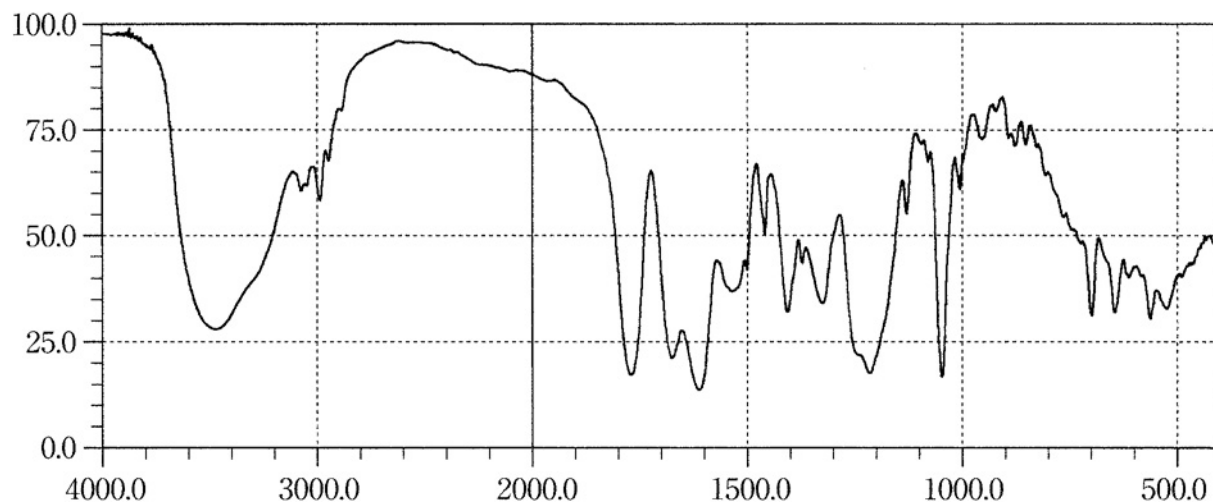
Preparation of sample: Potassium bromide disk method

Spironolactone

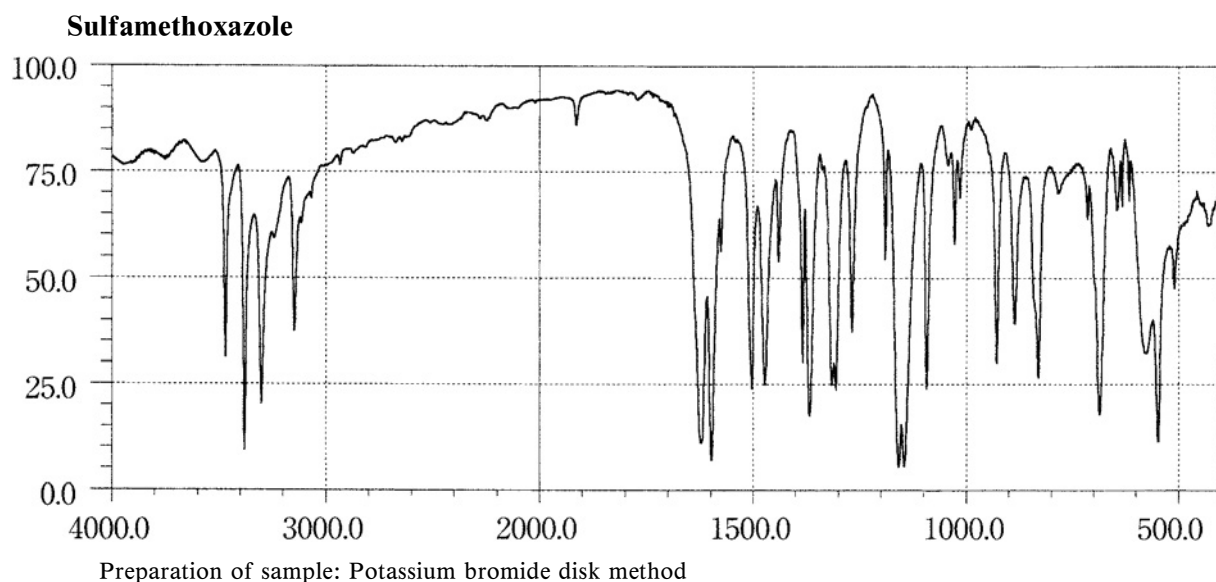
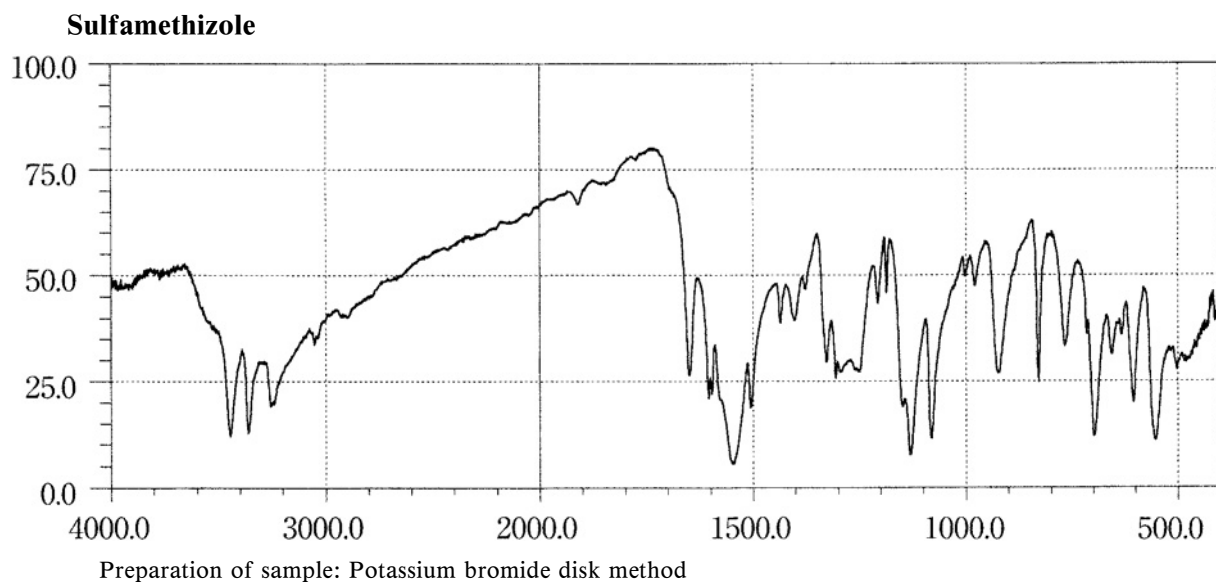
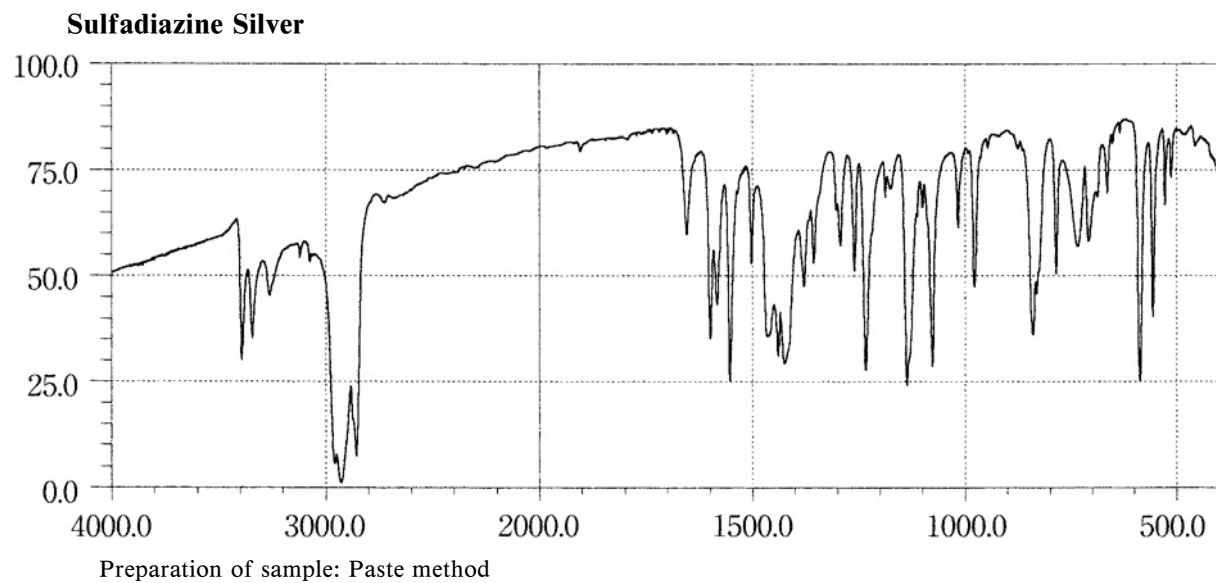
Preparation of sample: Potassium bromide disk method

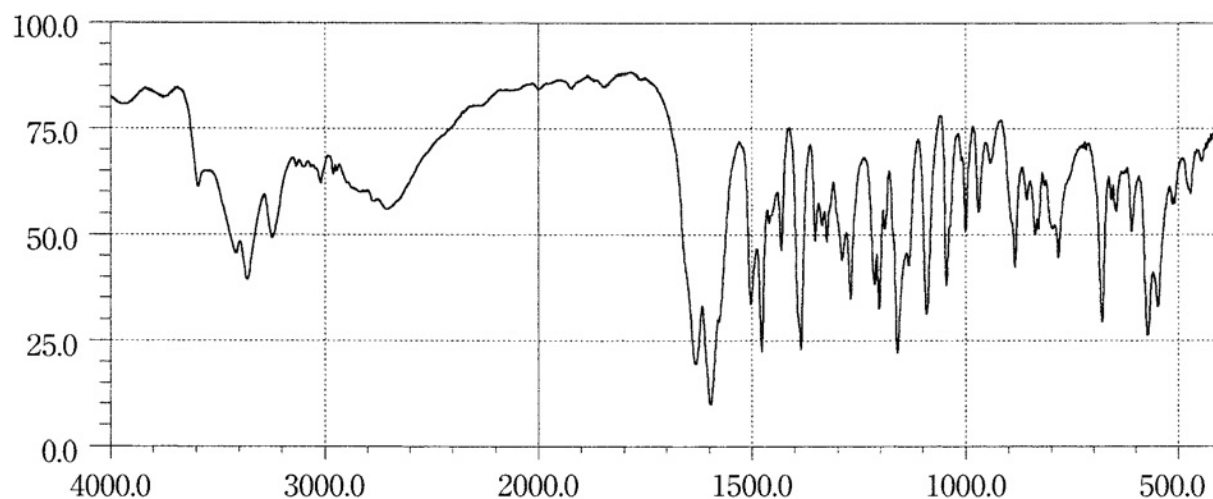
Sulbactam Sodium

Preparation of sample: Potassium bromide disk method

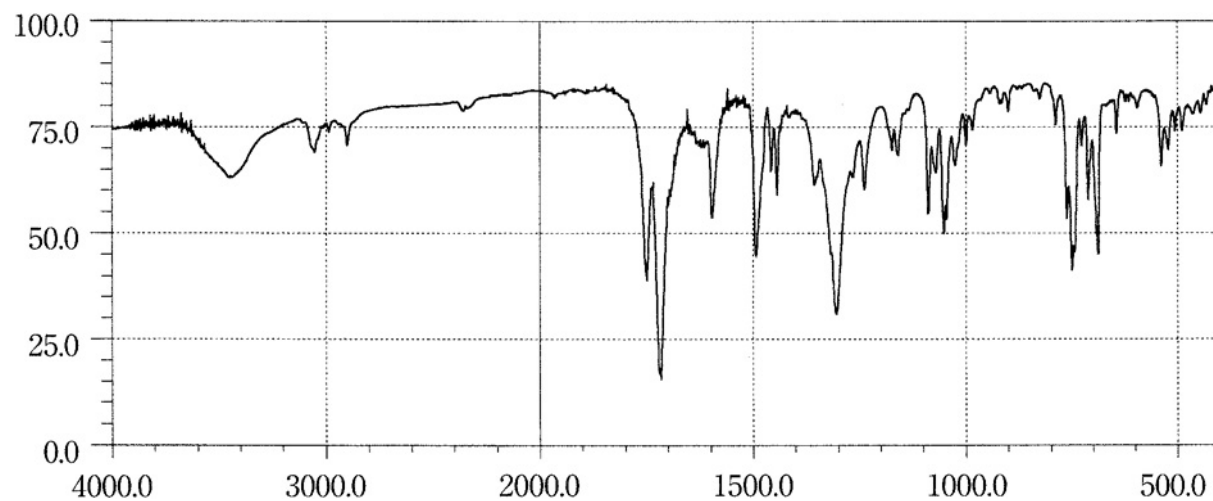
Sulbencillin Sodium

Preparation of sample: Potassium bromide disk method

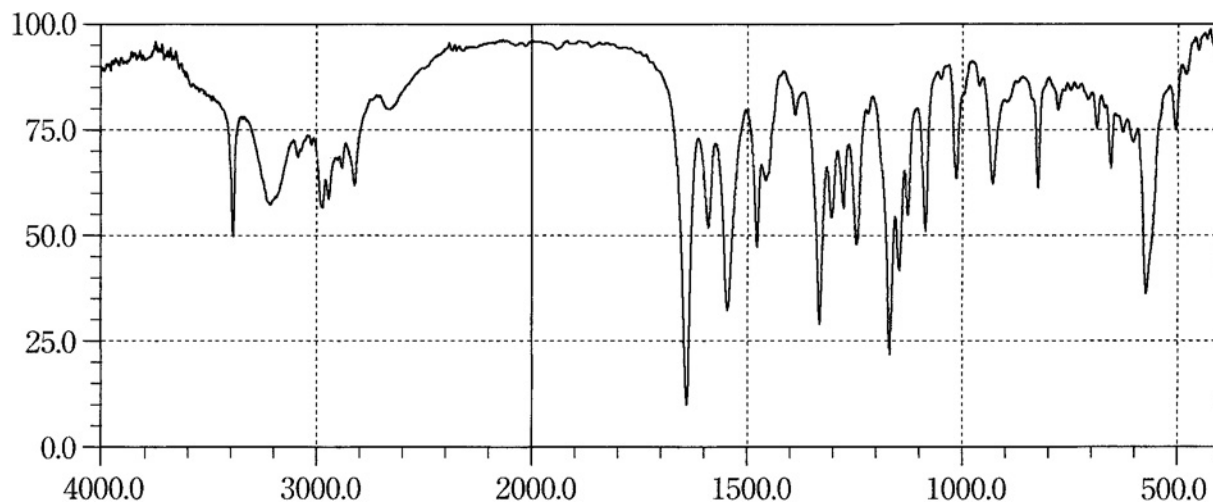


Sulfamonomethoxine Hydrate

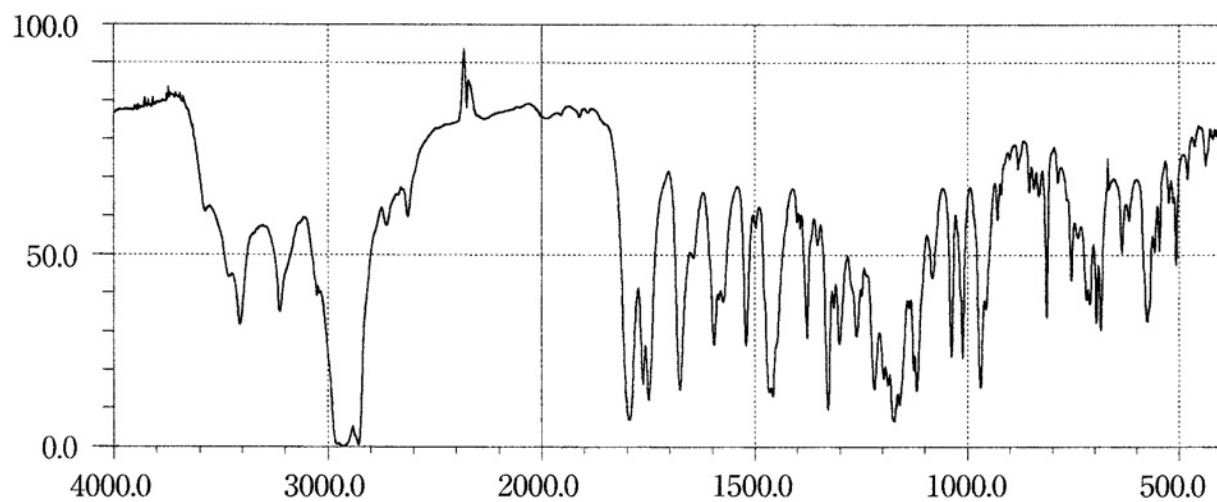
Preparation of sample: Potassium bromide disk method

Sulfinpyrazone

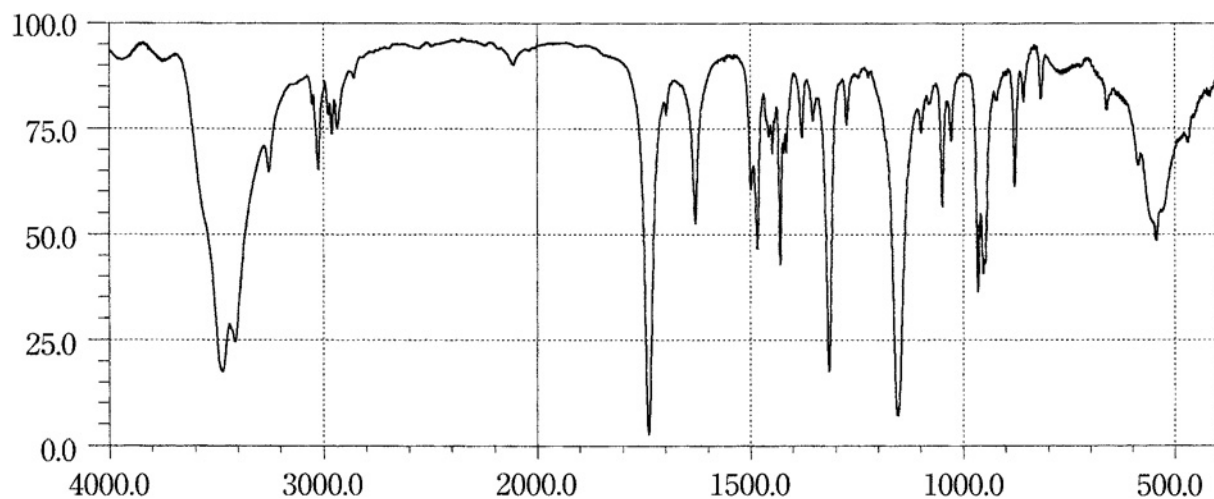
Preparation of sample: Potassium bromide disk method

Sulpiride

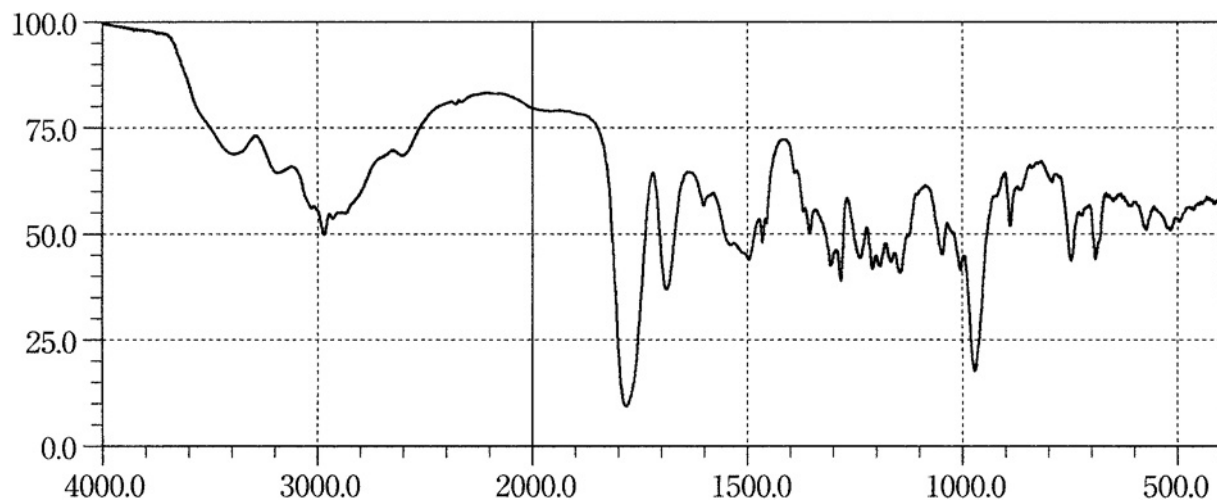
Preparation of sample: Potassium bromide disk method

Sultamicillin Tosilate Hydrate

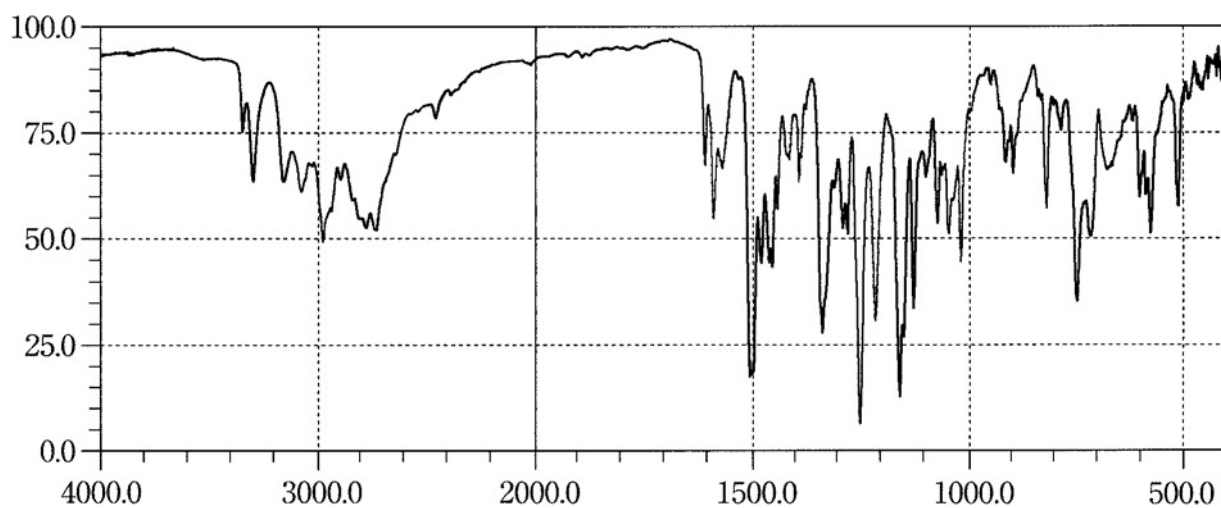
Preparation of sample: Paste method

Suxamethonium Chloride Hydrate

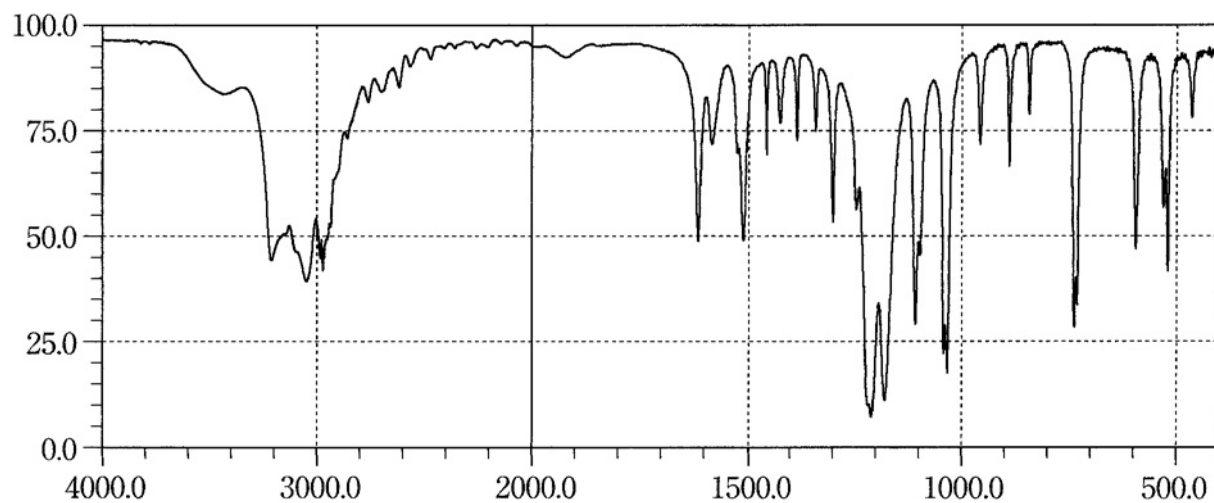
Preparation of sample: Potassium bromide disk method

Talampicillin Hydrochloride

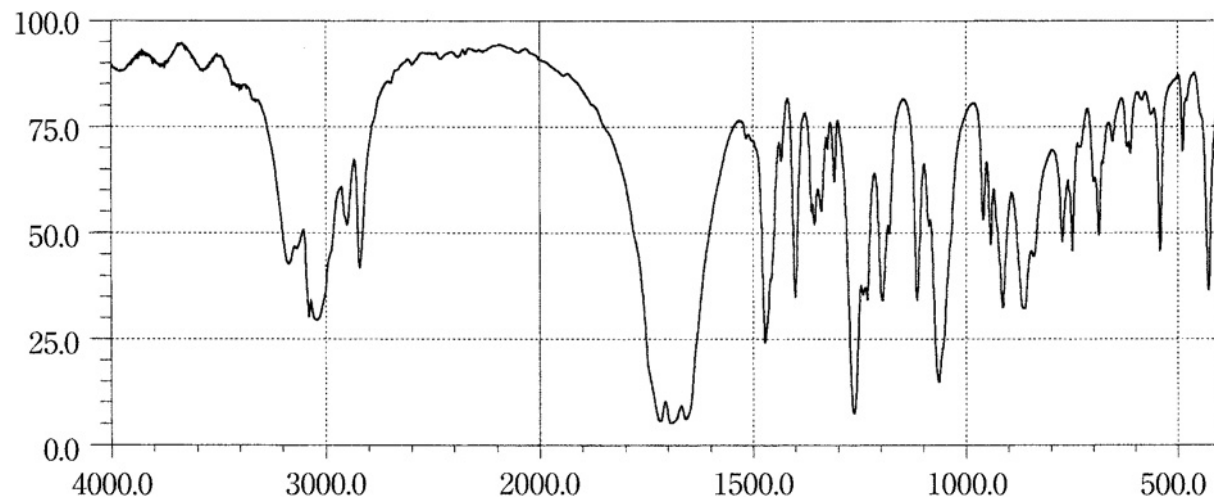
Preparation of sample: Potassium bromide disk method

Tamsulosin Hydrochloride

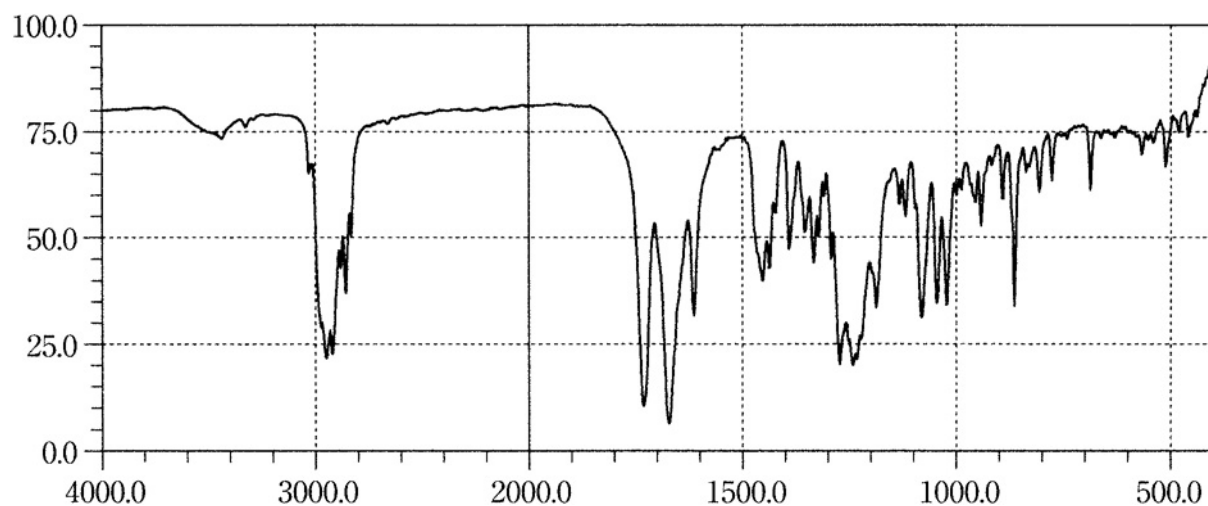
Preparation of sample: Potassium chloride disk method

Taurine

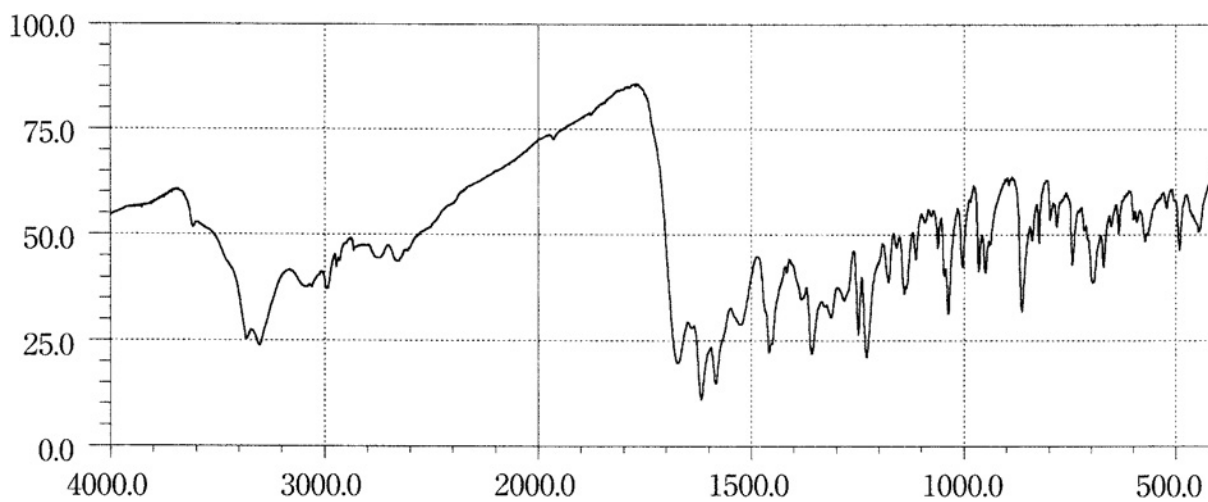
Preparation of sample: Potassium bromide disk method

Tegafur

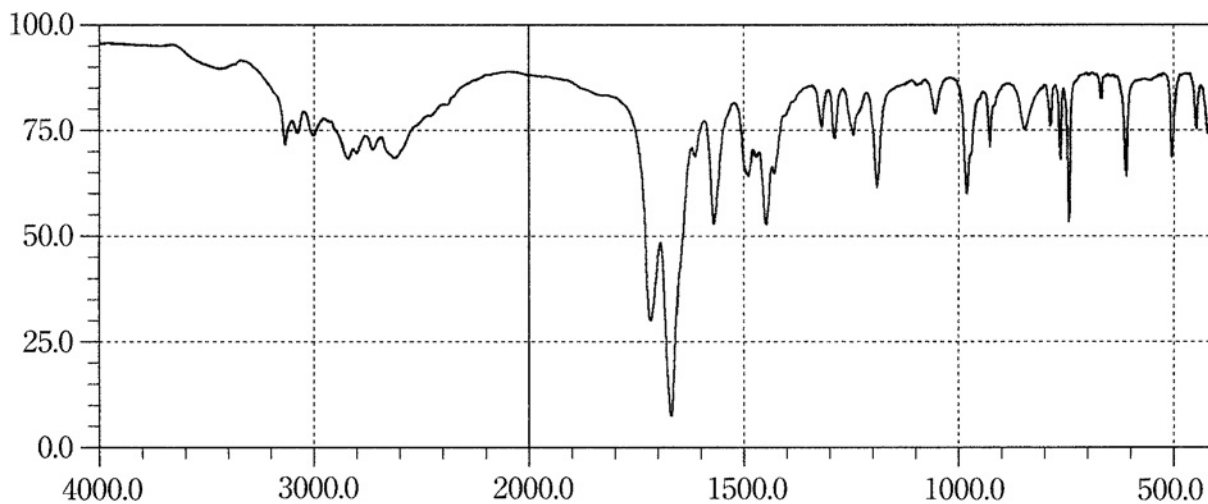
Preparation of sample: Potassium bromide disk method

Testosterone Propionate

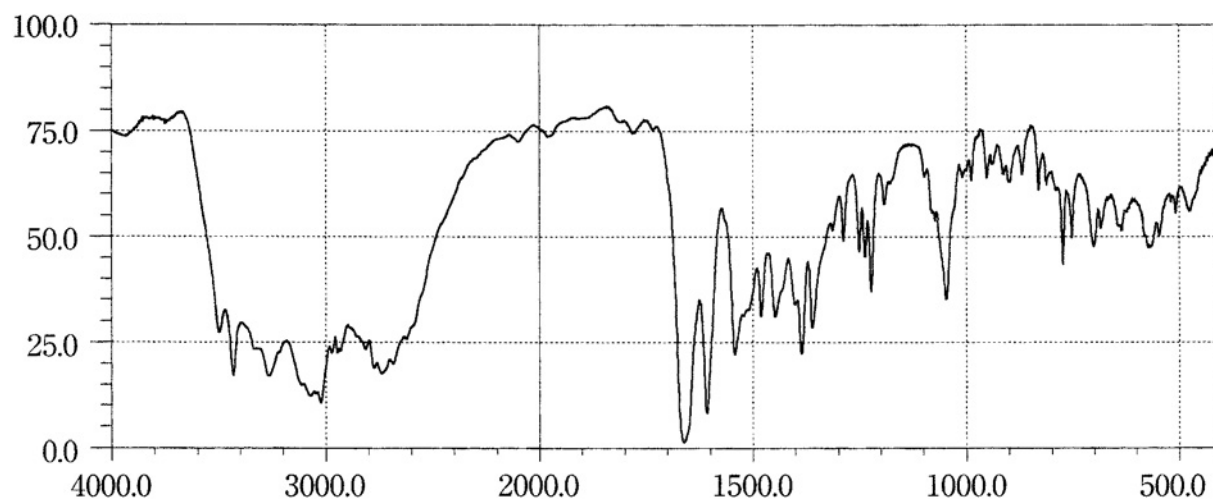
Preparation of sample: Potassium bromide disk method

Tetracycline Hydrochloride

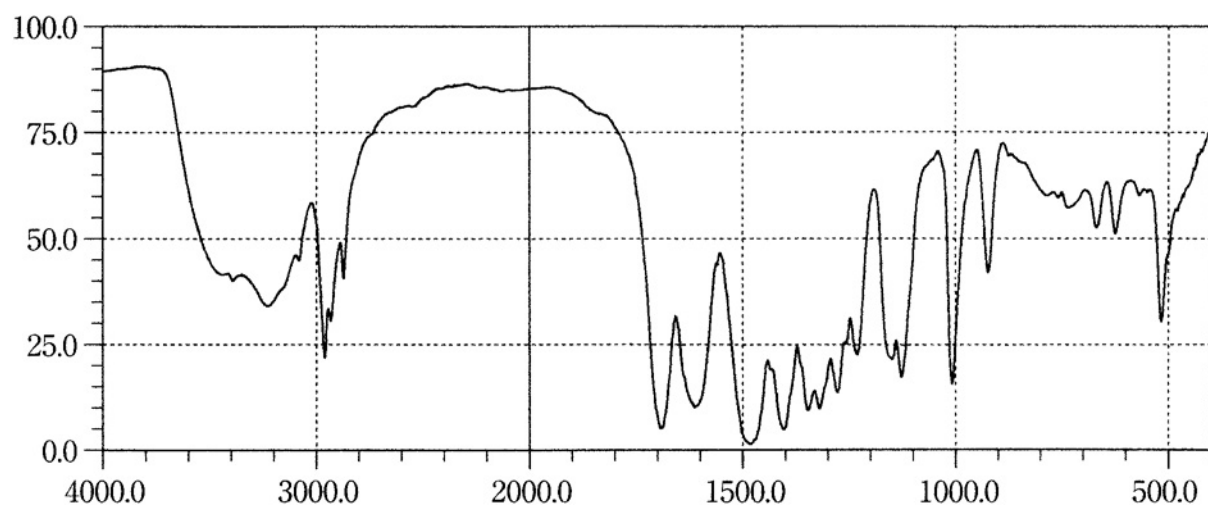
Preparation of sample: Potassium chloride disk method

Theophylline

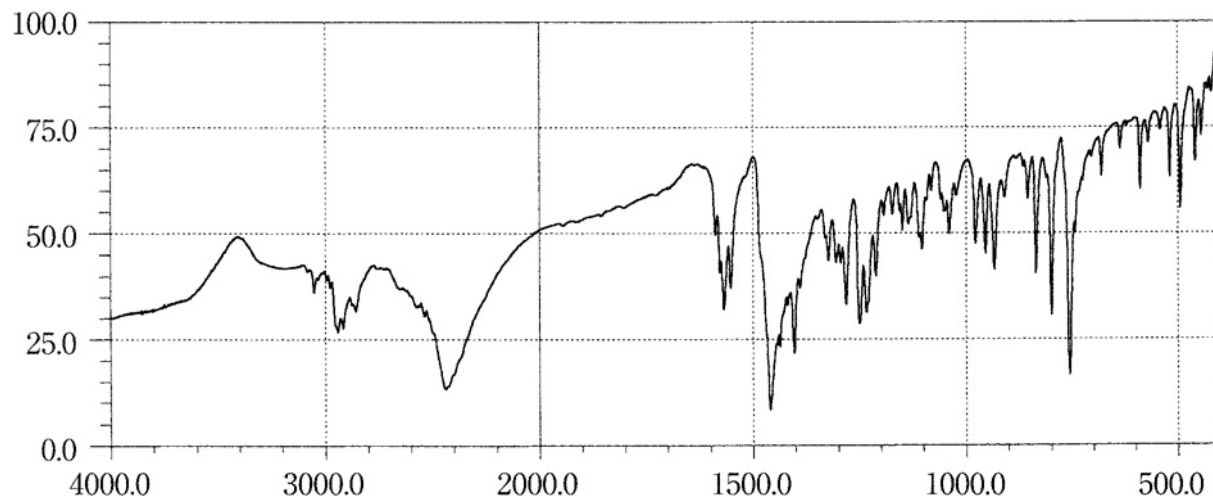
Preparation of sample: Potassium bromide disk method

Thiamine Chloride Hydrochloride

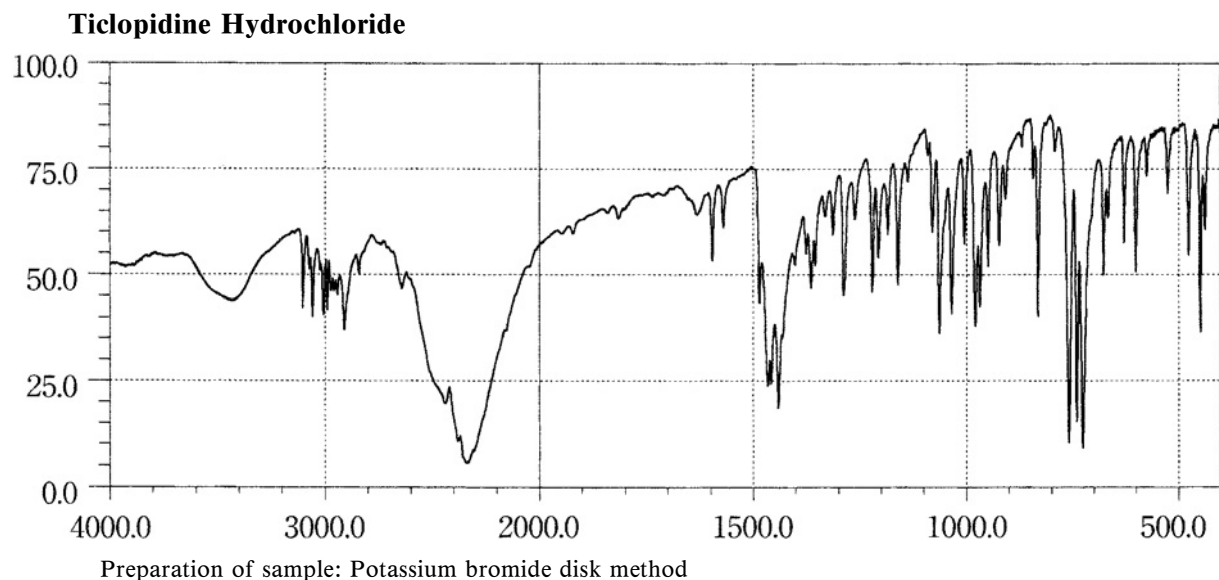
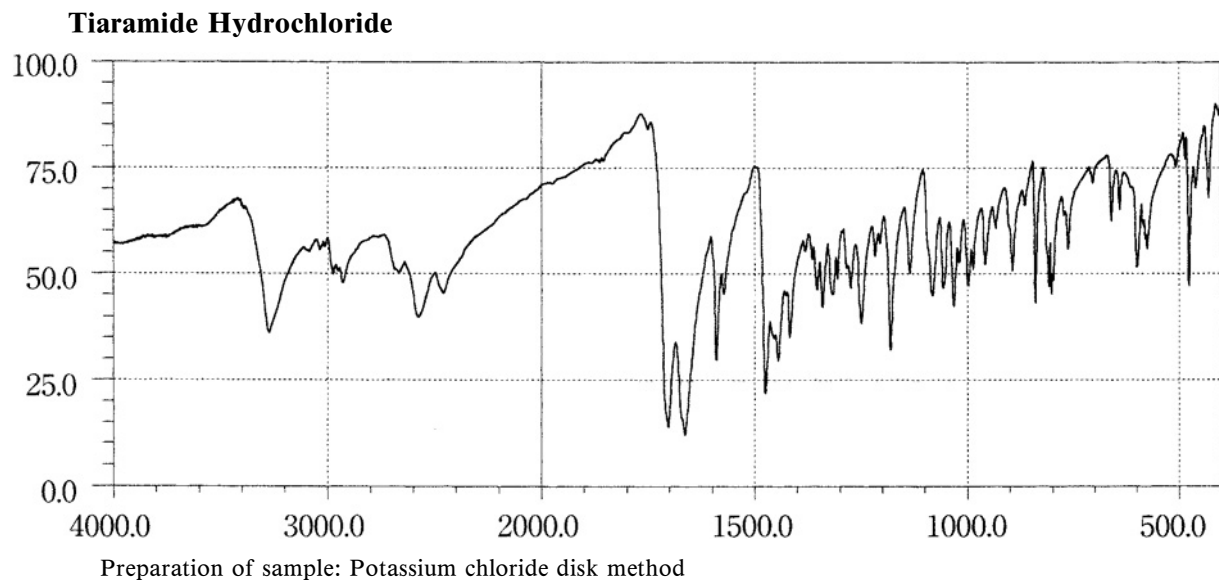
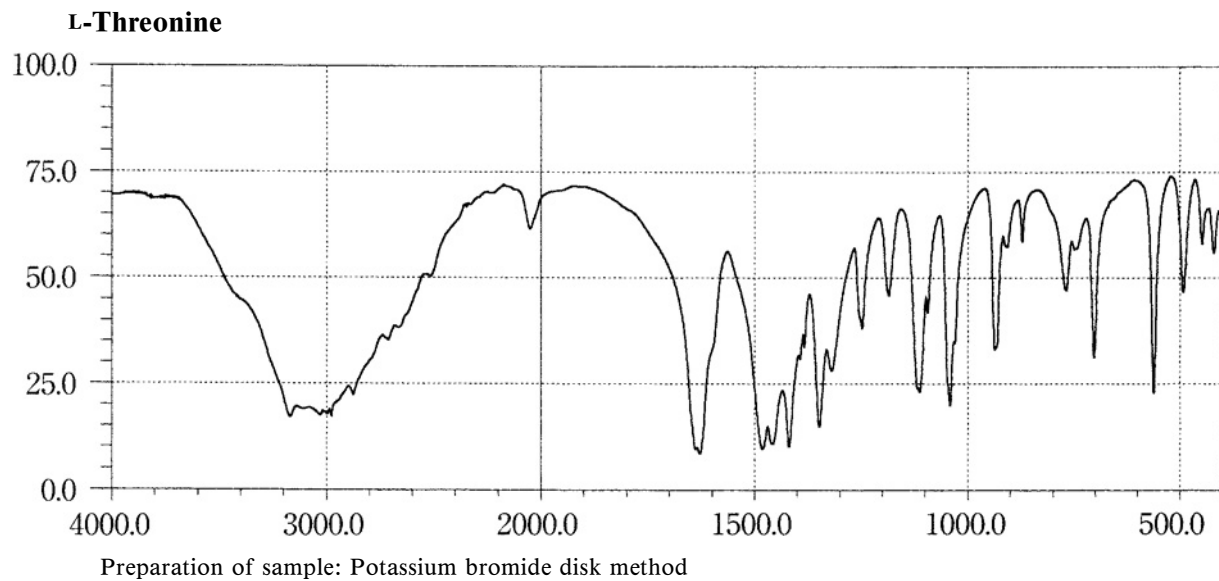
Preparation of sample: Potassium bromide disk method

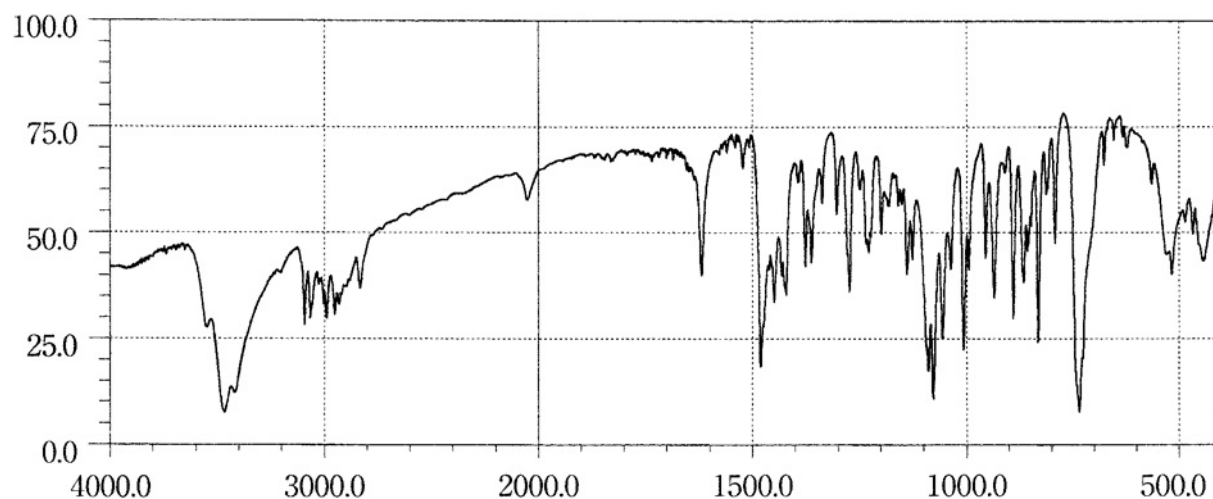
Thiamylal Sodium

Preparation of sample: Potassium bromide disk method

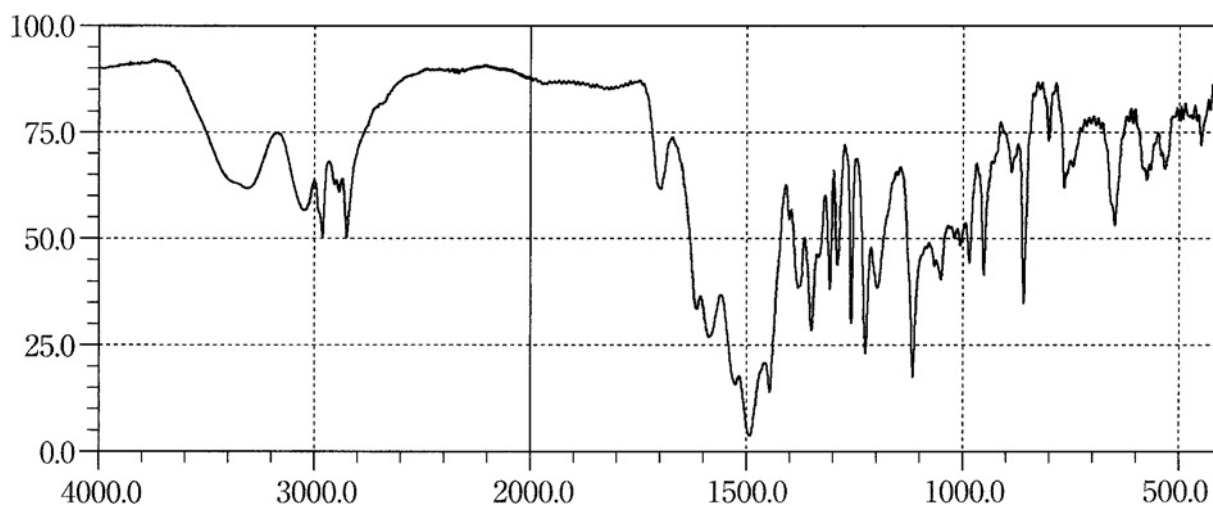
Thioridazine Hydrochloride

Preparation of sample: Potassium chloride disk method

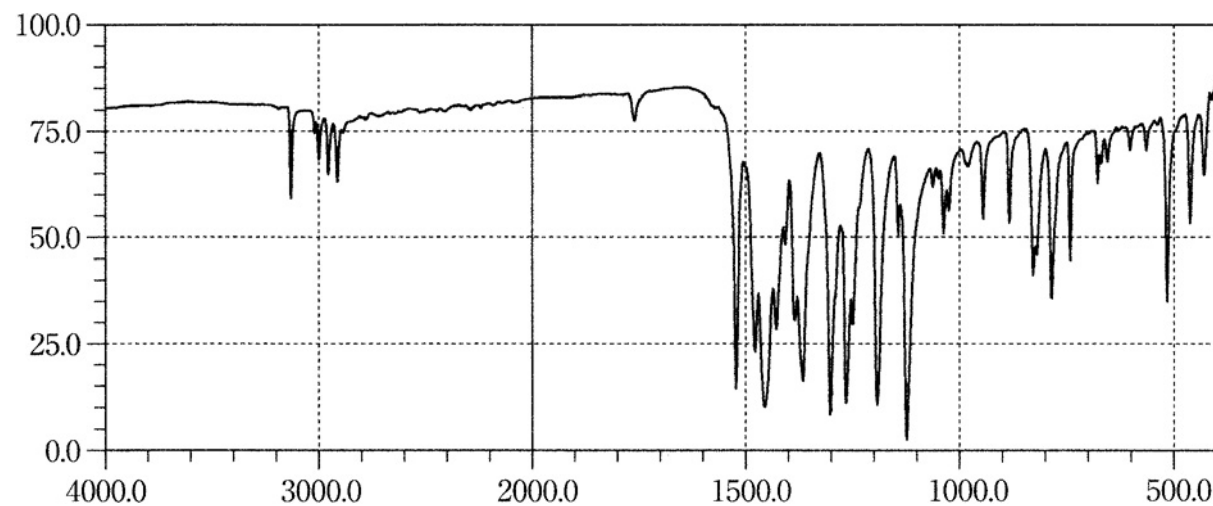


Timepidium Bromide Hydrate

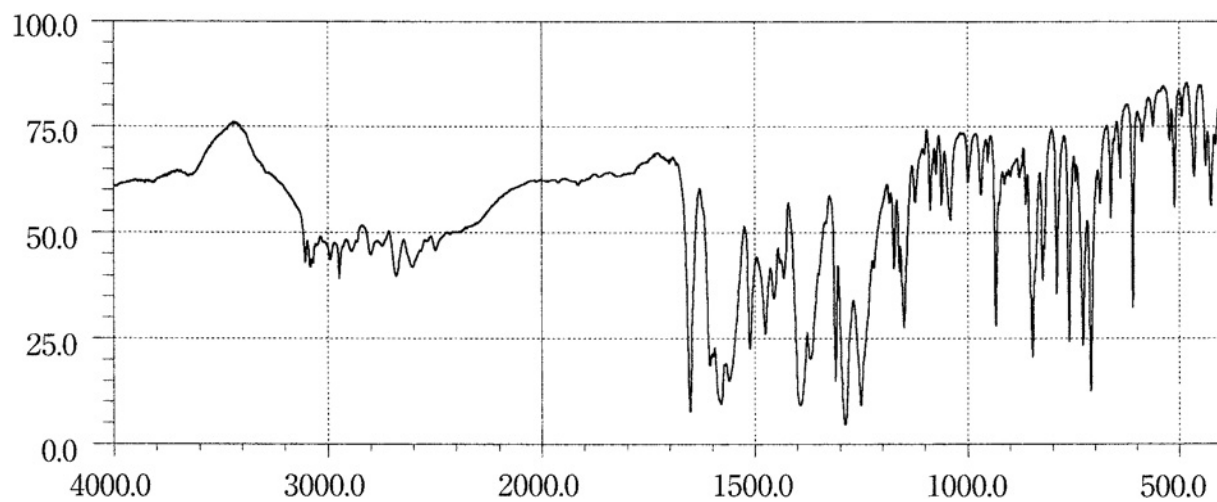
Preparation of sample: Potassium bromide disk method

Timolol Maleate

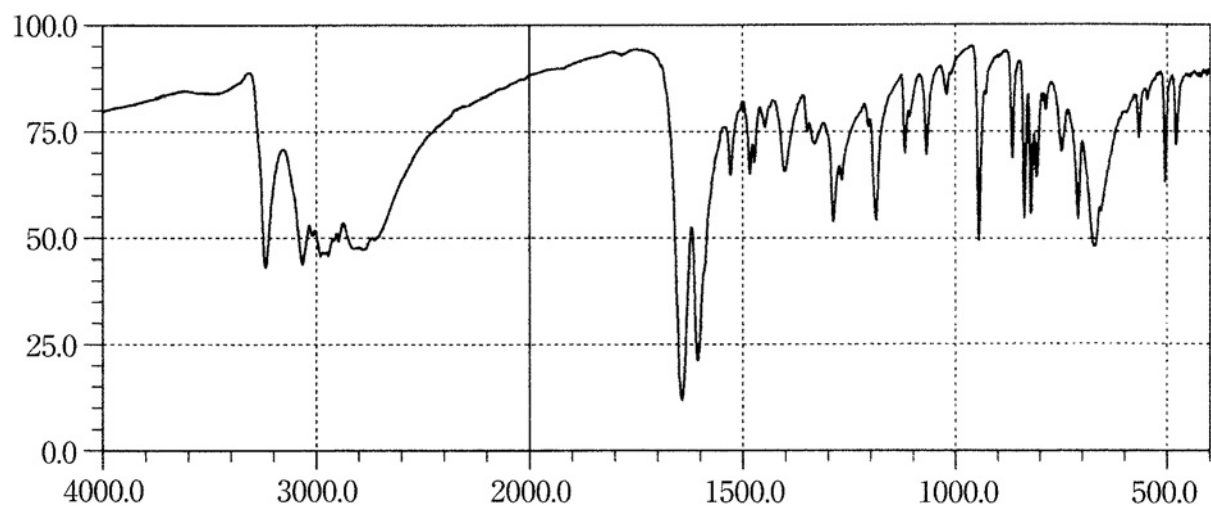
Preparation of sample: Potassium bromide disk method

Tinidazole

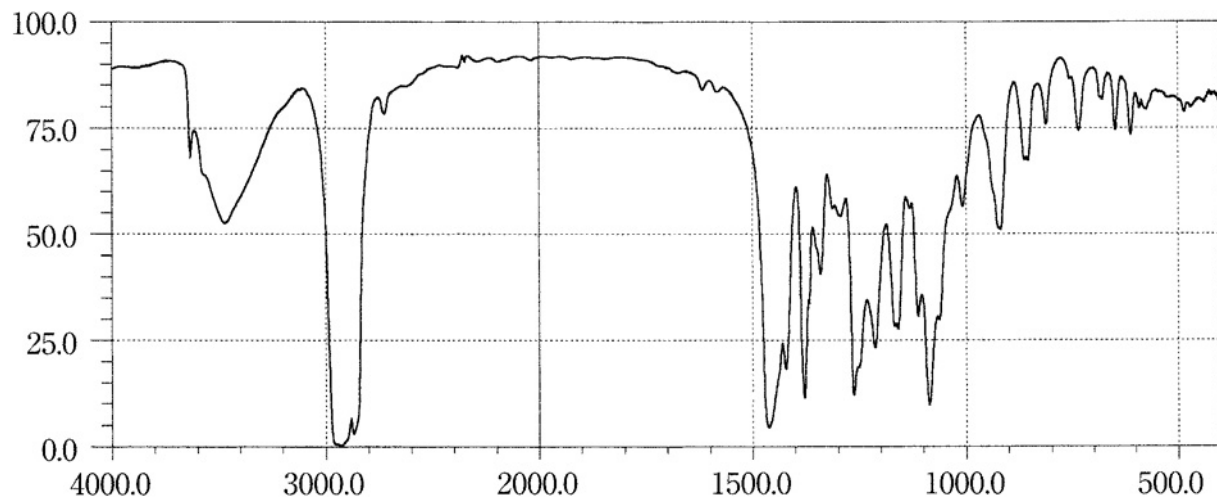
Preparation of sample: Potassium bromide disk method

Tipepidine Hibenzate

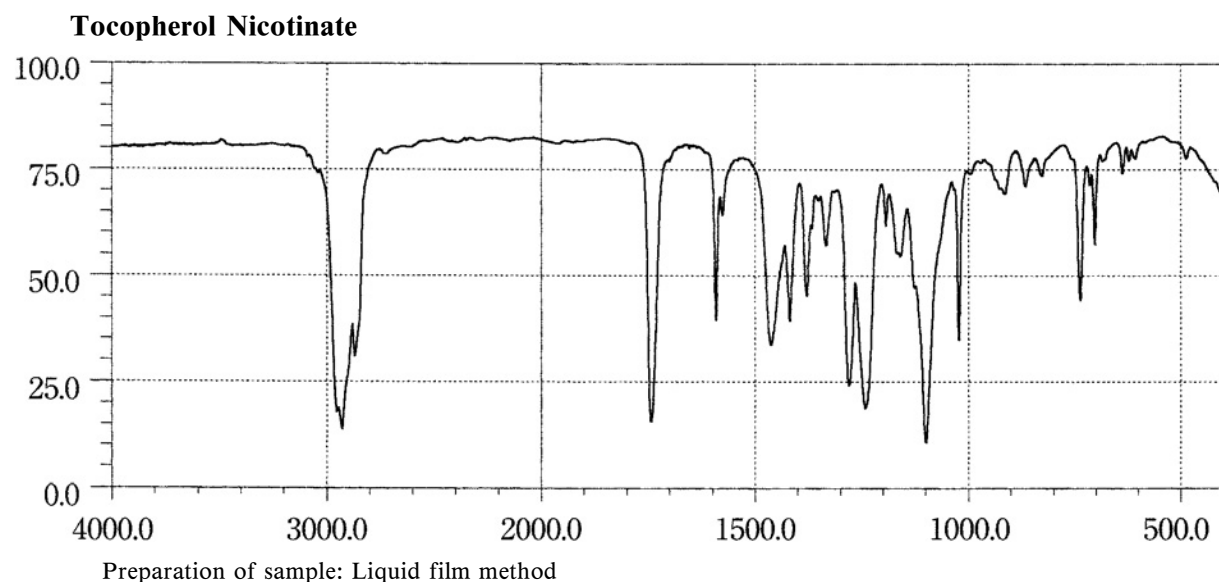
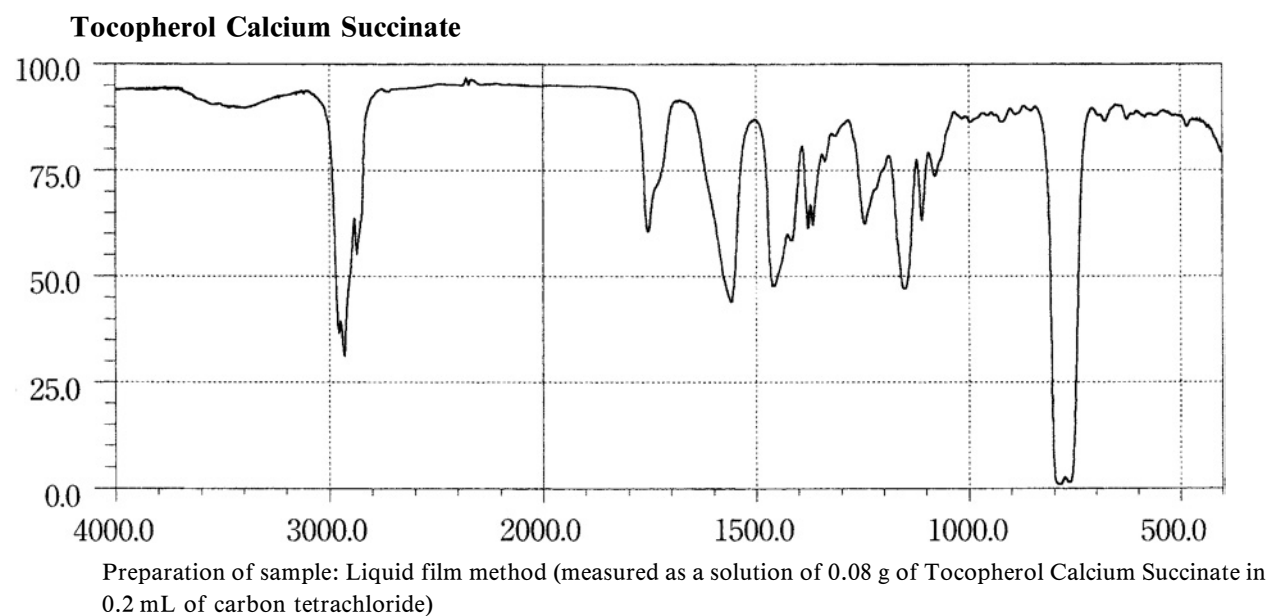
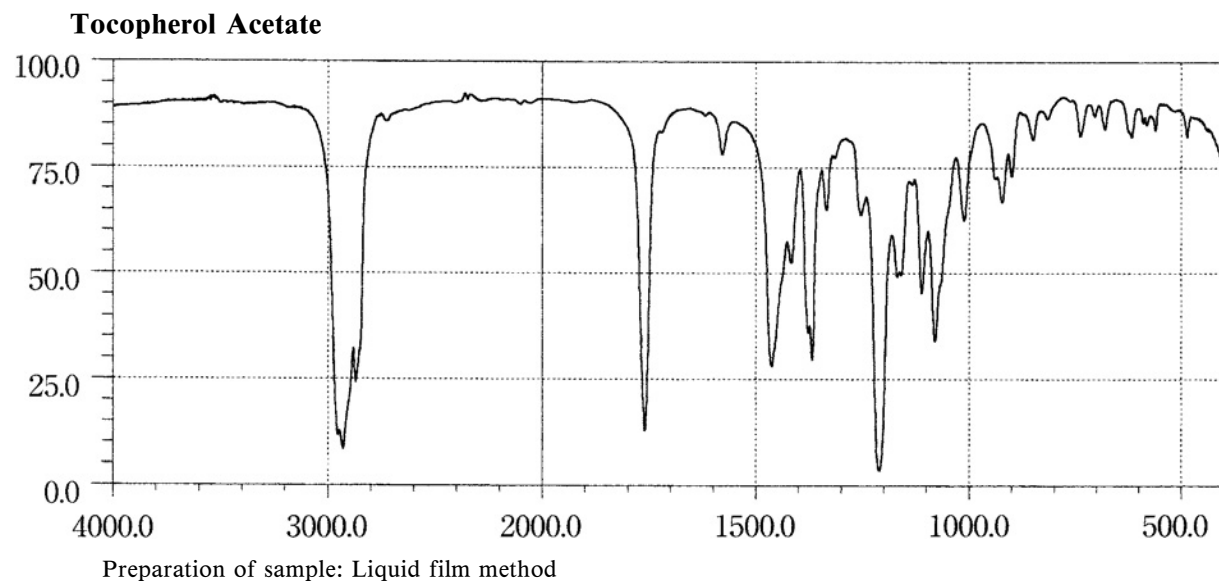
Preparation of sample: Potassium bromide disk method

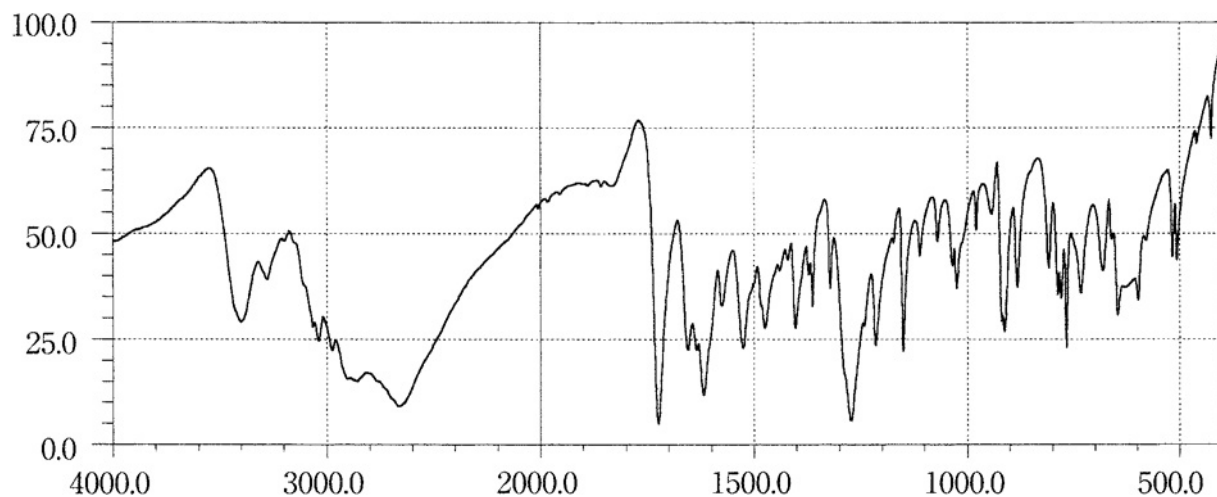
Tizanidine Hydrochloride

Preparation of sample: Potassium chloride disk method

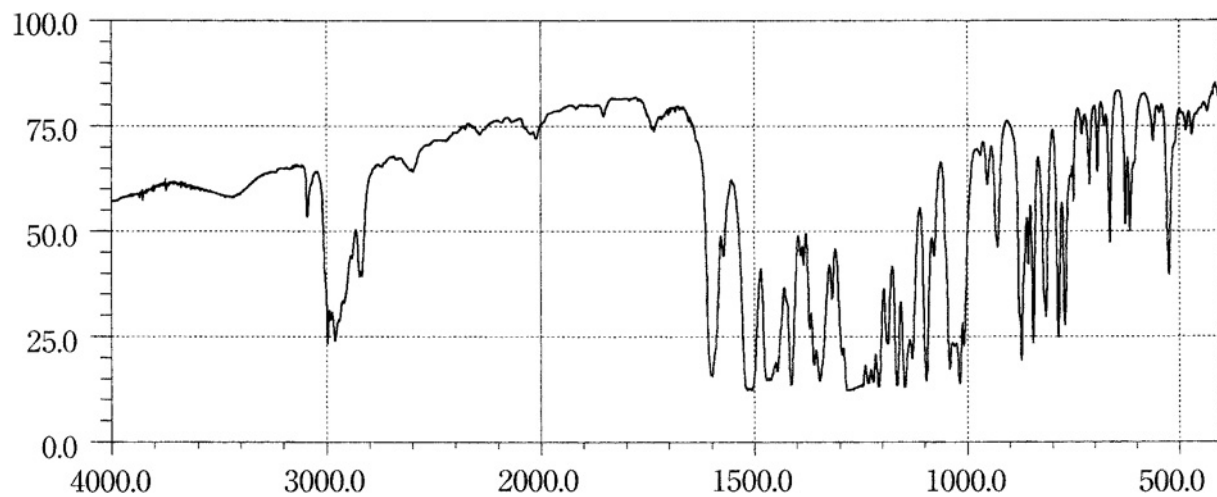
Tocopherol

Preparation of sample: Liquid film method

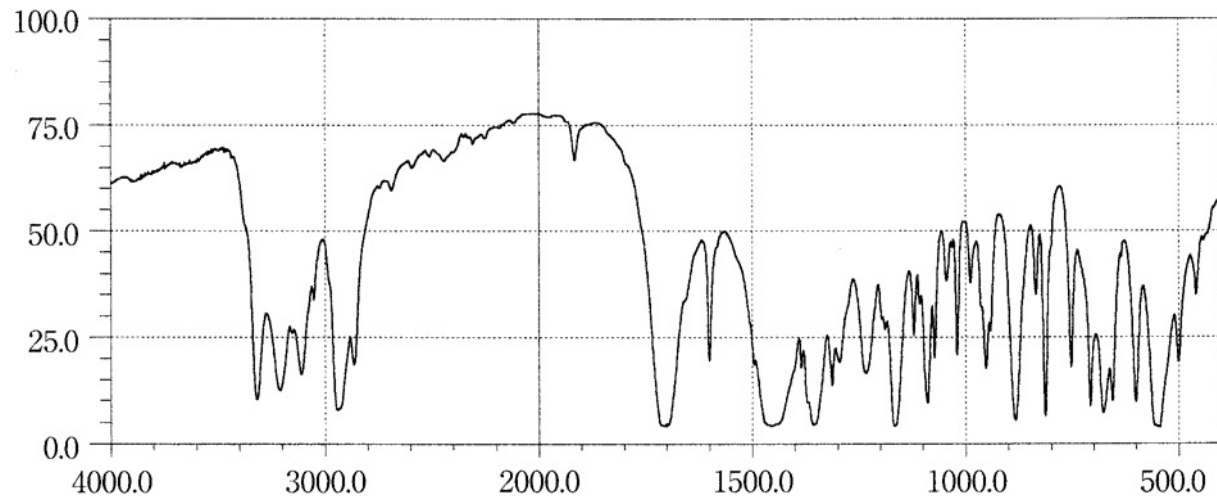


Todalazine Hydrochloride Hydrate

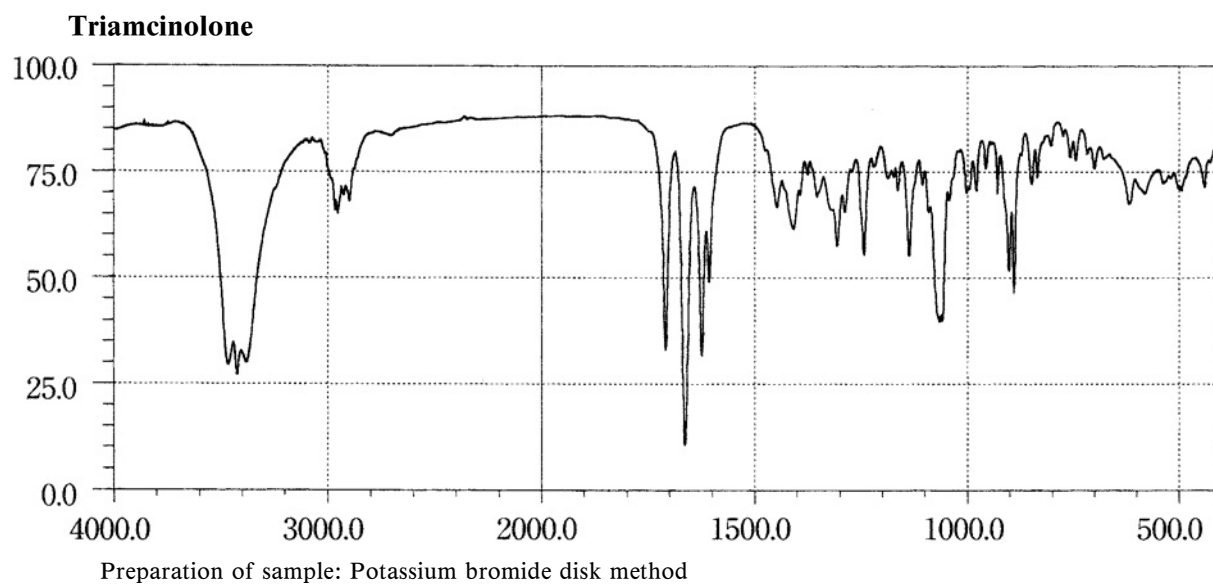
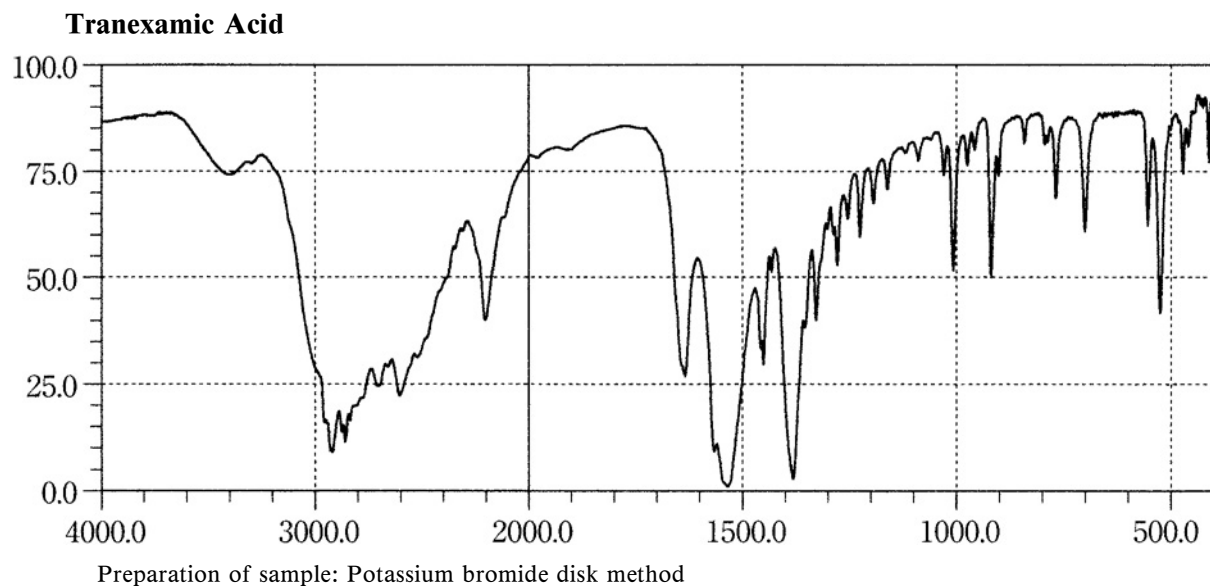
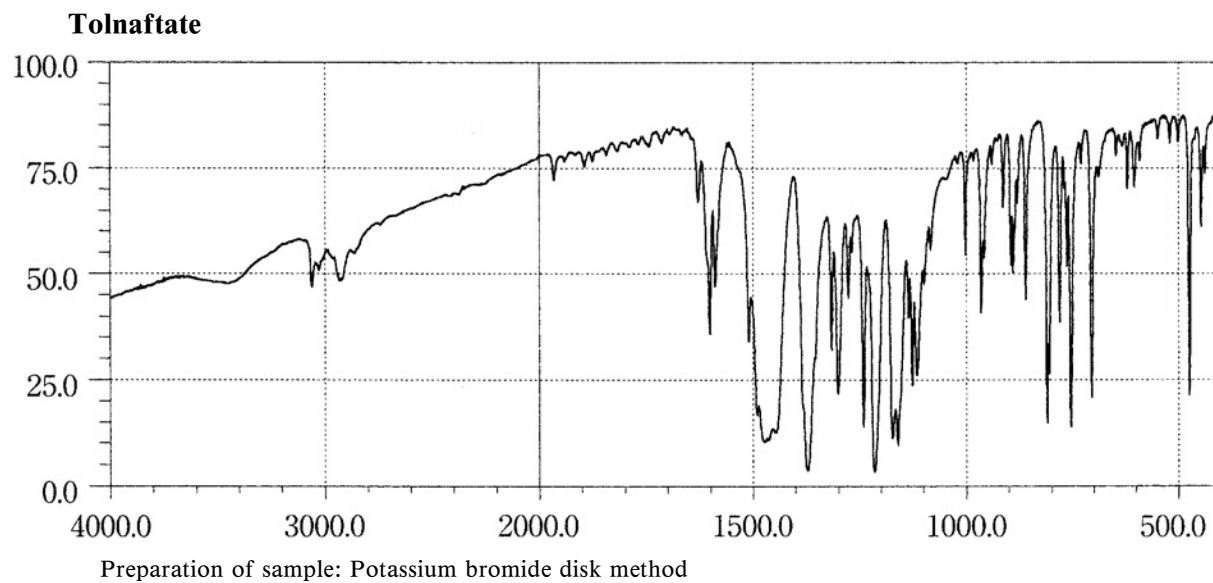
Preparation of sample: Potassium chloride disk method

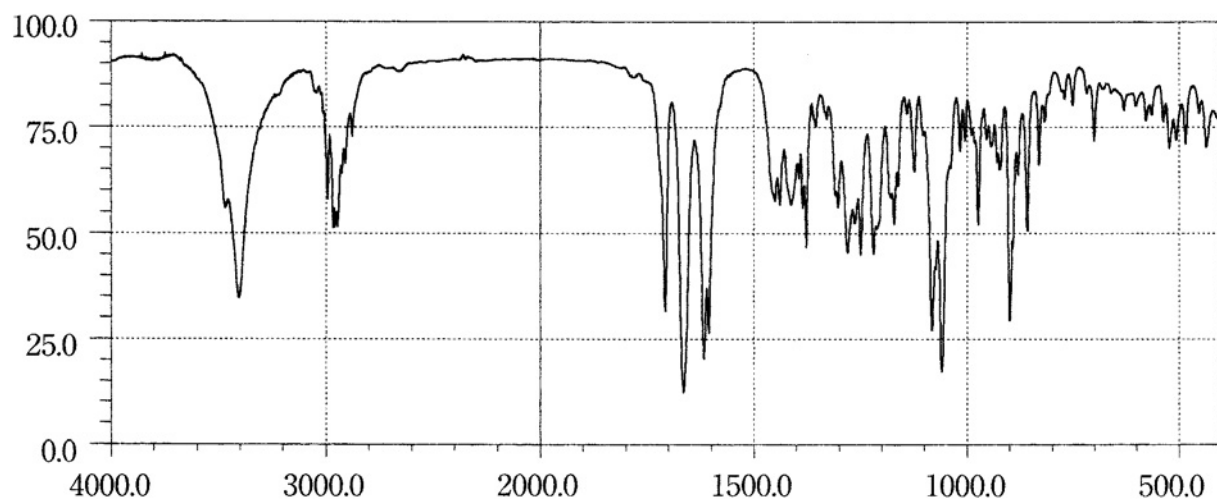
Tofisopam

Preparation of sample: Potassium bromide disk method

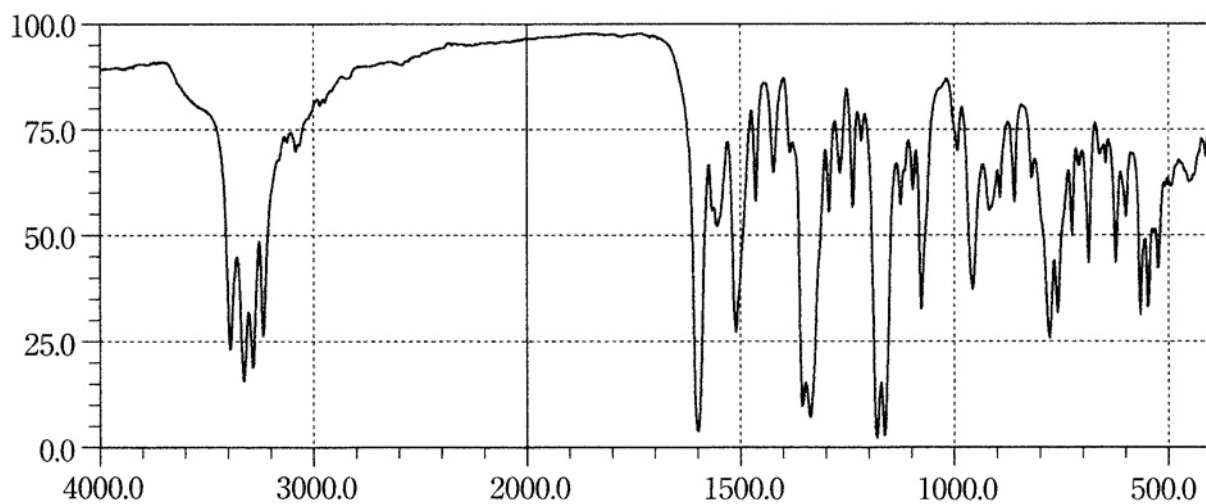
Tolazamide

Preparation of sample: Potassium bromide disk method

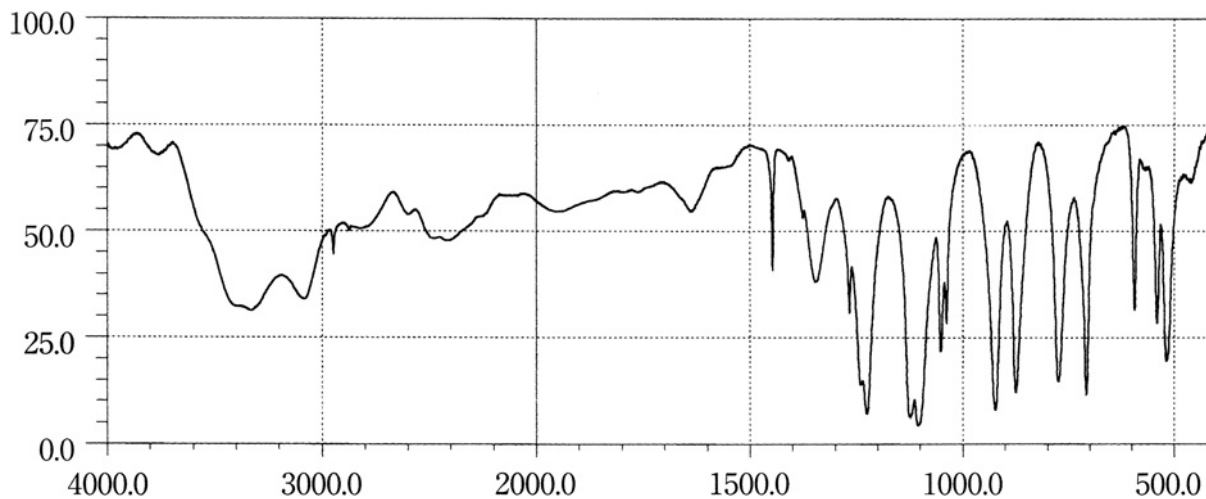


Triamcinolone Acetonide

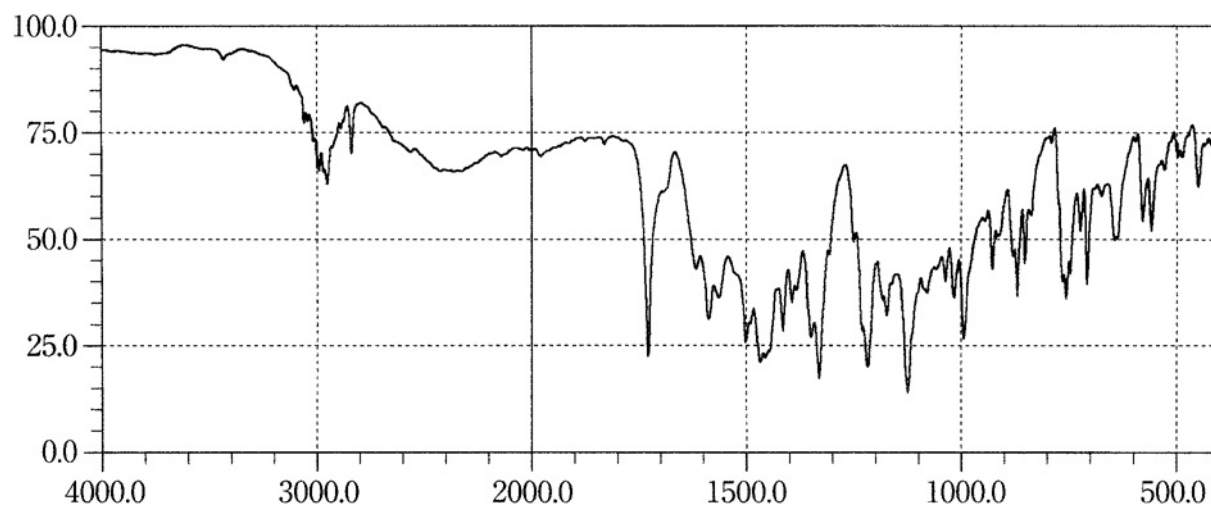
Preparation of sample: Potassium bromide disk method

Trichlormethiazide

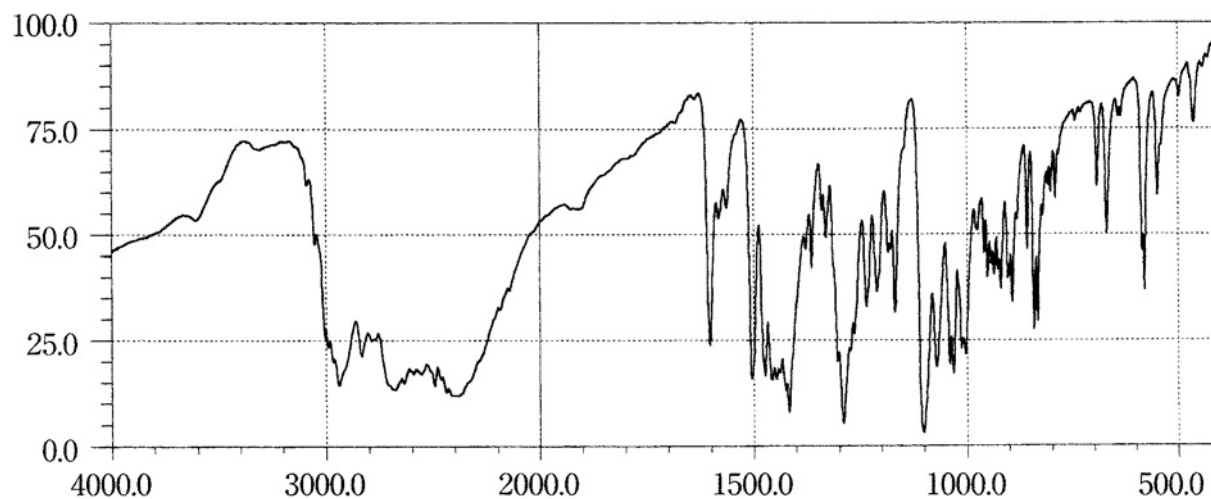
Preparation of sample: Potassium bromide disk method

Triclofos Sodium

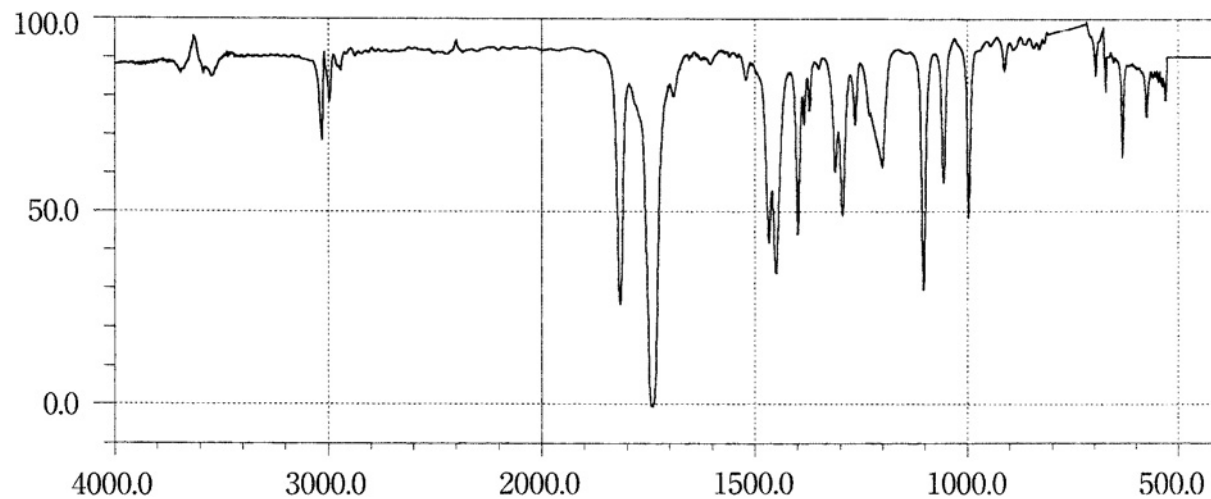
Preparation of sample: Potassium bromide disk method

Trimebutine Maleate

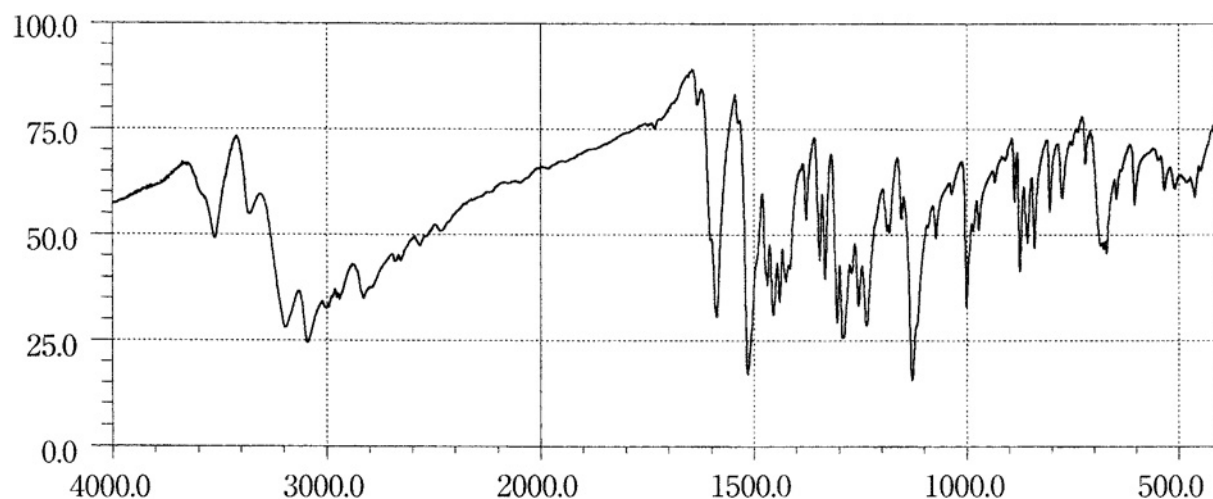
Preparation of sample: Potassium bromide disk method

Trimetazidine Hydrochloride

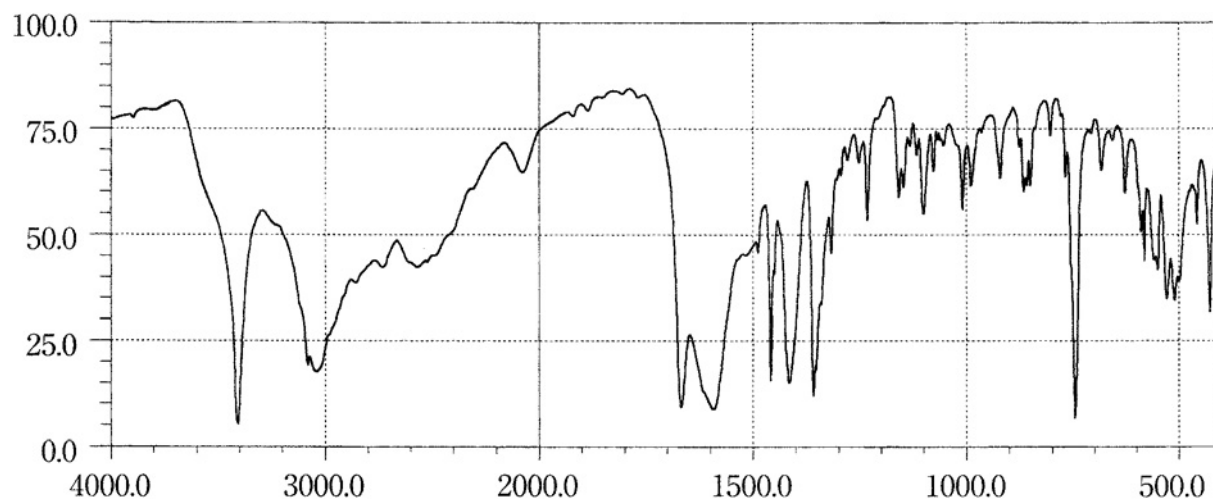
Preparation of sample: Potassium chloride disk method

Trimethadione

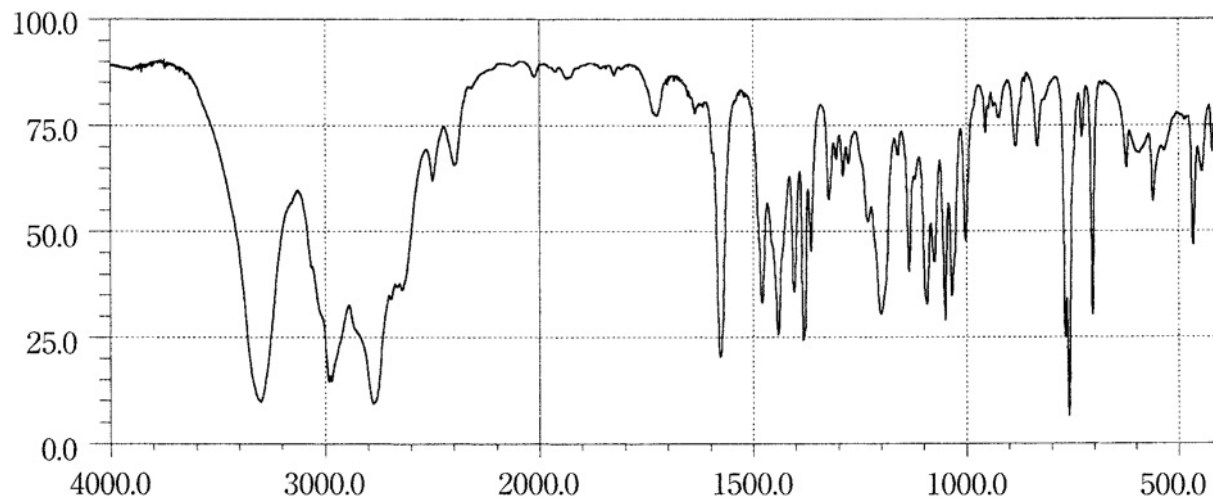
Preparation of sample: Solution method (measured as a solution in chloroform)

Trimetoquinol Hydrochloride Hydrate

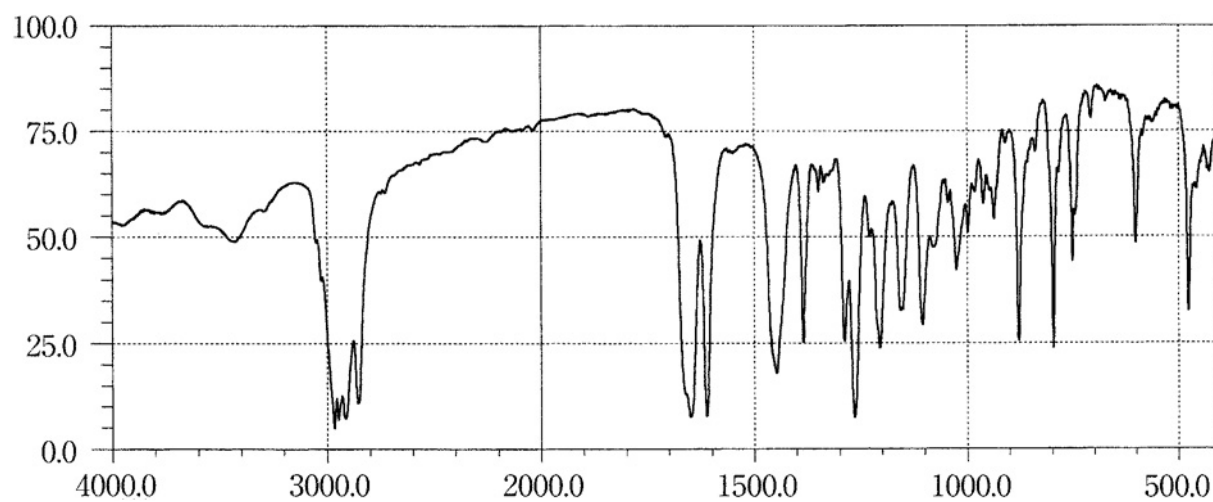
Preparation of sample: Potassium chloride disk method

L-Tryptophan

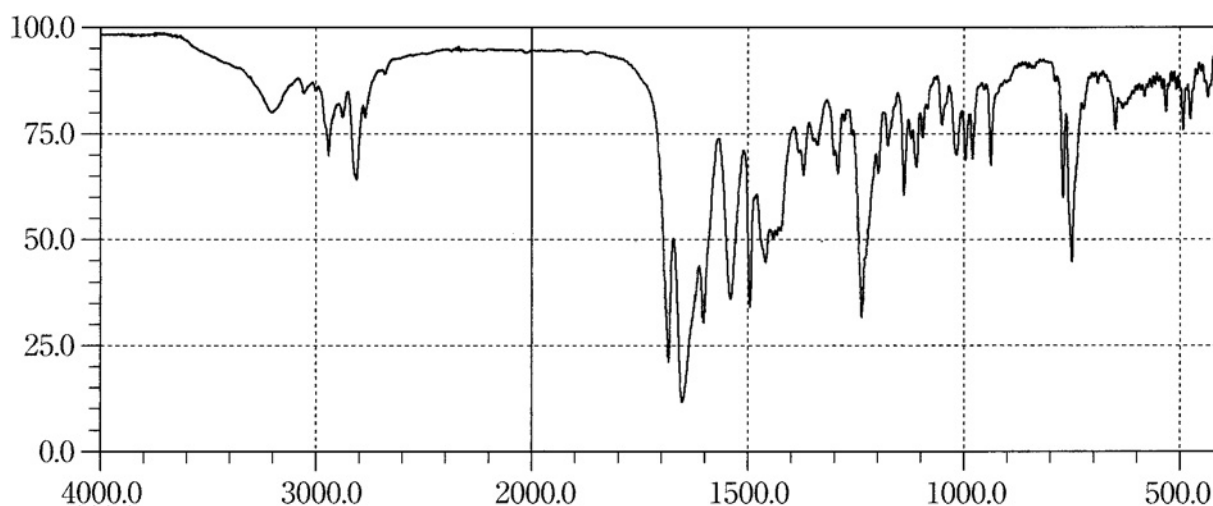
Preparation of sample: Potassium bromide disk method

Tulobuterol Hydrochloride

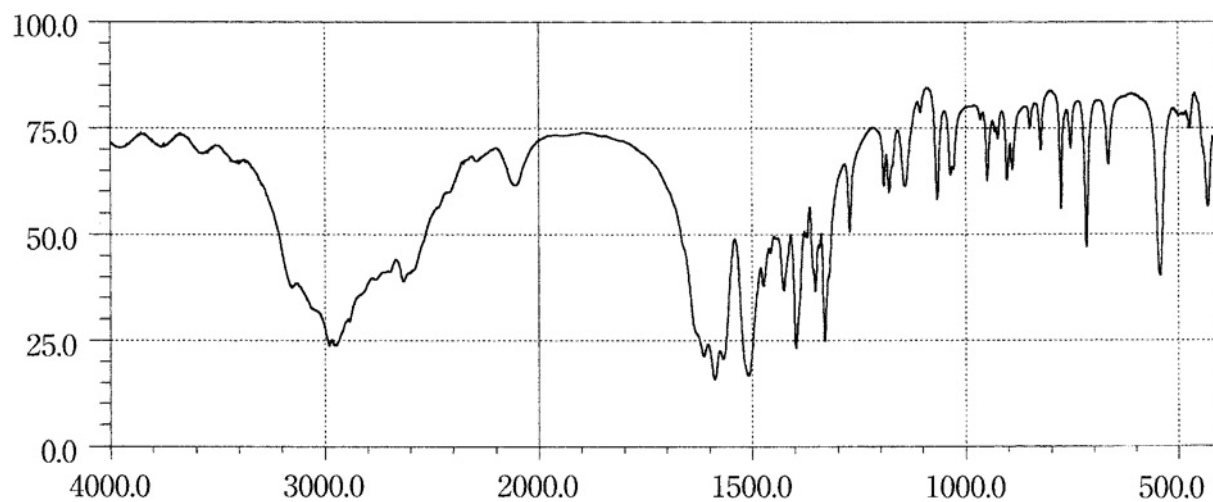
Preparation of sample: Potassium bromide disk method

Ubidecarenone

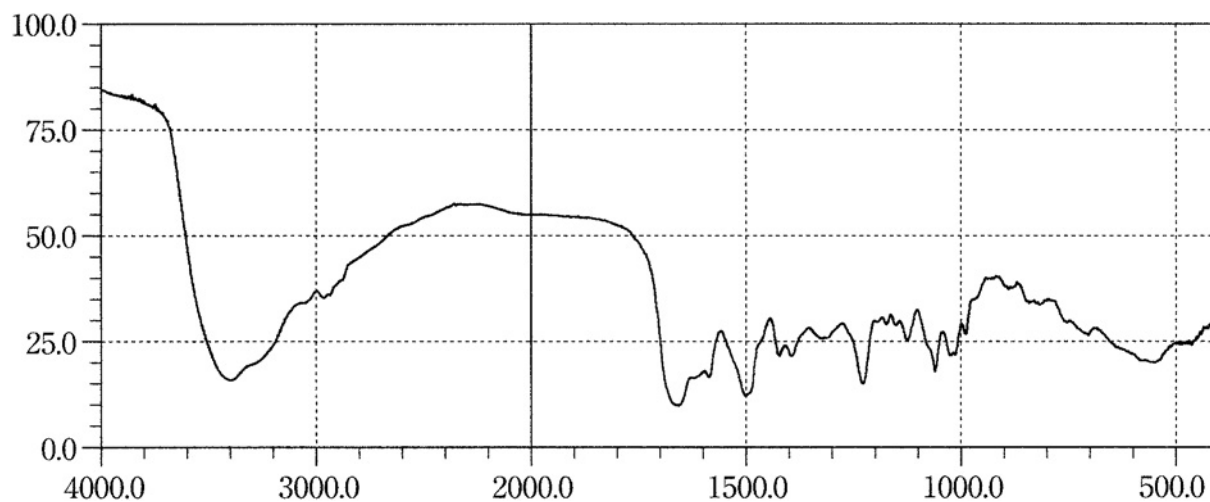
Preparation of sample: Potassium bromide disk method

Urapidil

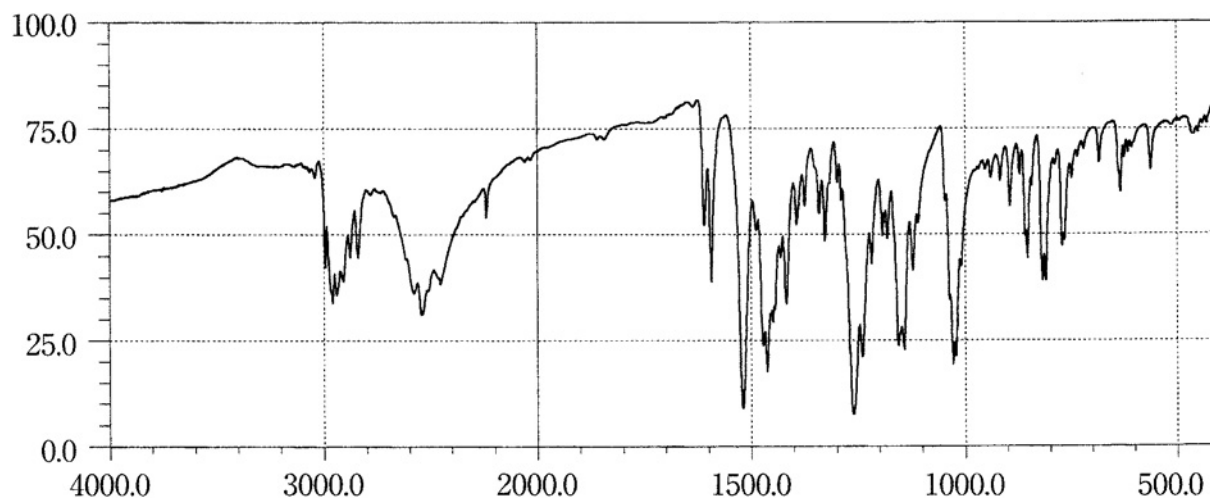
Preparation of sample: Potassium bromide disk method

L-Valine

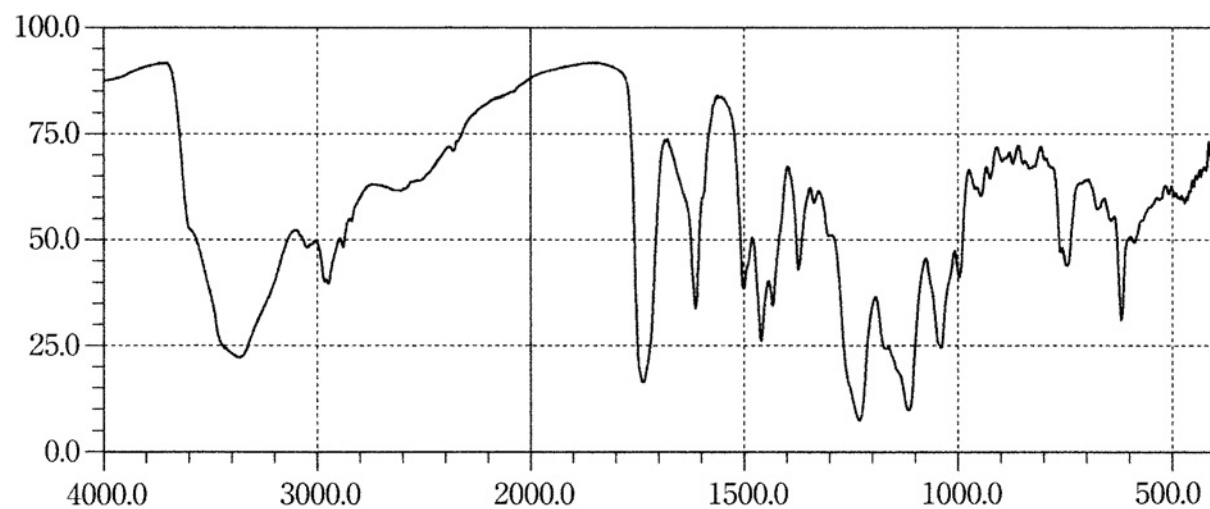
Preparation of sample: Potassium bromide disk method

Vancomycin Hydrochloride

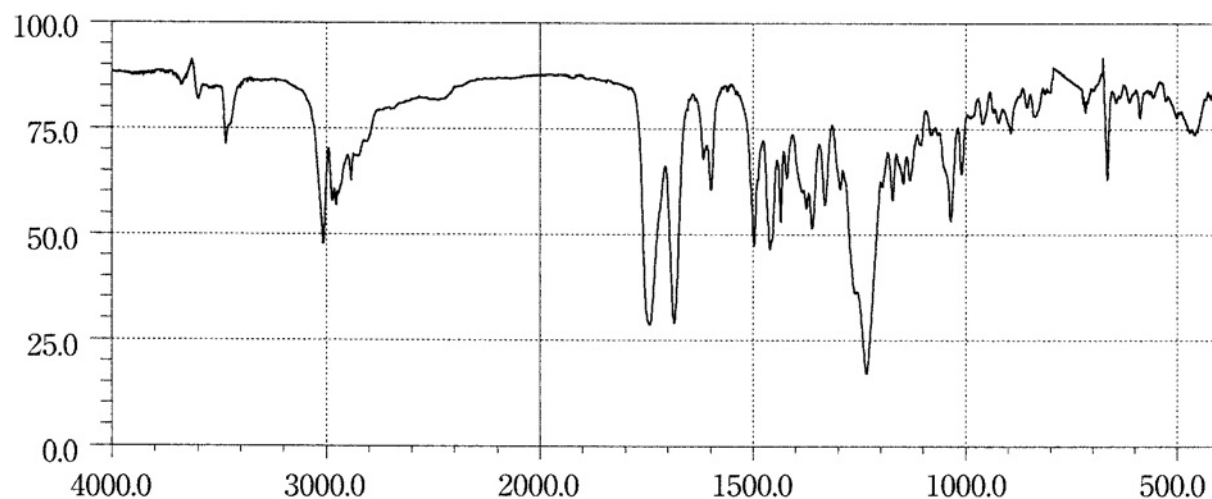
Preparation of sample: Potassium bromide disk method

Verapamil Hydrochloride

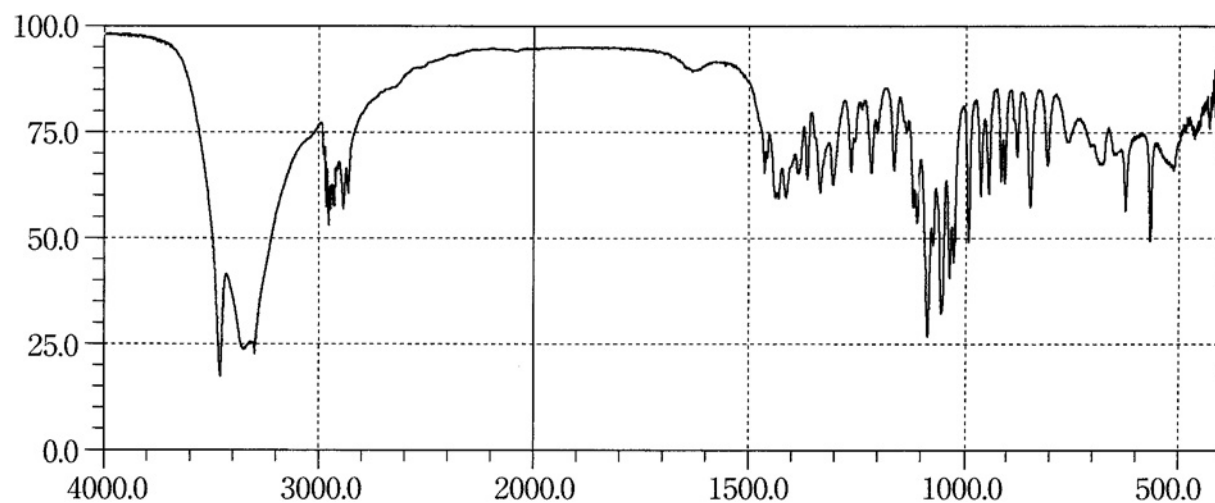
Preparation of sample: Potassium chloride disk method

Vinblastine Sulfate

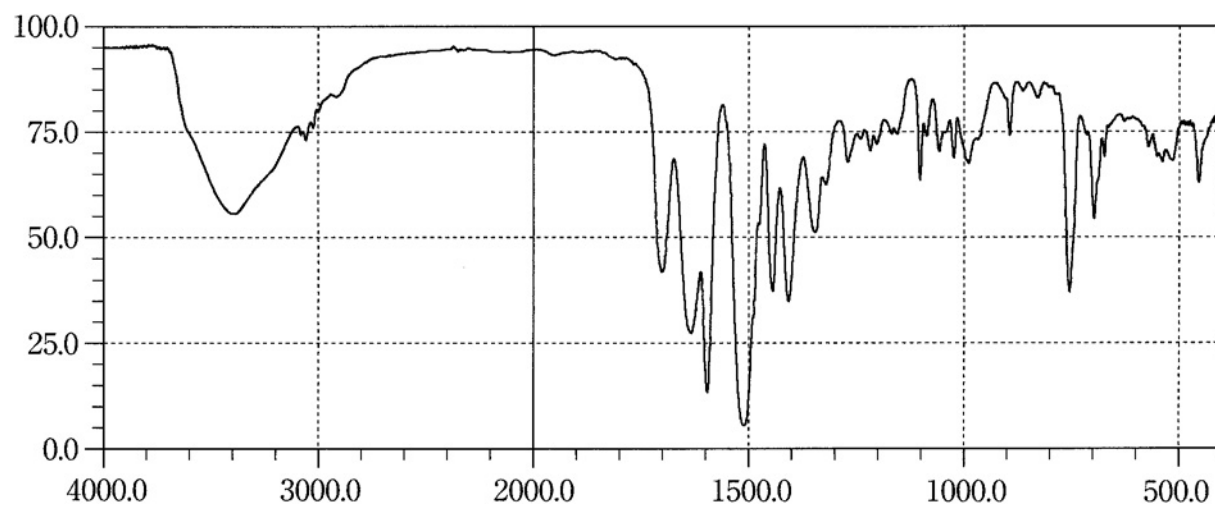
Preparation of sample: Potassium bromide disk method

Vincristine Sulfate

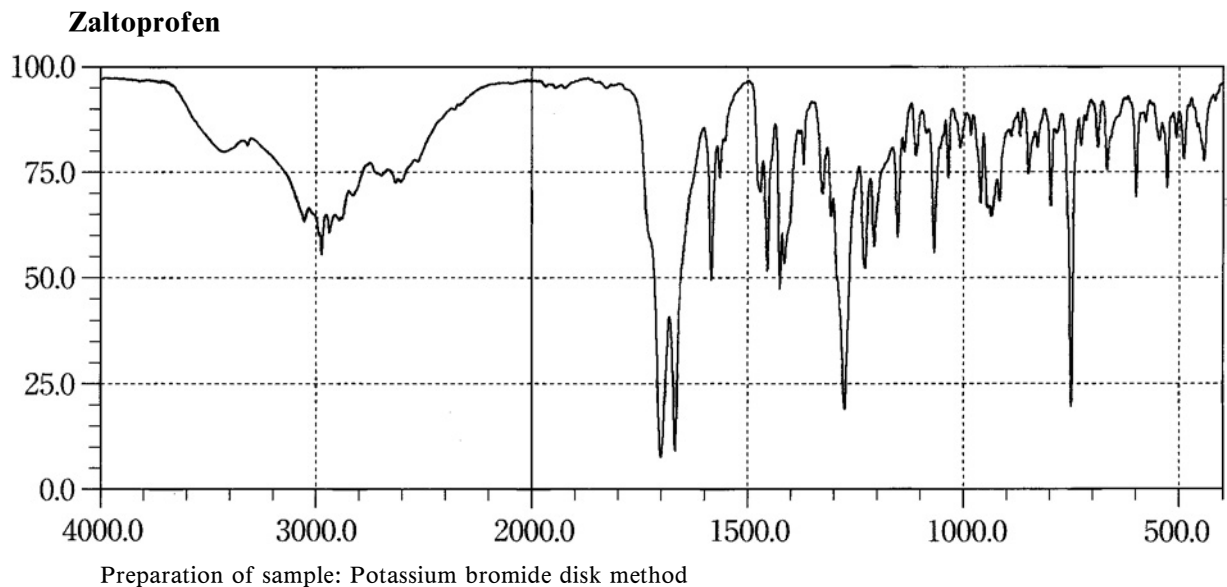
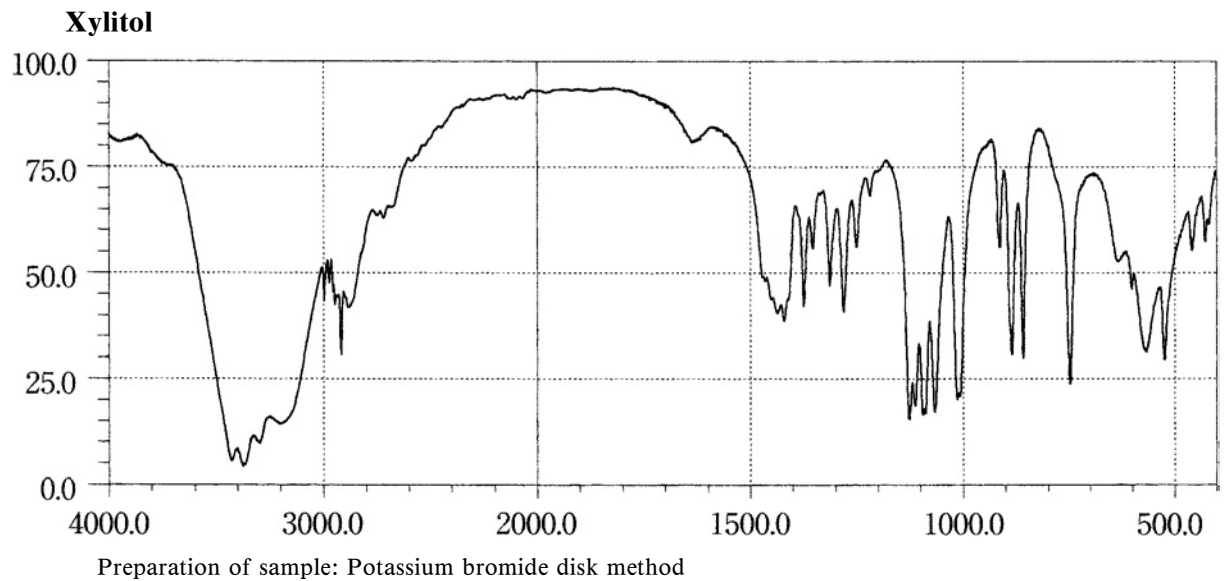
Preparation of sample: Solution method (measured as a solution in chloroform)

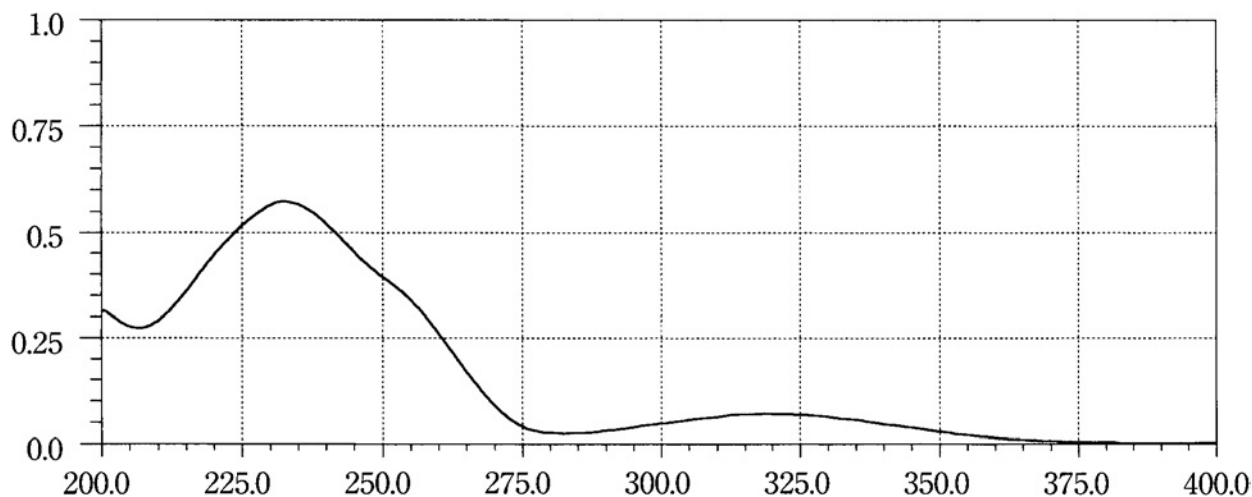
Voglibose

Preparation of sample: Potassium bromide disk method

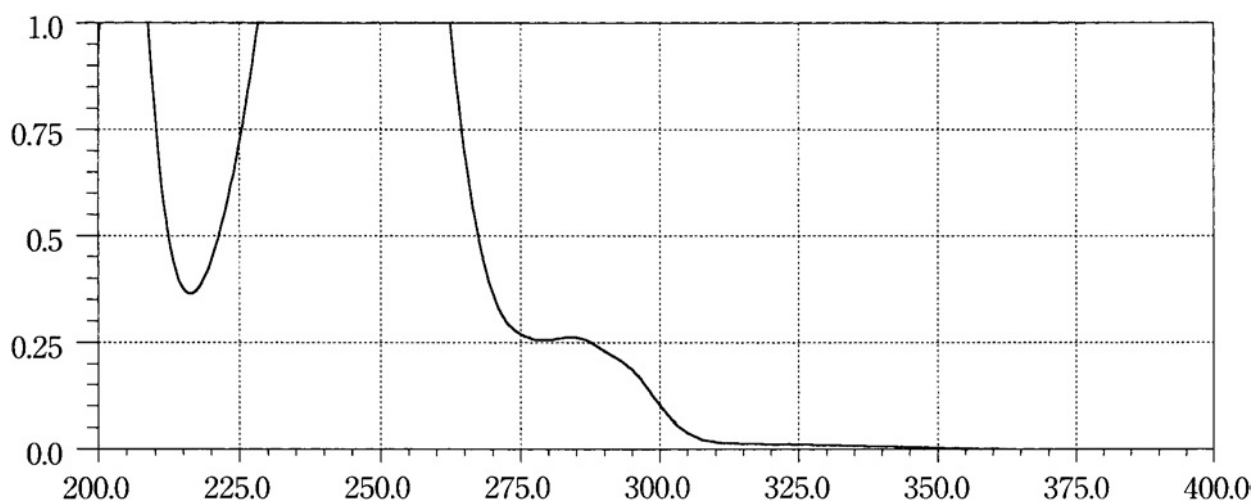
Warfarin Potassium

Preparation of sample: Potassium bromide disk method

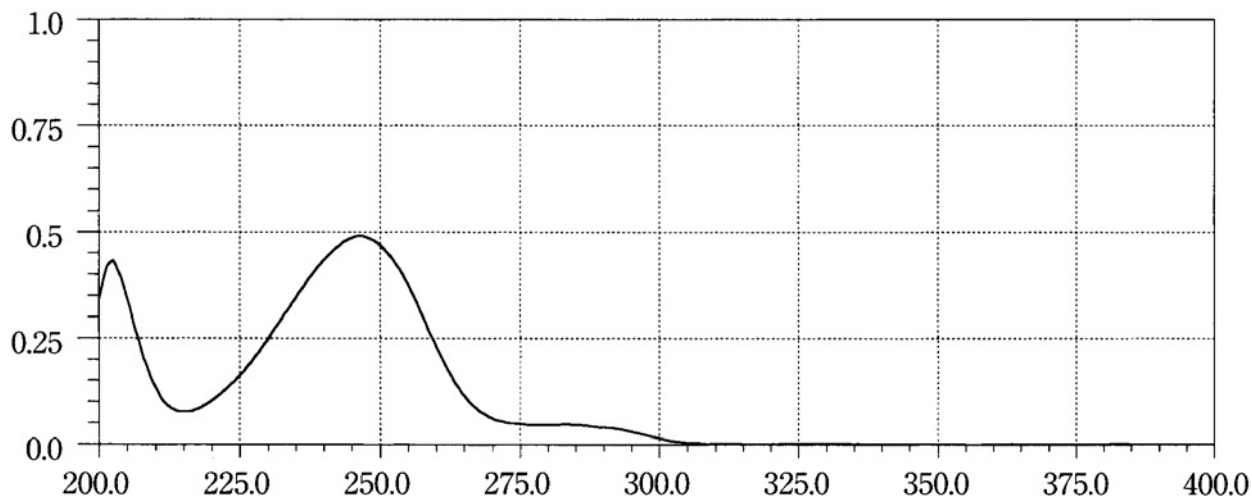


Acetutolol Hydrochloride

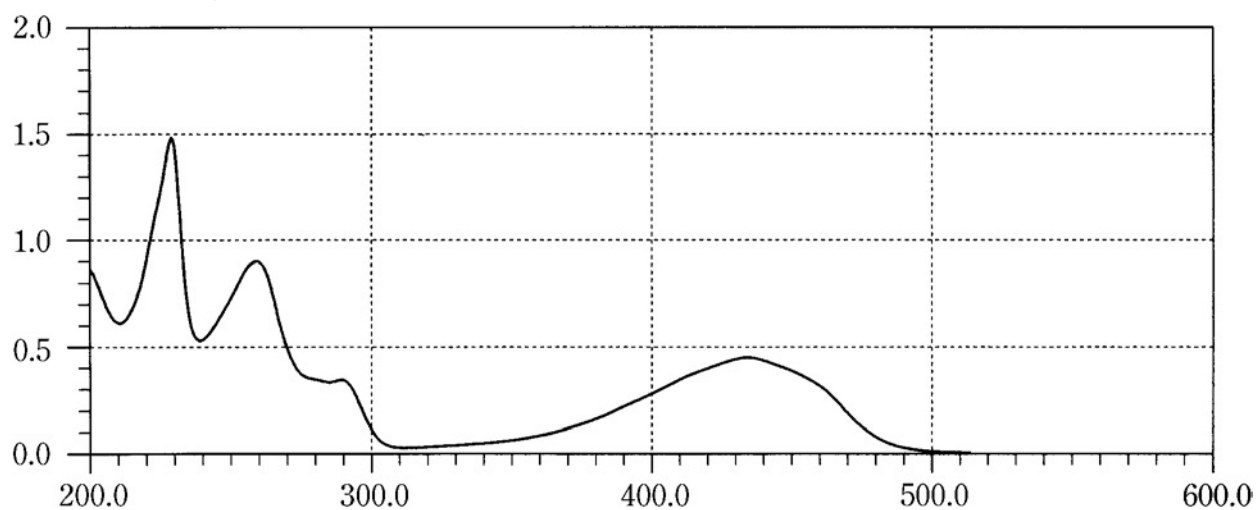
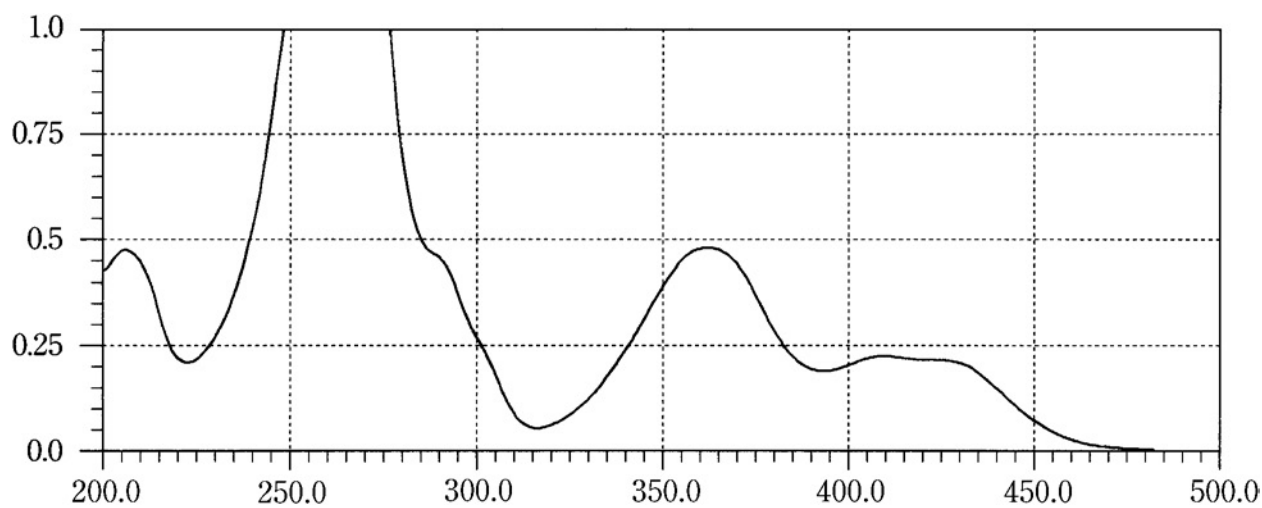
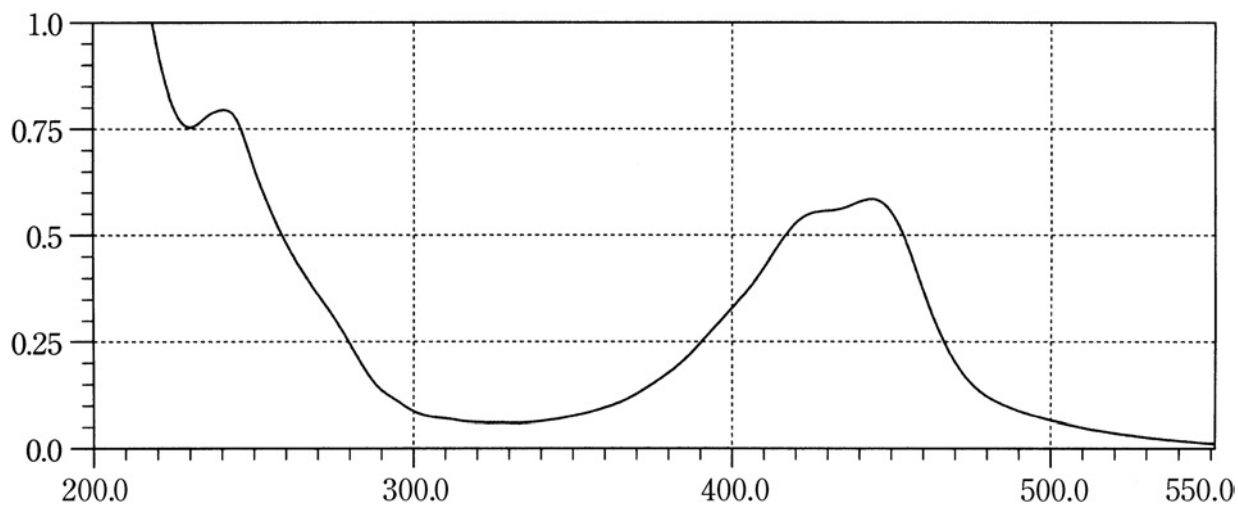
A solution in 0.01 mol/L hydrochloric acid TS (1 in 100,000)

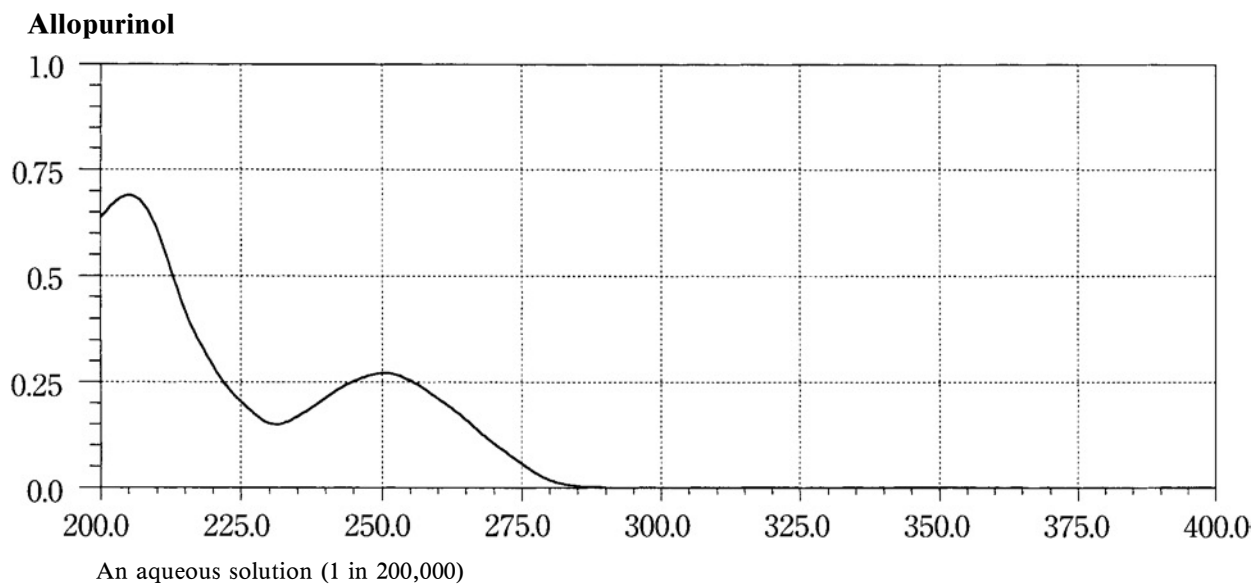
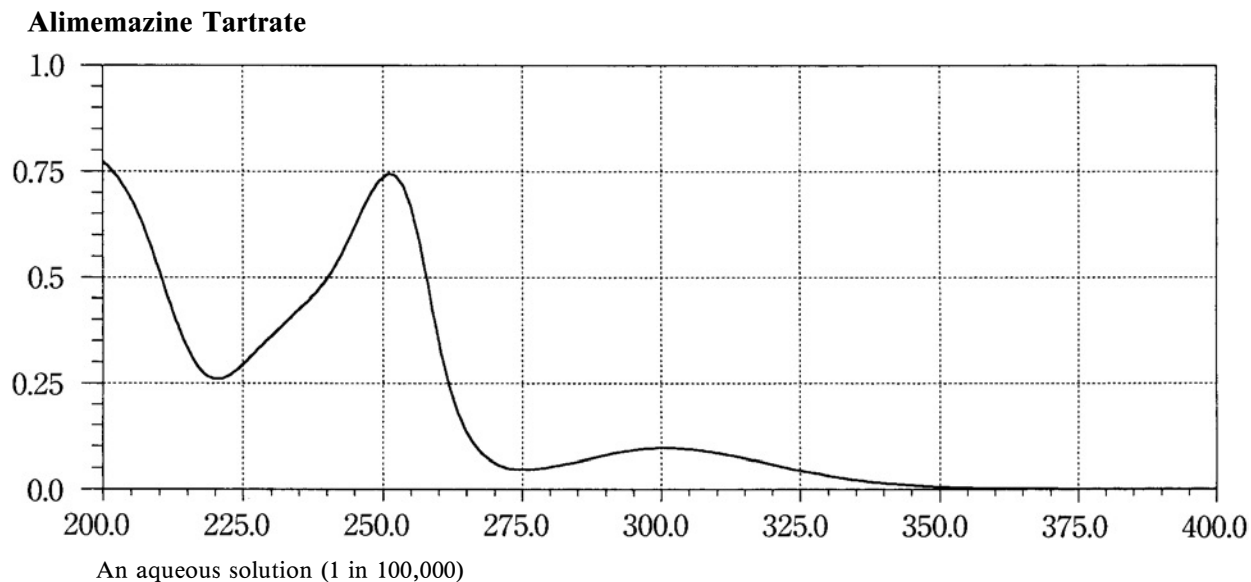
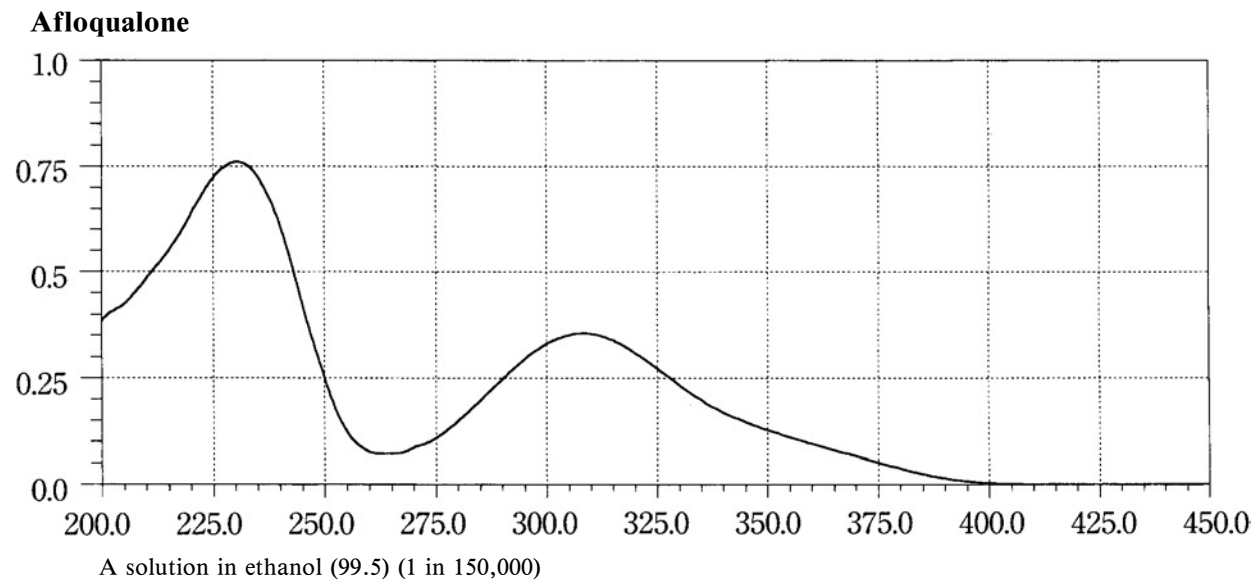
Acetohexamide 1

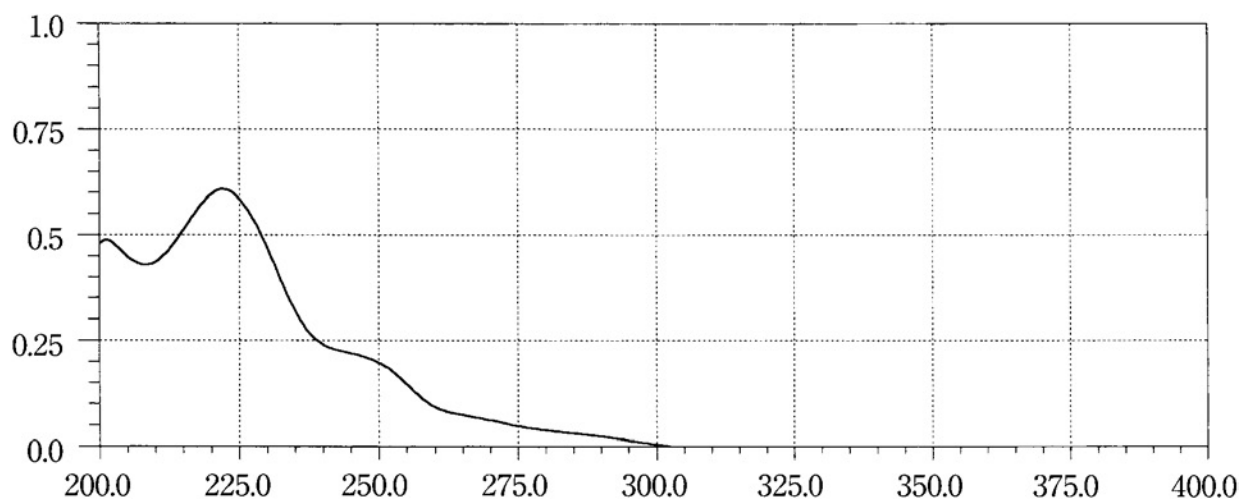
A solution prepared as follows: Dissolve 0.10 g in 100 mL of methanol. To 5 mL of this solution add 20 mL of 0.5 mol/L hydrochloric acid TS and 75 mL of methanol (Sample solution 1).

Acetohexamide 2

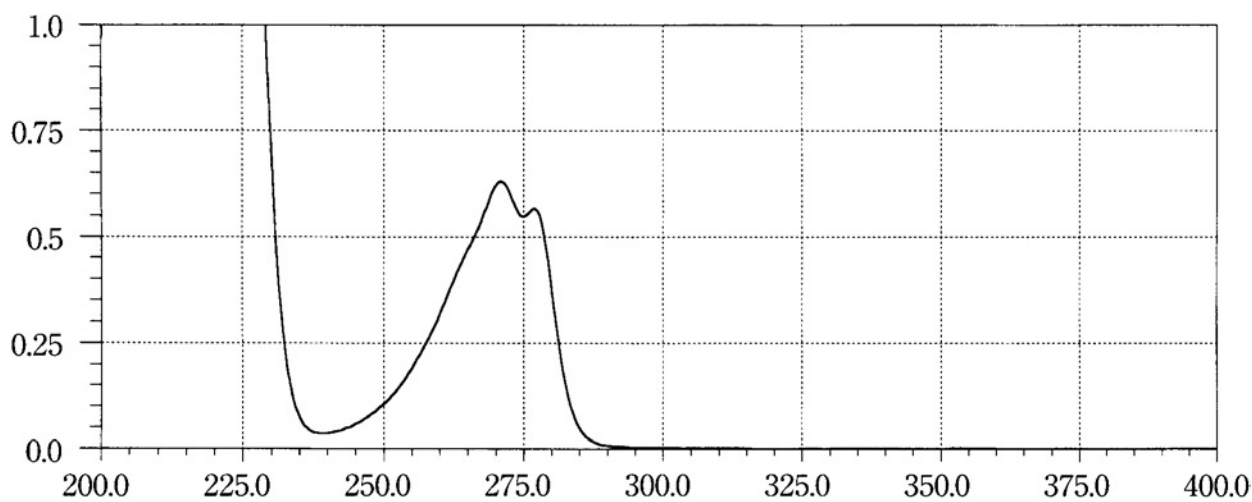
A solution prepared as follows: To 10 mL of Sample solution 1 add methanol to make 50 mL (Sample solution 2).

Aclarubicin Hydrochloride**Acrinol Hydrate****Actinomycin D**

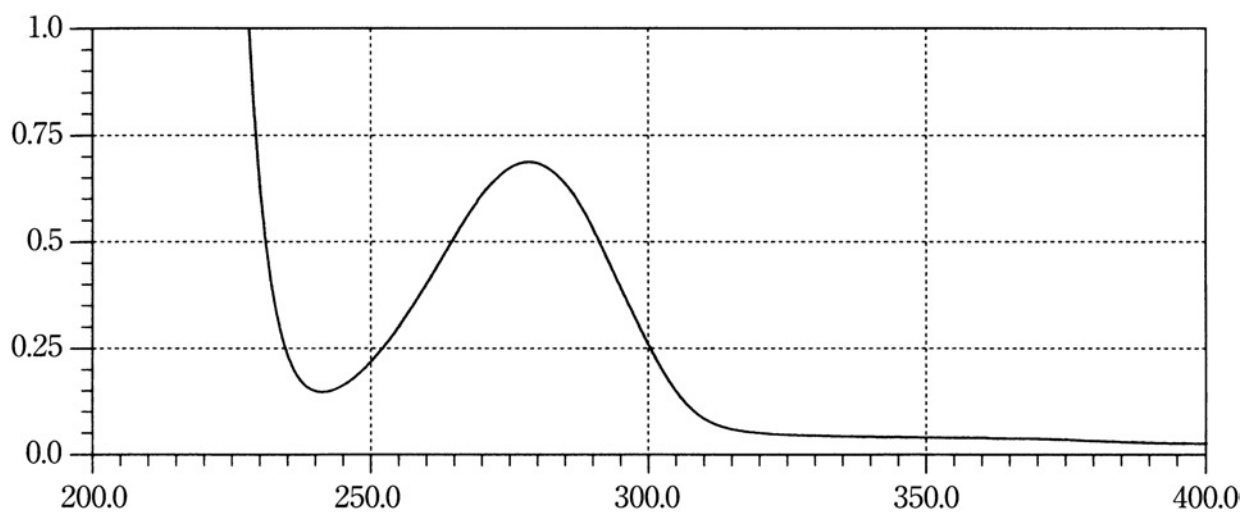


Alprazolam

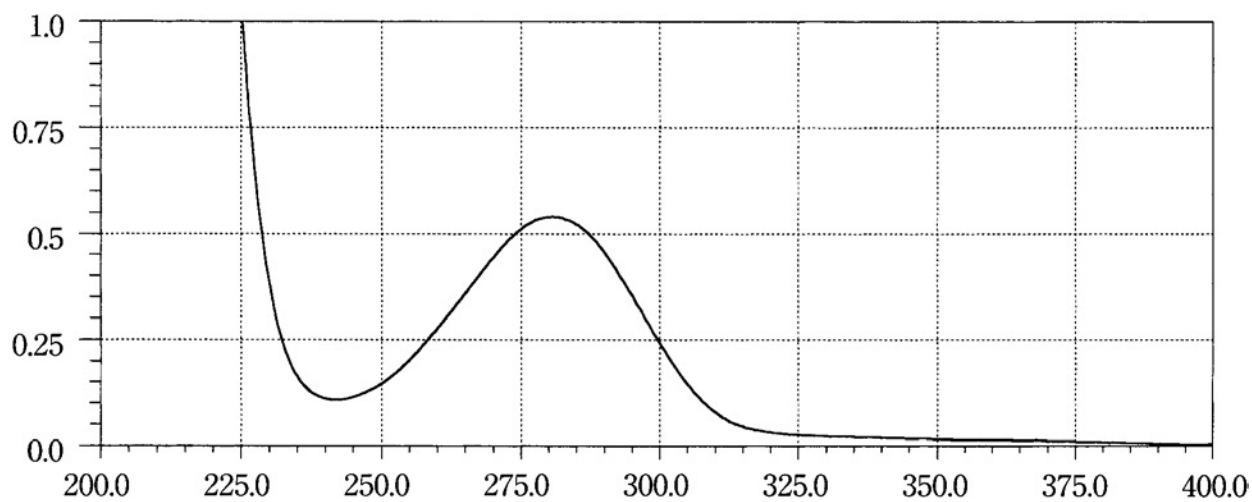
A solution in ethanol (95) (1 in 200,000)

Alprenolol Hydrochloride

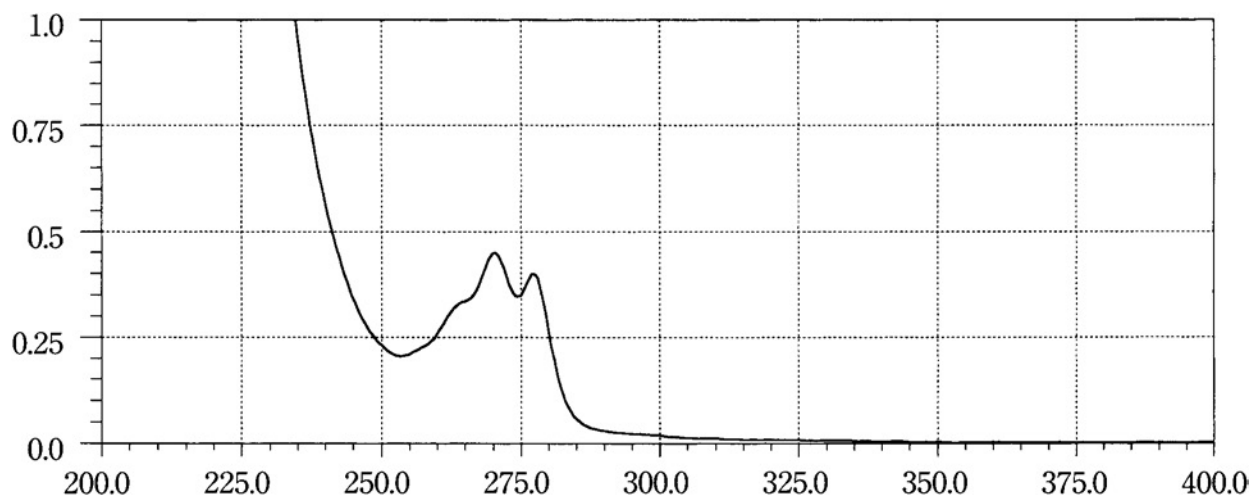
A solution in ethanol (95) (1 in 10,000)

Alprostadiol

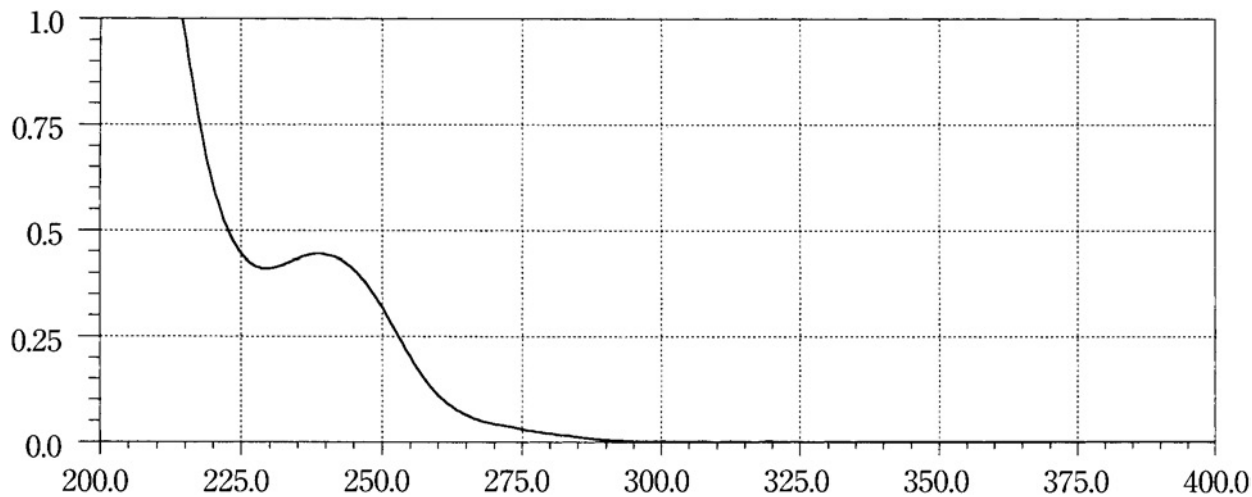
A solution prepared as follows: To 10 mL of a solution in ethanol (99.5) (1 in 100,000) add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes.

Alprostadil Alfadex

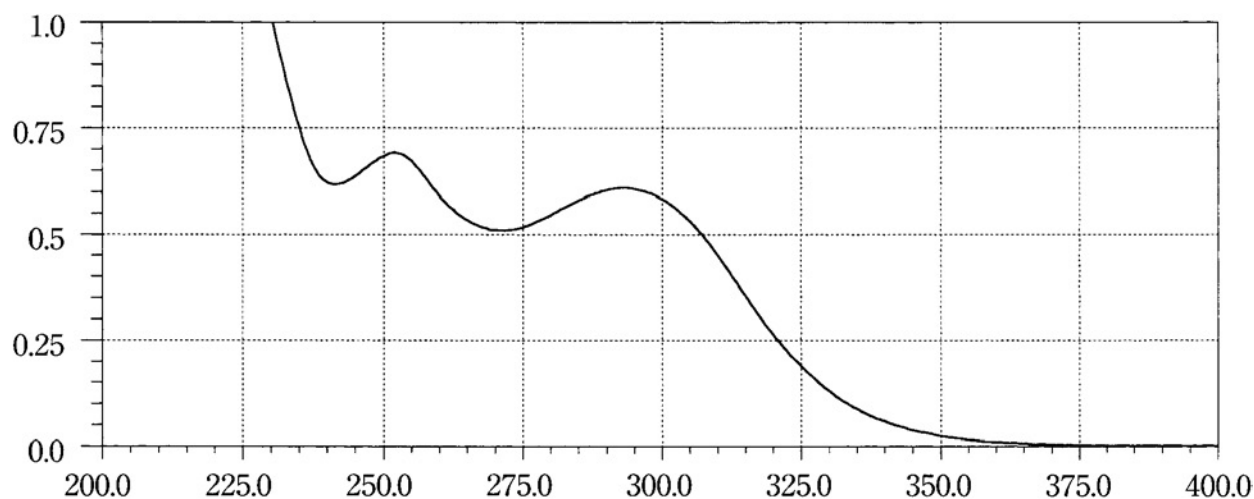
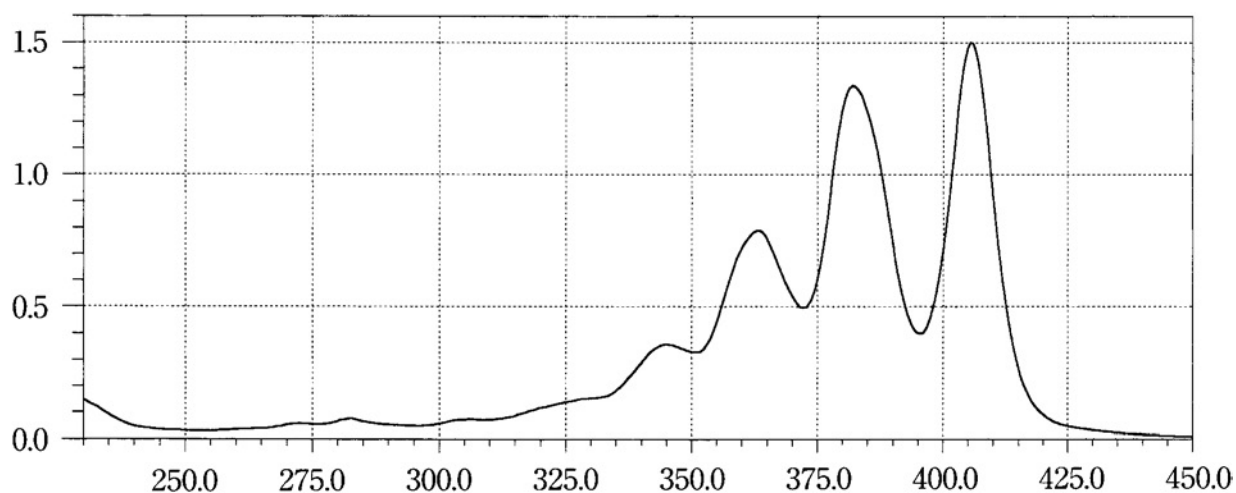
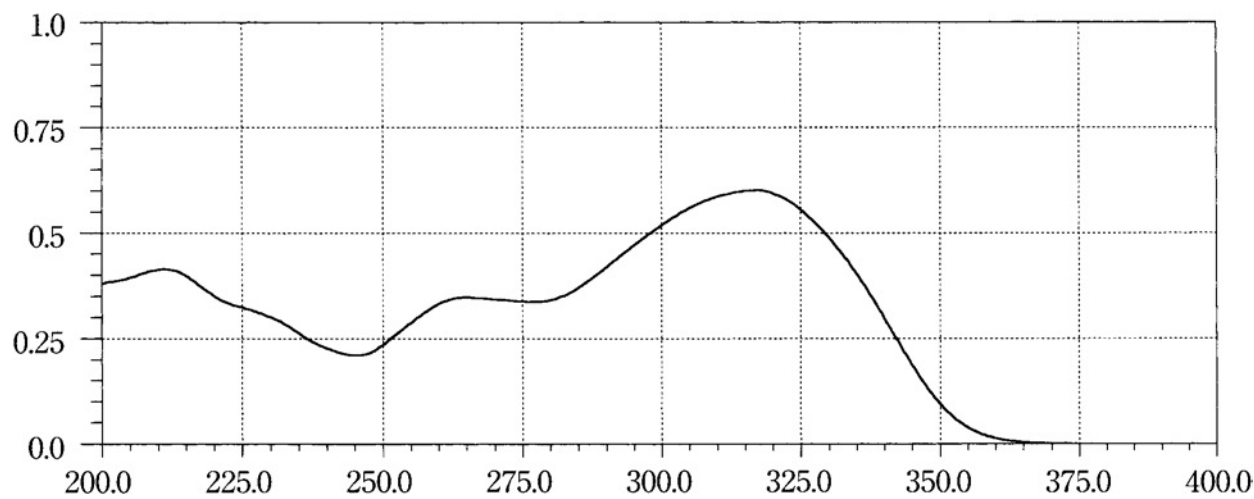
A solution prepared as follows: To 10 mL of a solution in dilute ethanol (3 in 10,000) add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes.

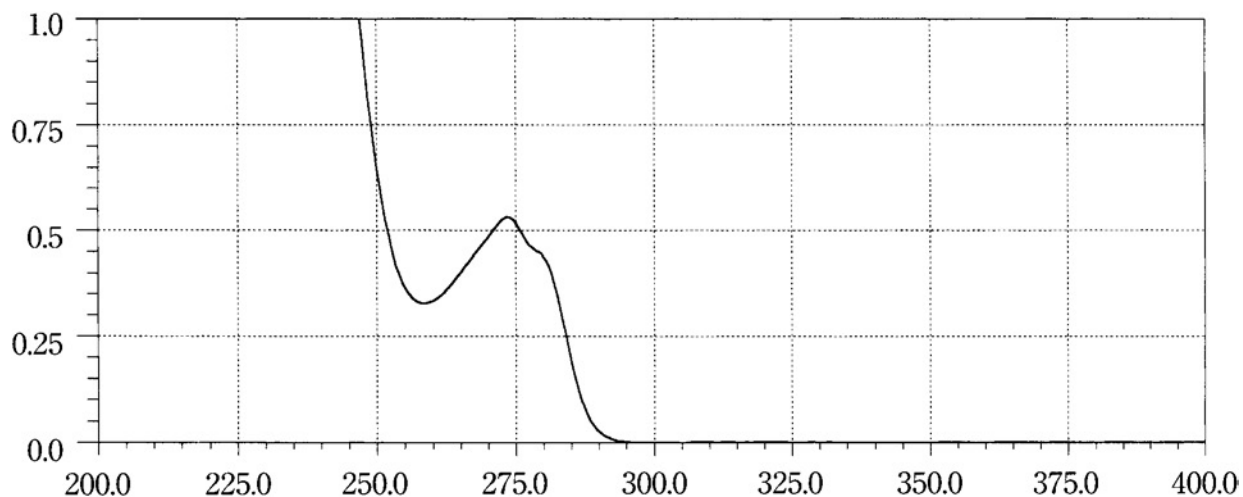
Ambenonium Chloride

A solution in methanol (1 in 5000)

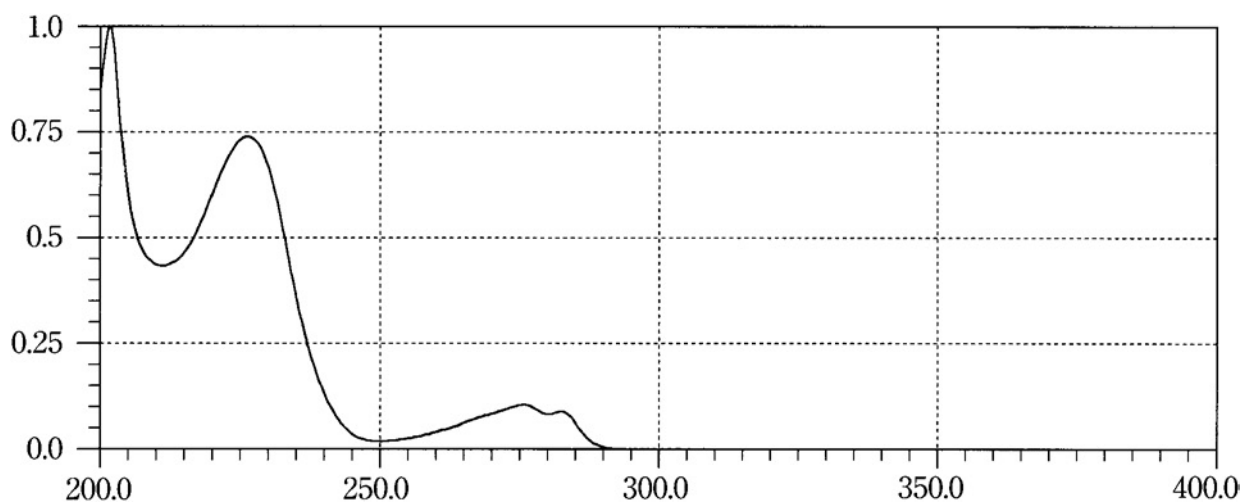
Amitriptyline Hydrochloride

An aqueous solution (1 in 100,000)

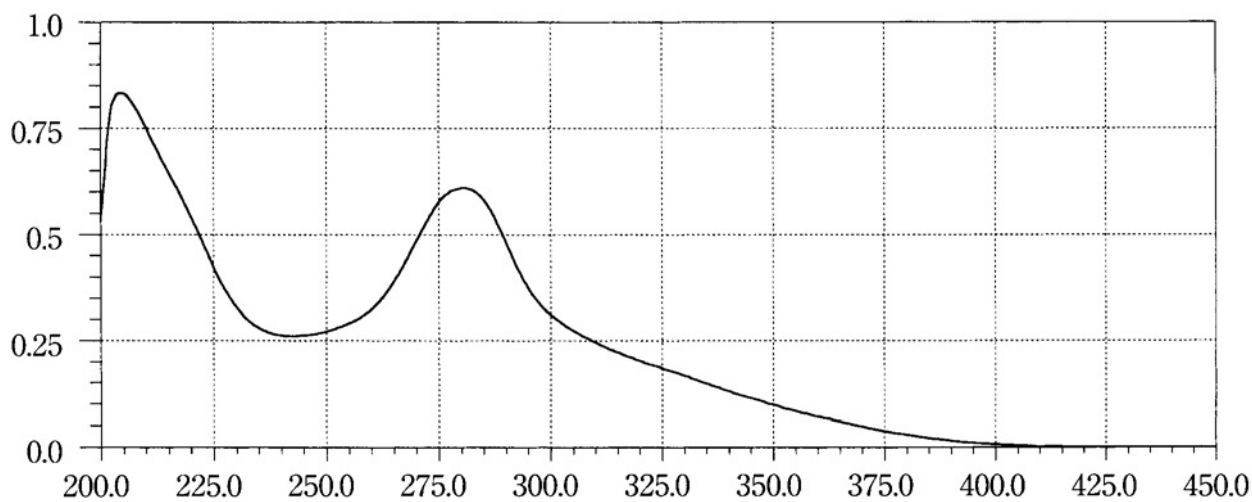
Amoxapine**Amphotericin B****Arotinolol Hydrochloride**

Aspoxicillin Hydrate

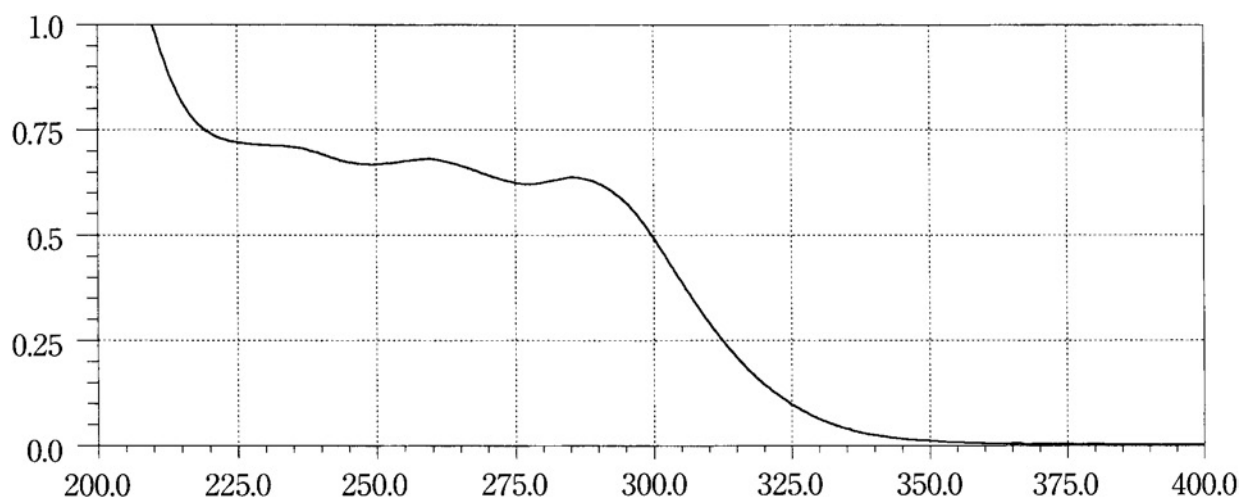
An aqueous solution (1 in 4000)

Atenolol

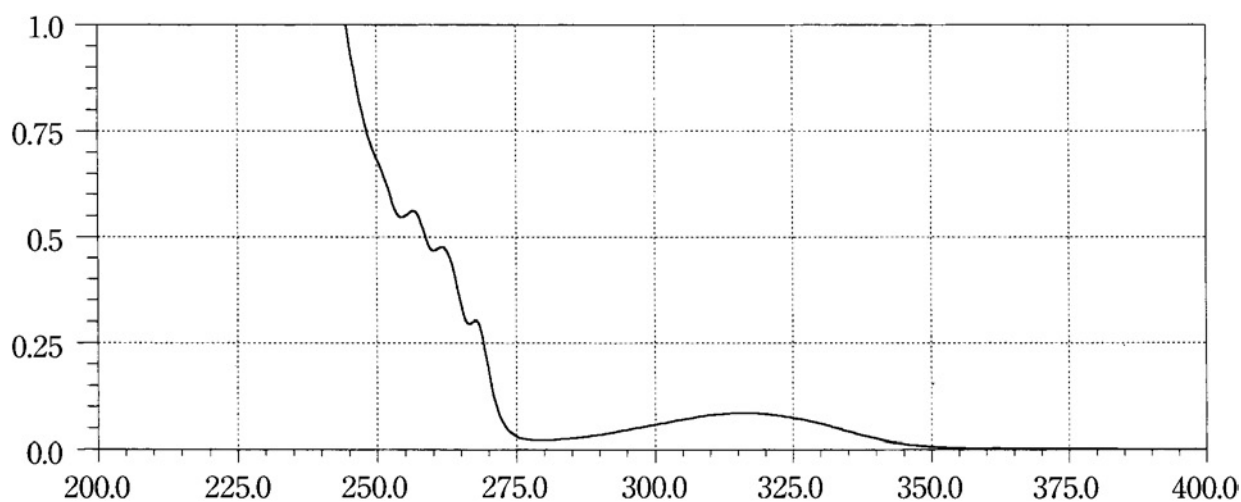
A solution in methanol (1 in 50,000)

Azathioprine

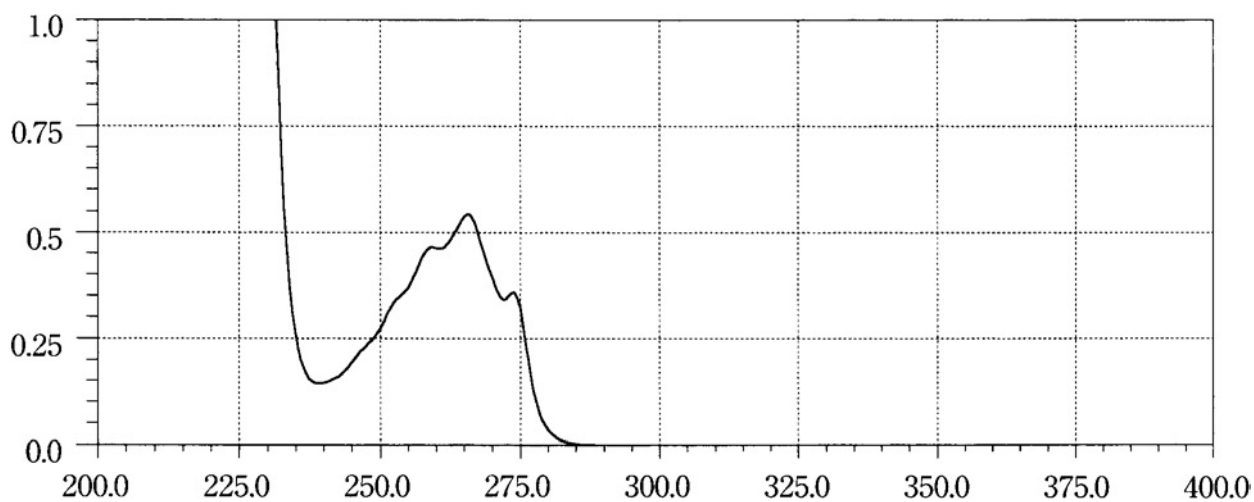
A solution prepared as follows: To 5 mL of a solution in 2 mol/L hydrochloric acid TS (1 in 10,000) add water to make 50 mL.

Aztreonam

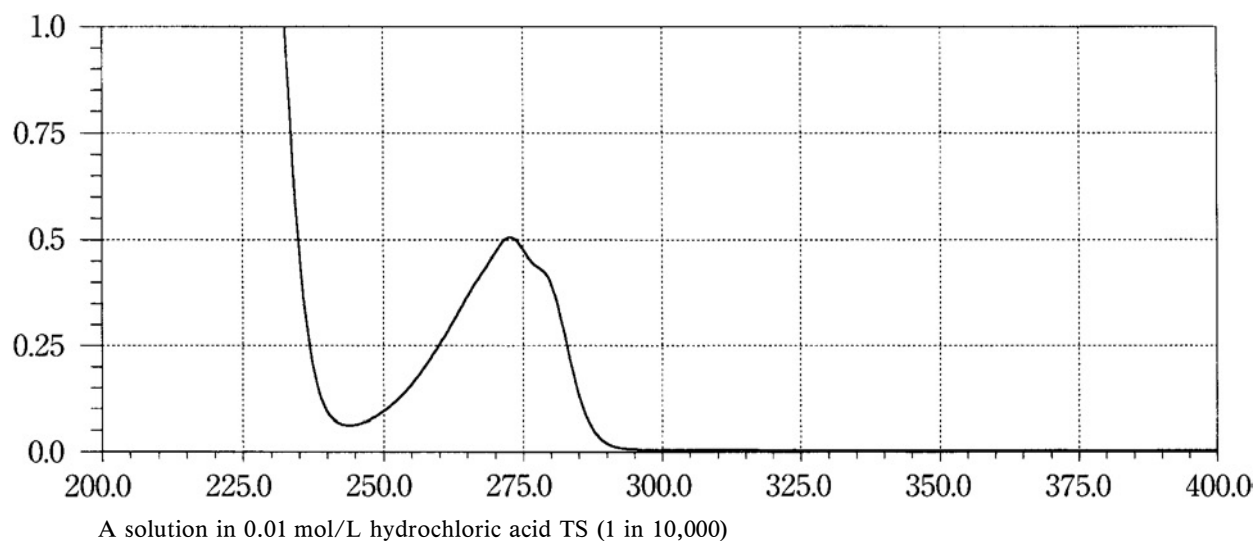
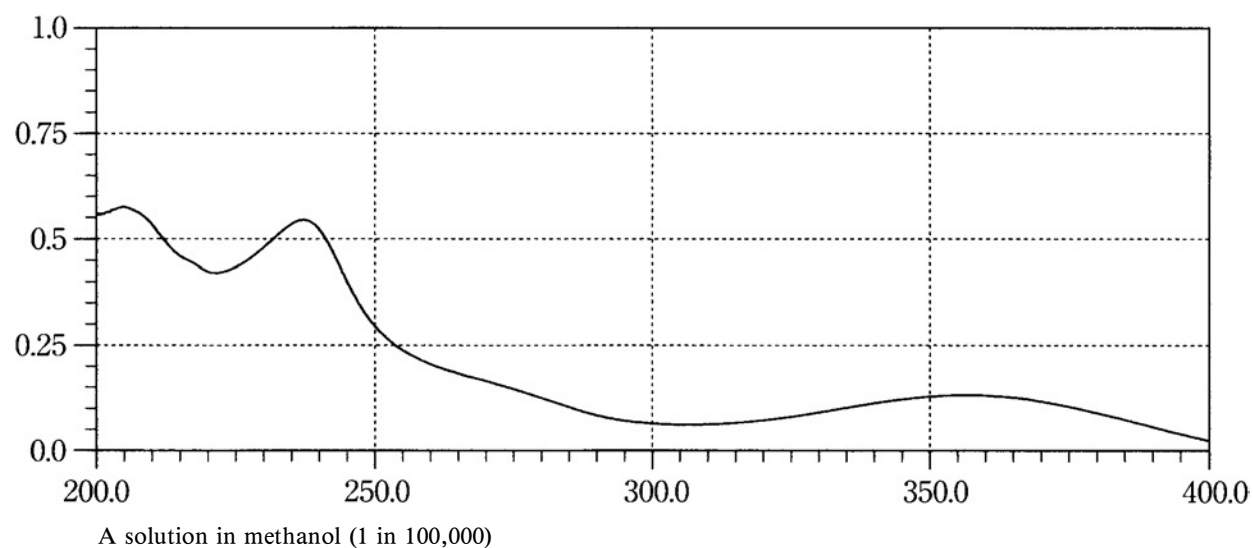
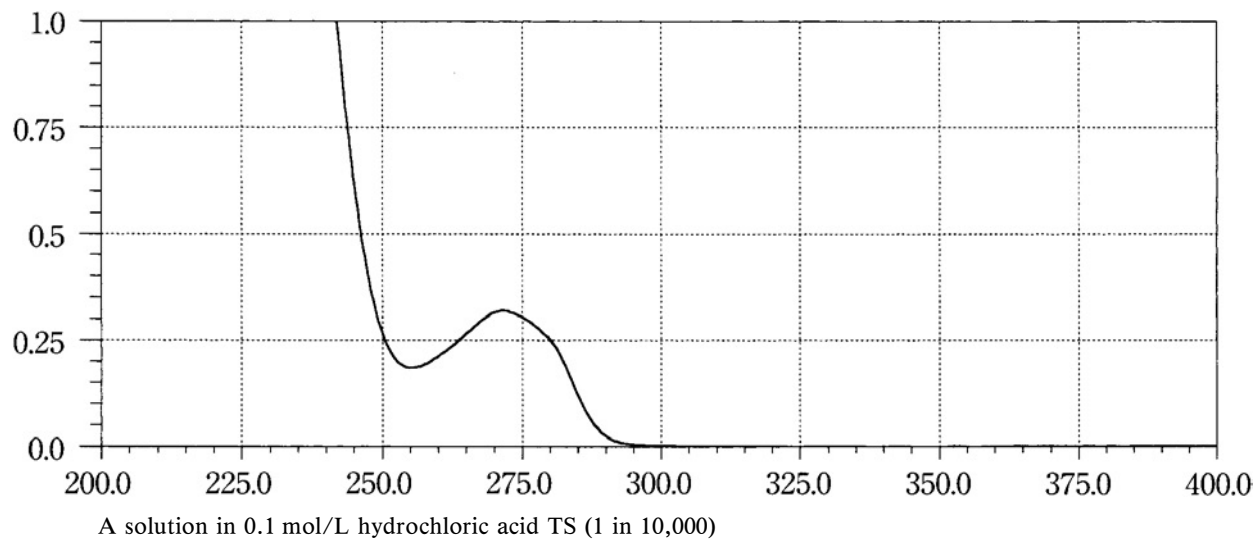
An aqueous solution (3 in 100,000)

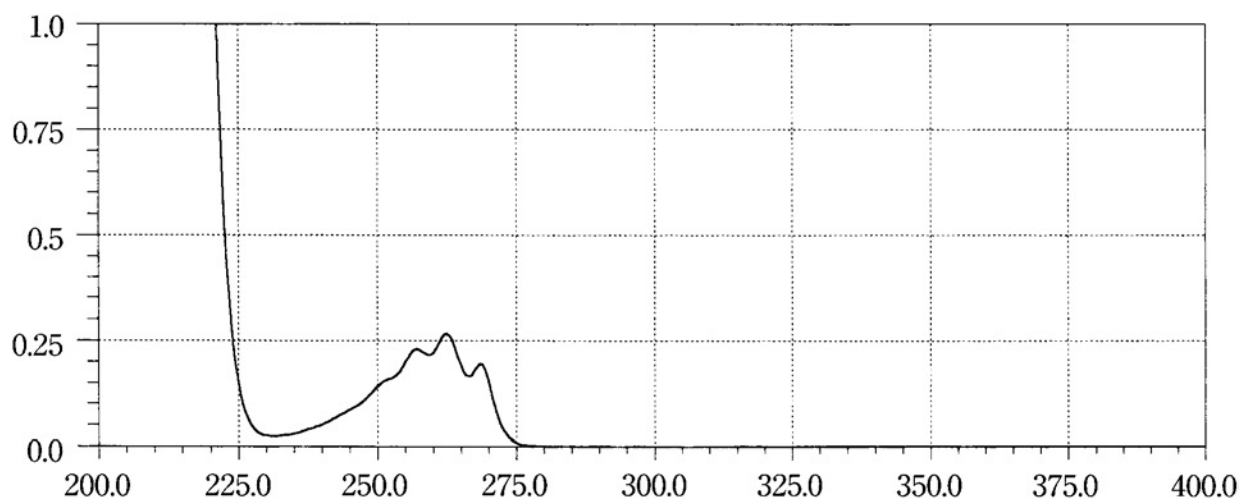
Bacampicillin Hydrochloride

A solution in methanol (1 in 1000)

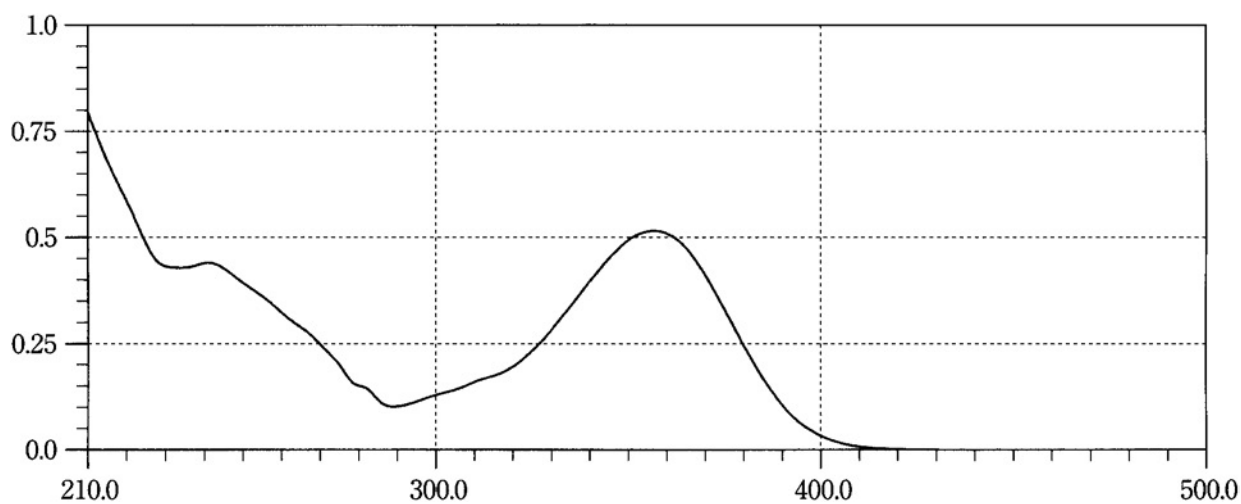
Baclofen

A solution in 0.1 mol/L hydrochloric acid TS (1 in 2000)

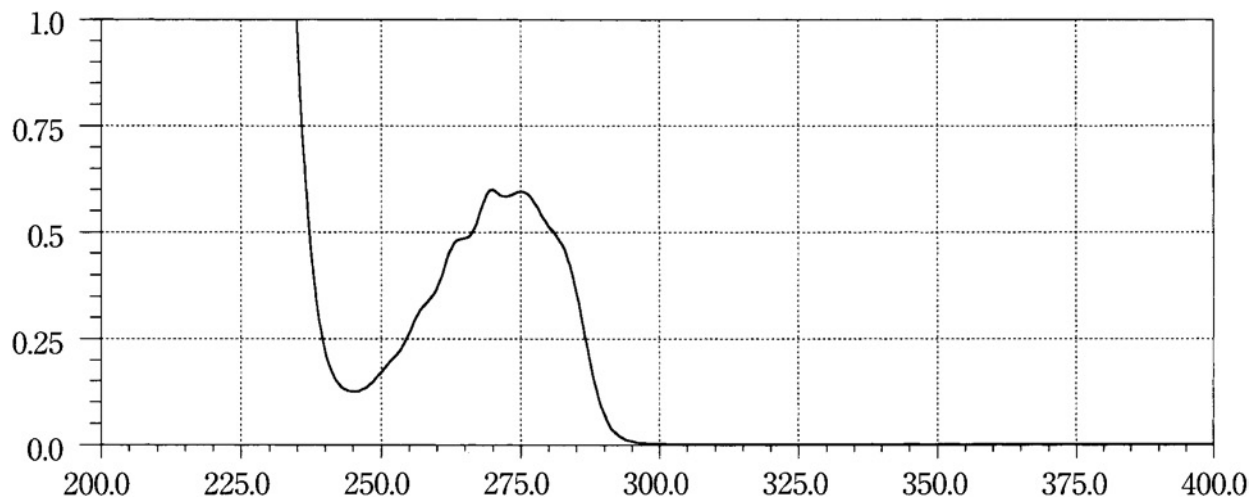
Bamethan Sulfate**Benidipine Hydrochloride****Benserazide Hydrochloride**

Benzalkonium Chloride

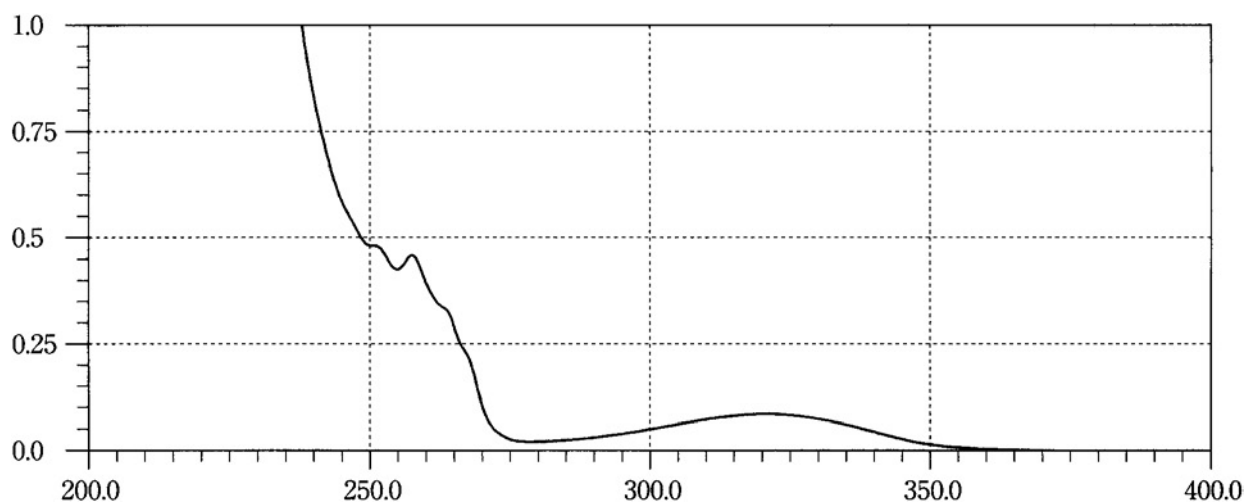
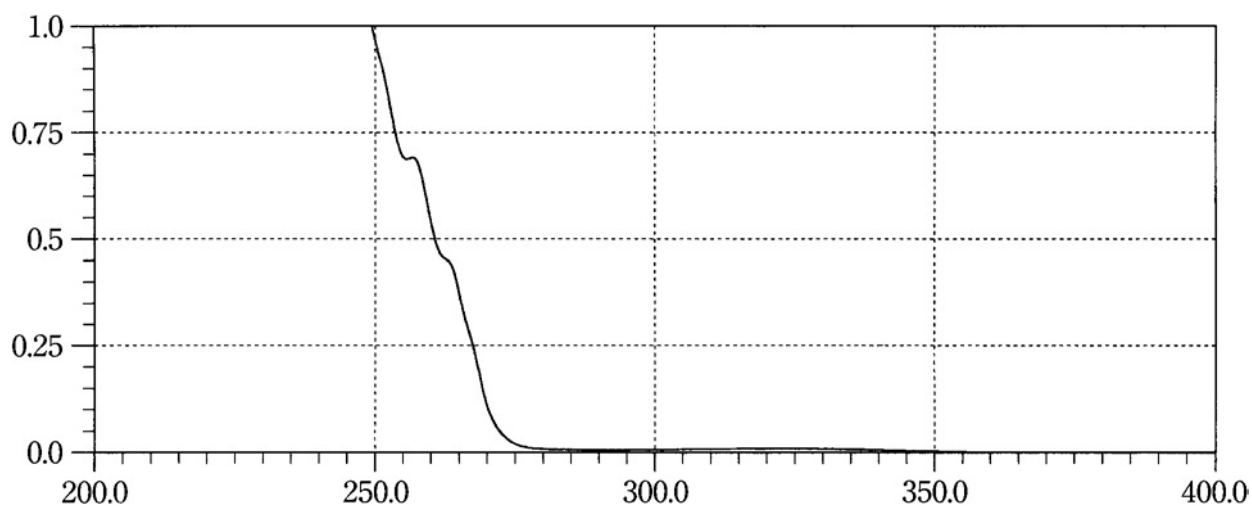
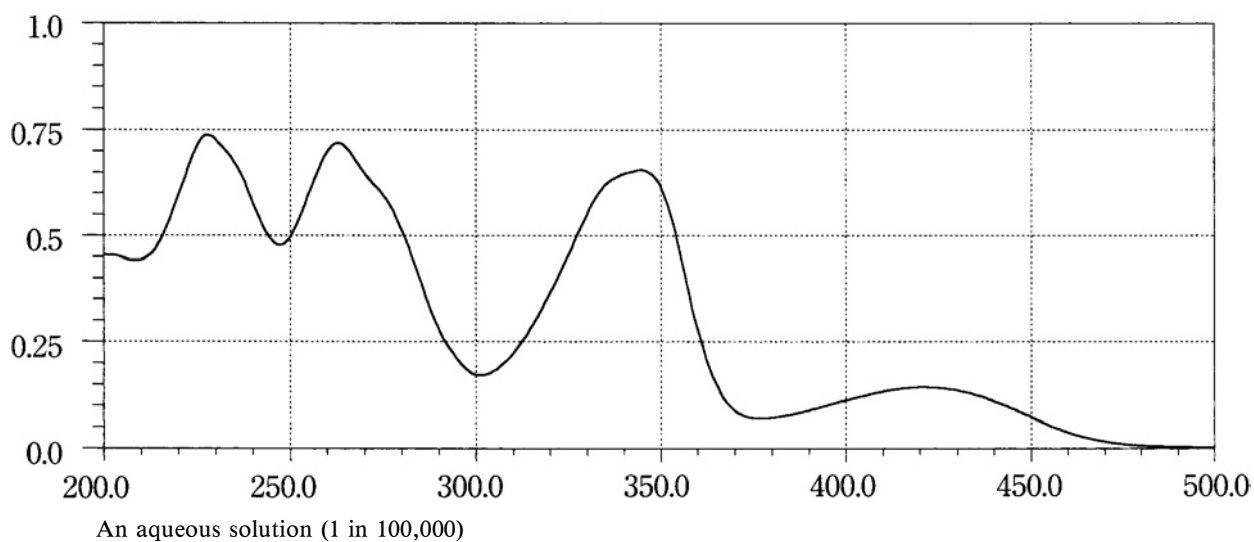
A solution in 0.1 mol/L hydrochloric acid (1 in 2000)

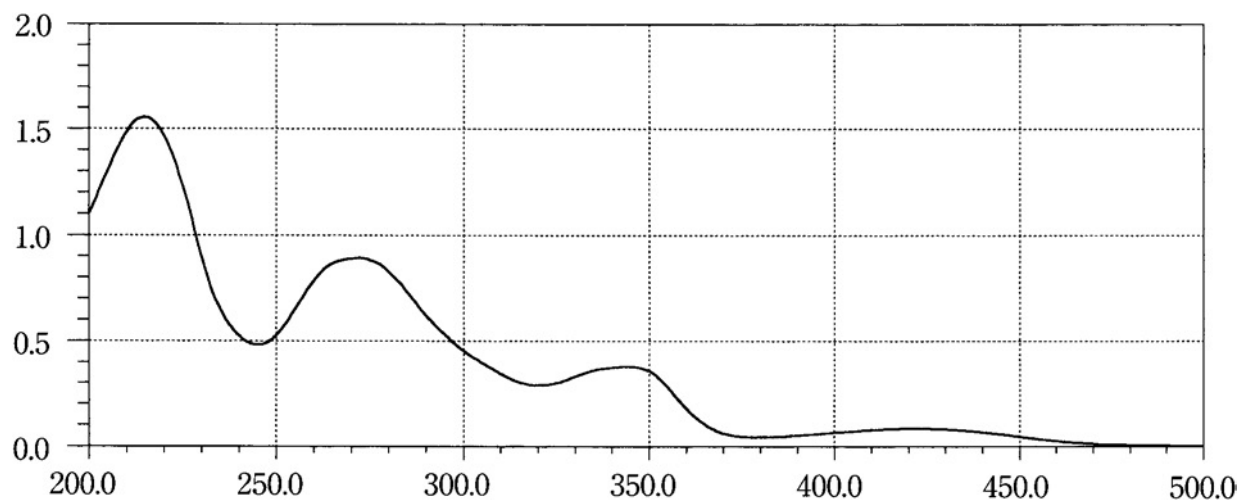
Benzbromarone

A solution in 0.01 mol/L sodium hydroxide TS (1 in 100,000)

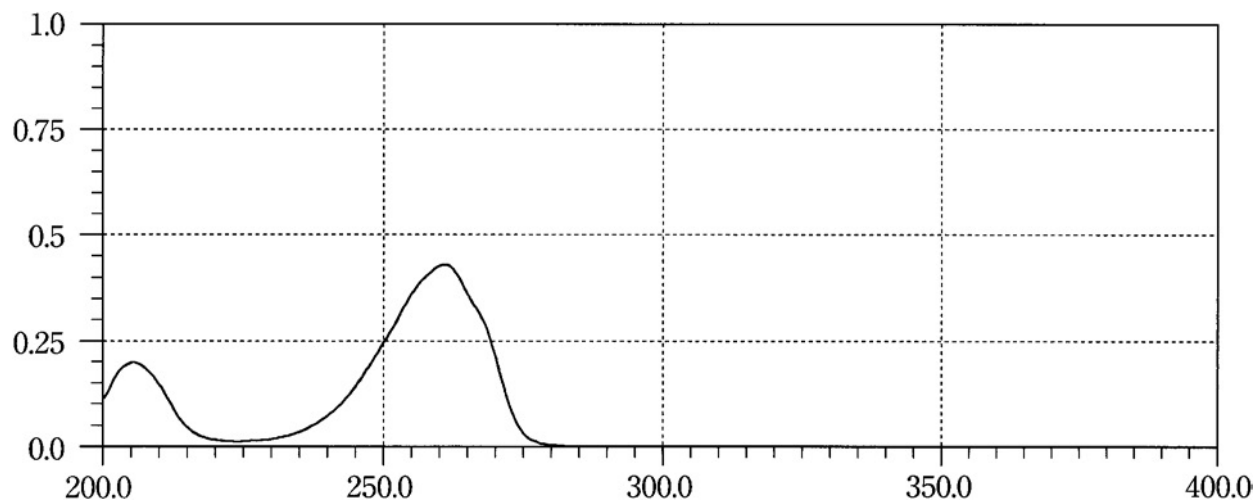
Benzethonium Chloride

A solution in 0.1 mol/L hydrochloric acid TS (1 in 5000)

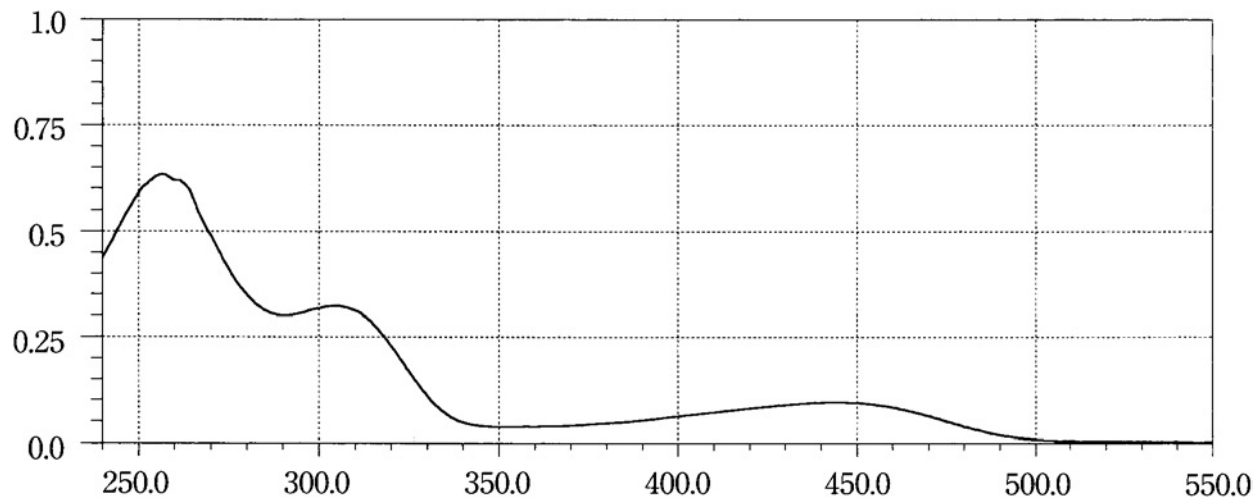
Benzylpenicillin Benzathine Hydrate**Benzylpenicillin Potassium****Berberine Chloride Hydrate**

Berberine Tannate

A solution prepared as follows: Dissolve 0.01 g in 10 mL of methanol and 0.4 mL of 1 mol/L hydrochloric acid TS, and add water to make 200 mL. To 8 mL of this solution add water to make 25 mL.

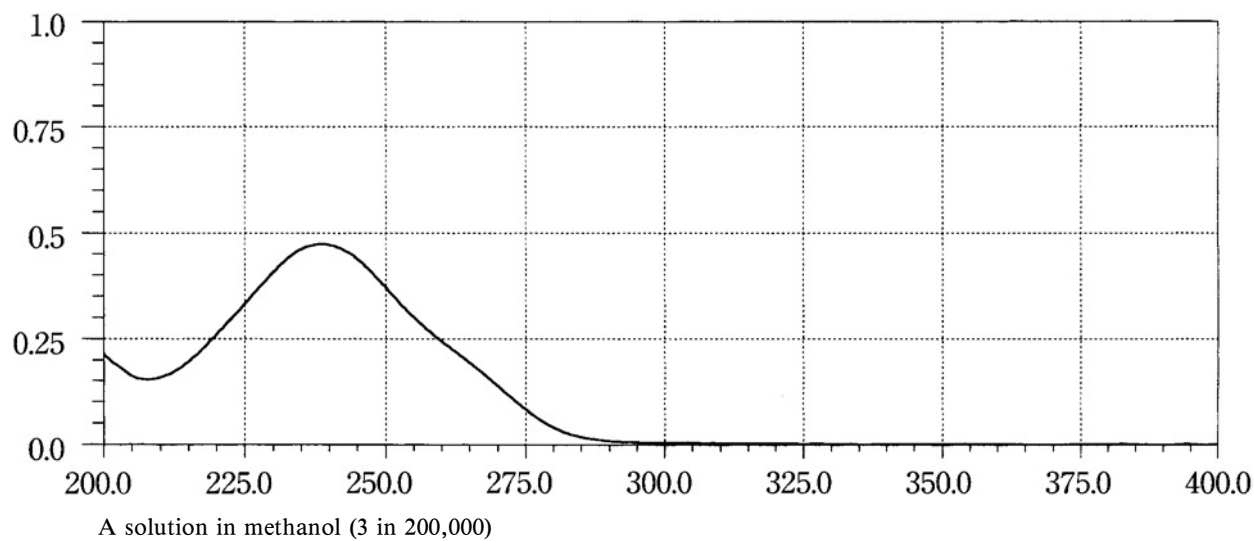
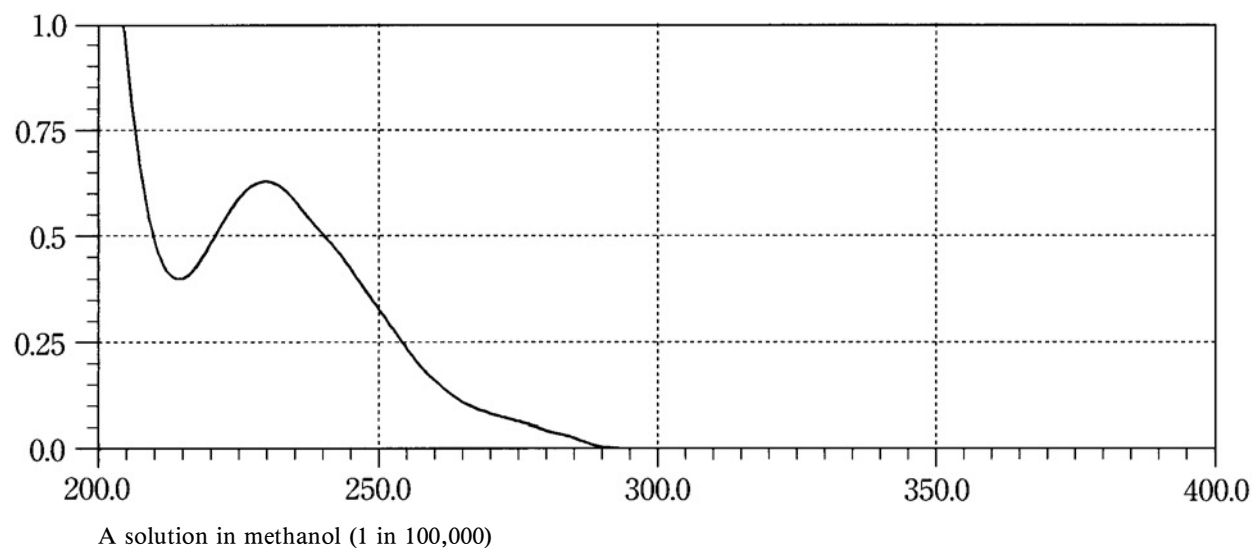
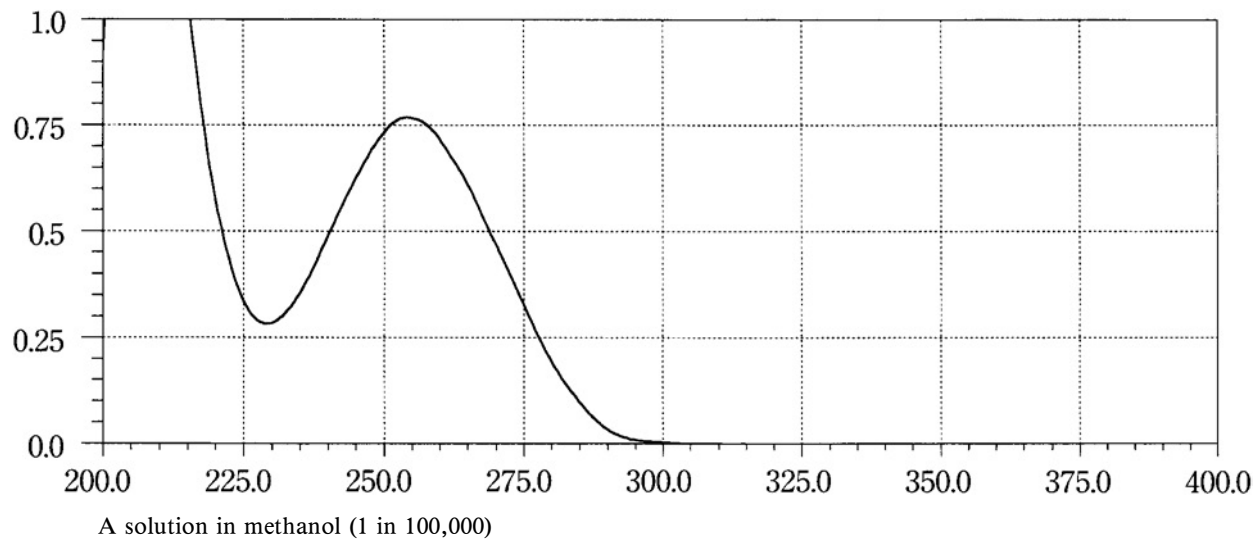
Betahistine Mesilate

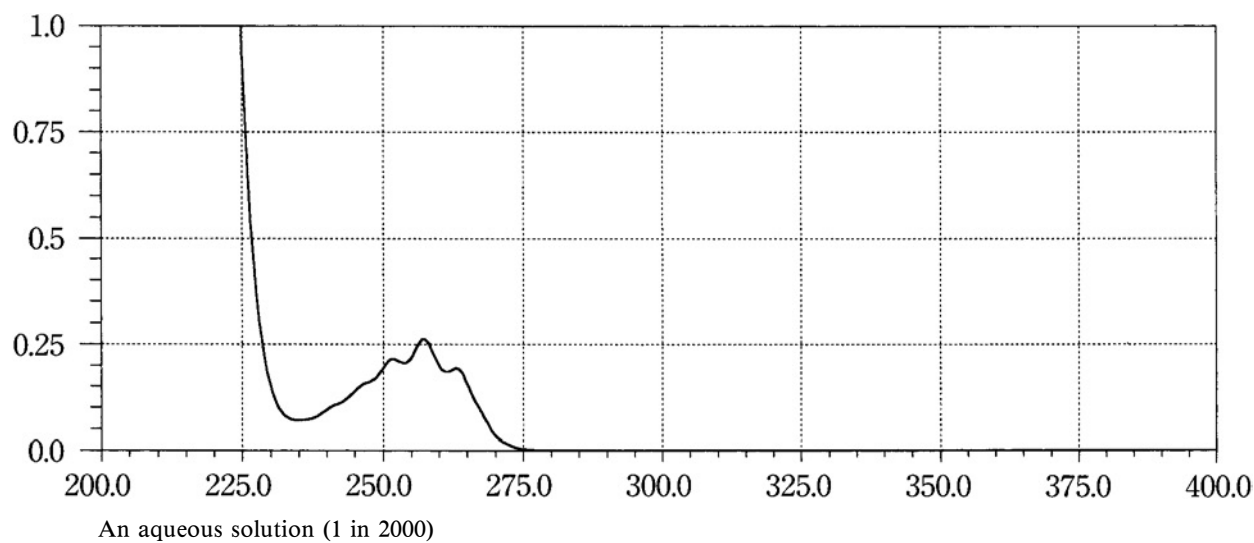
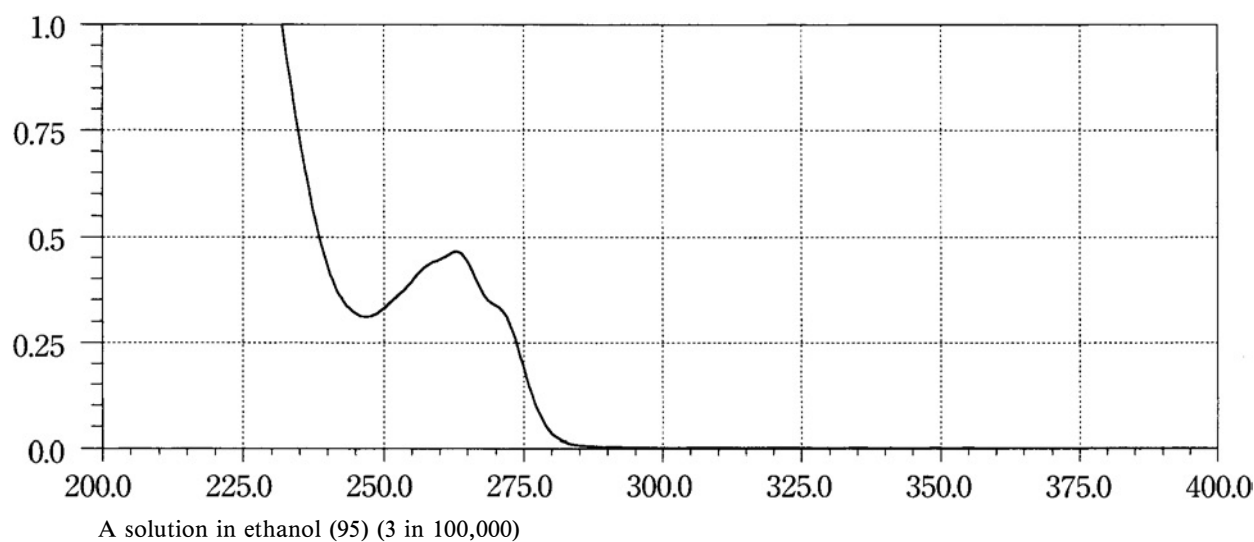
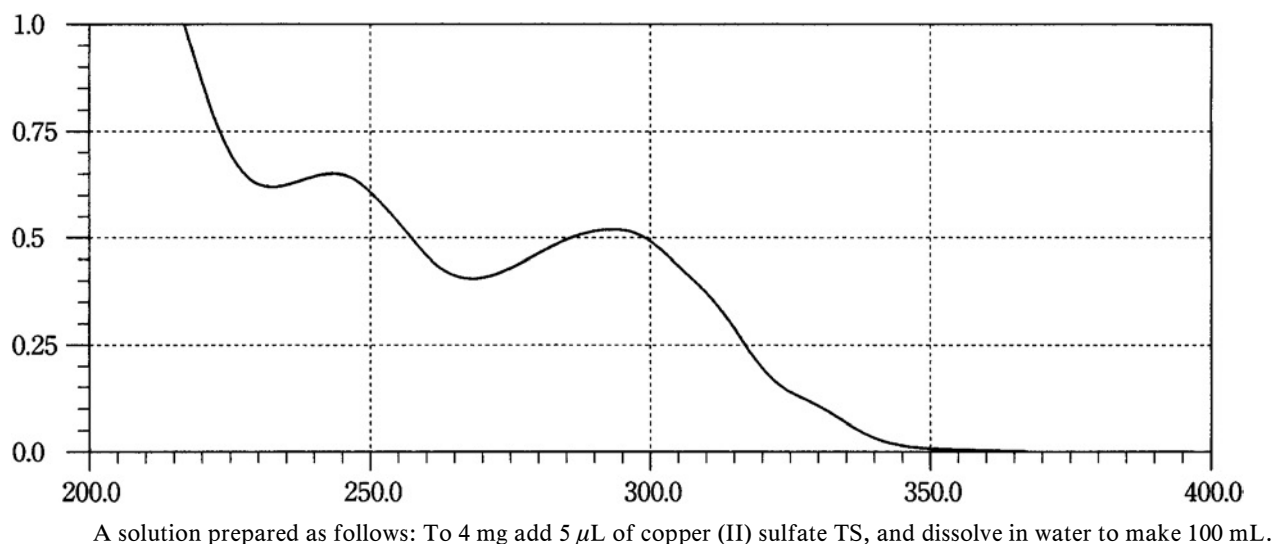
A solution in 0.1 mol/L hydrochloric acid TS (1 in 50,000)

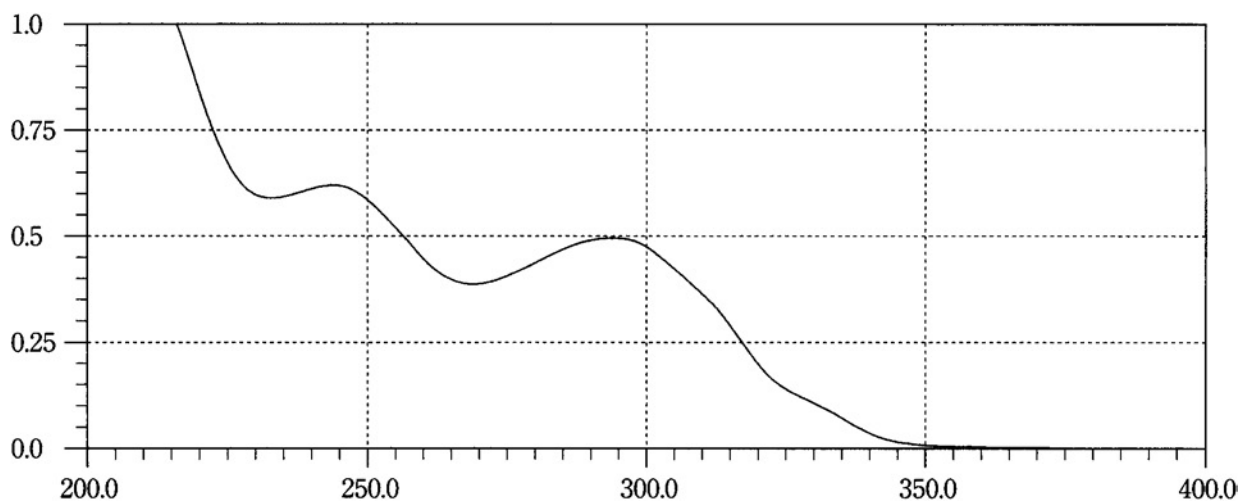
Betamethasone

A solution prepared as follows: Dissolve 1.0 mg in 10 mL of ethanol (95). To 2 mL of this solution, add 10 mL of phenylhydrazine hydrochloride TS, shake, heat in a water bath at 60°C for 20 minutes, and allow it to cool.

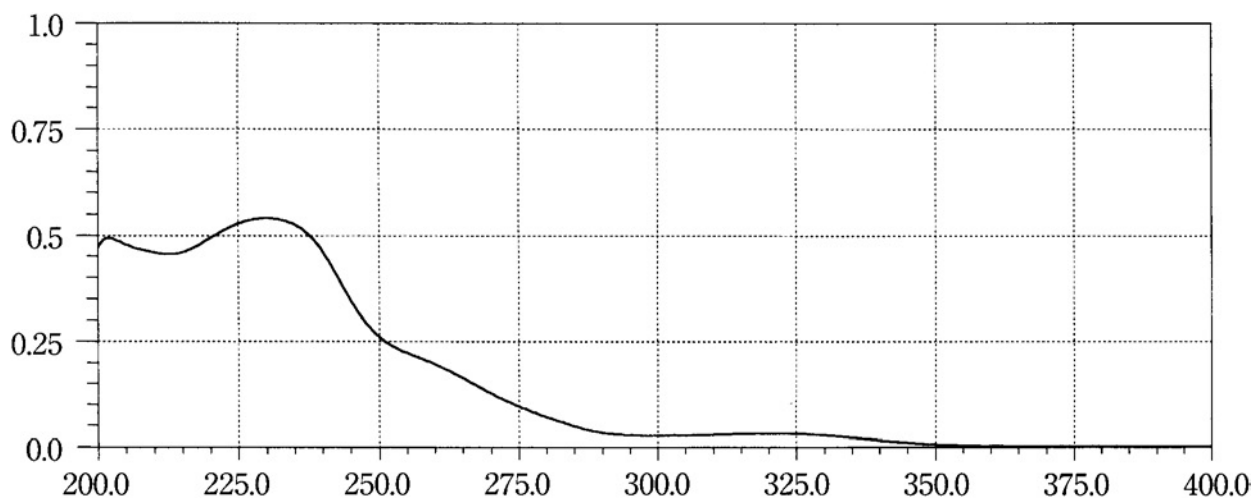
Blank: A solution obtained by proceeding in the same manner with 2.0 mL of ethanol (95).

Betamethasone Dipropionate**Bezafibrate****Bifonazole**

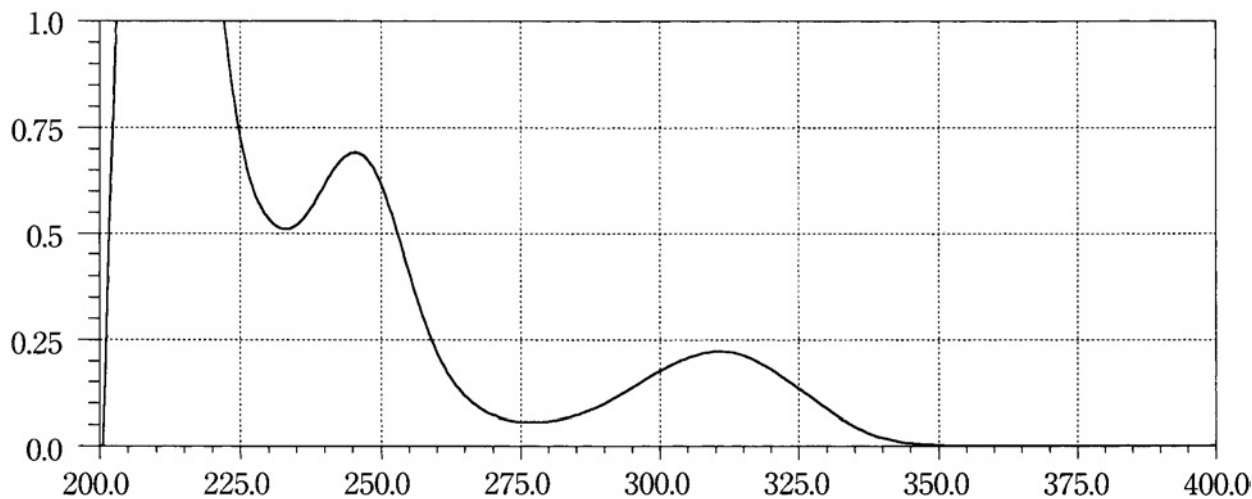
Biperiden Hydrochloride**Bisacodyl****Bleomycin Hydrochloride**

Bleomycin Sulfate

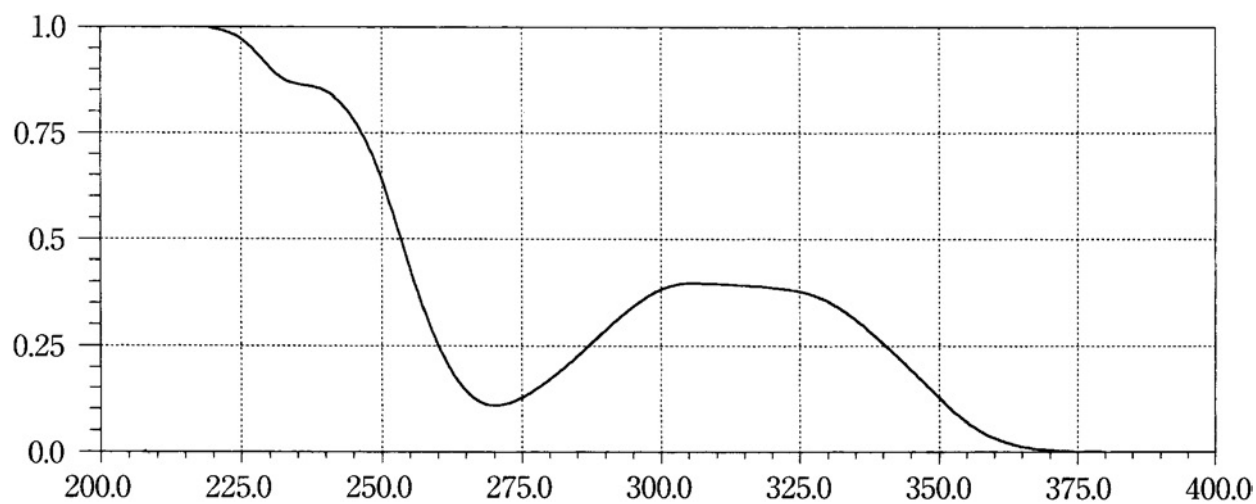
A solution prepared as follows: To 4 mg add 5 μ L of copper (II) sulfate TS, and dissolve in water to make 100 mL.

Bromazepam

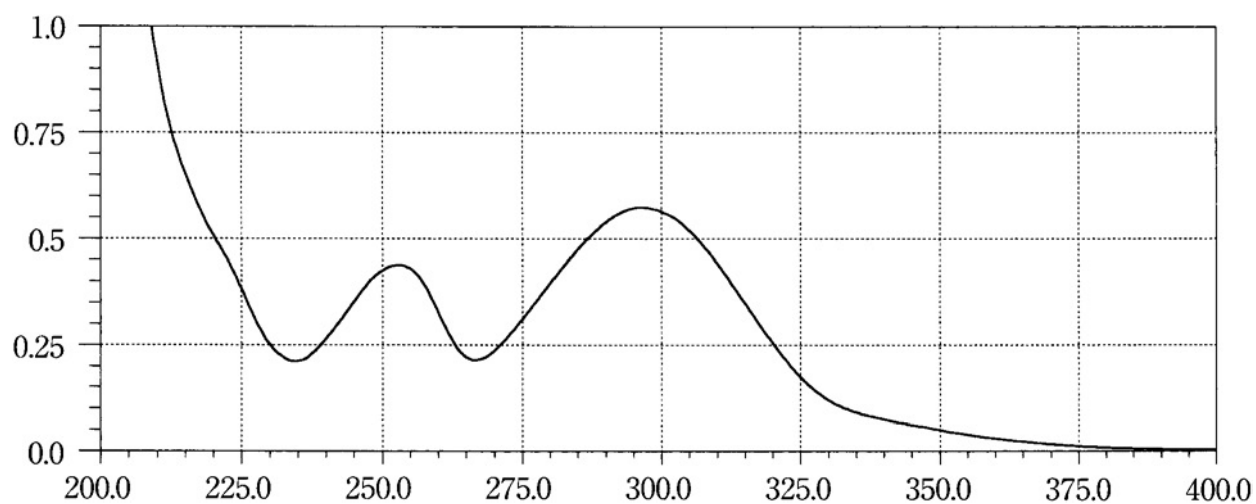
A solution in ethanol (99.5) (1 in 200,000)

Bromhexine Hydrochloride

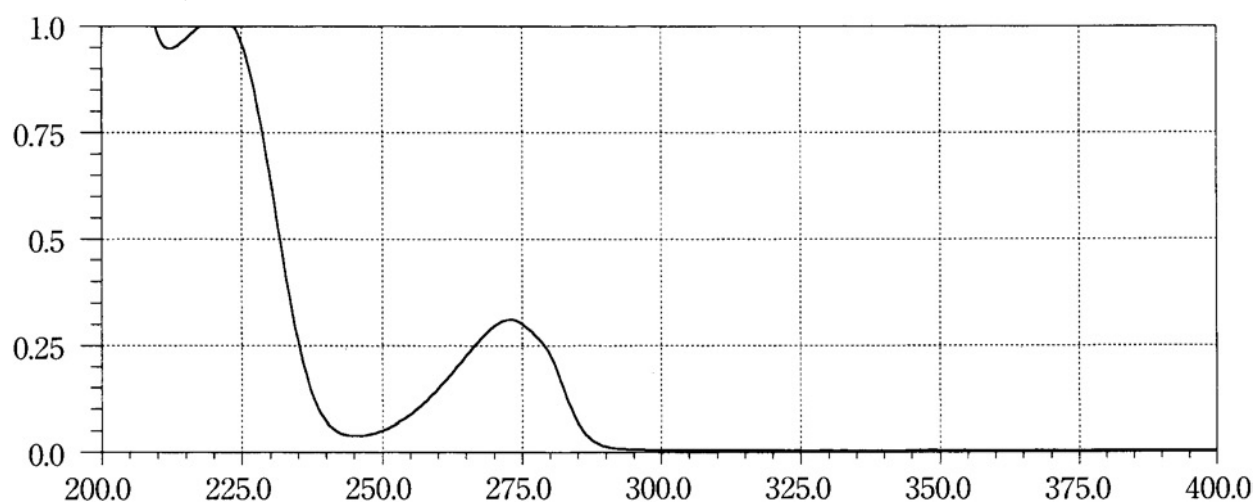
A solution in 0.01 mol/L hydrochloric acid TS (3 in 100,000)

Bromocriptine Mesilate

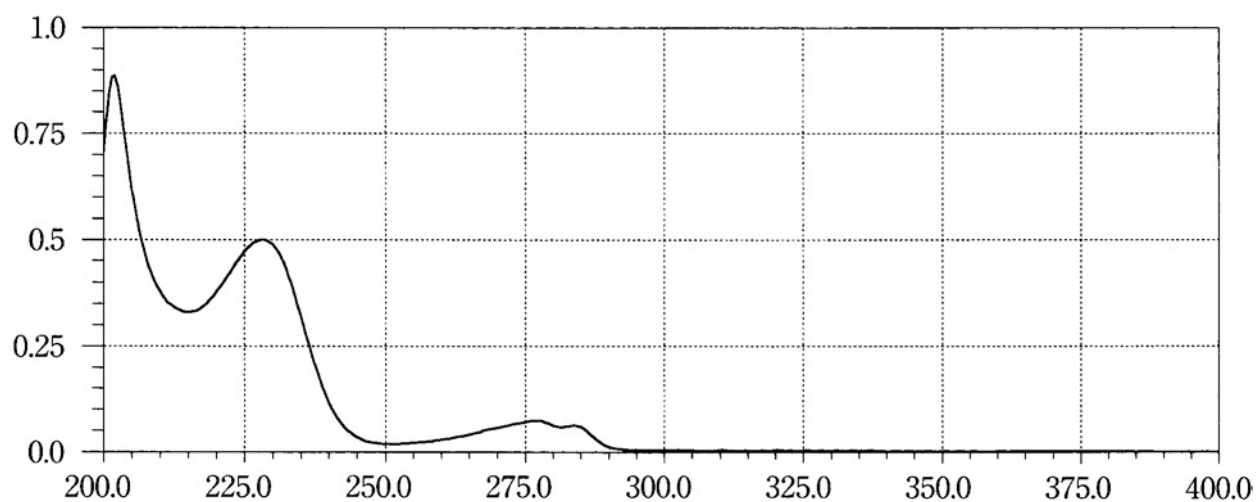
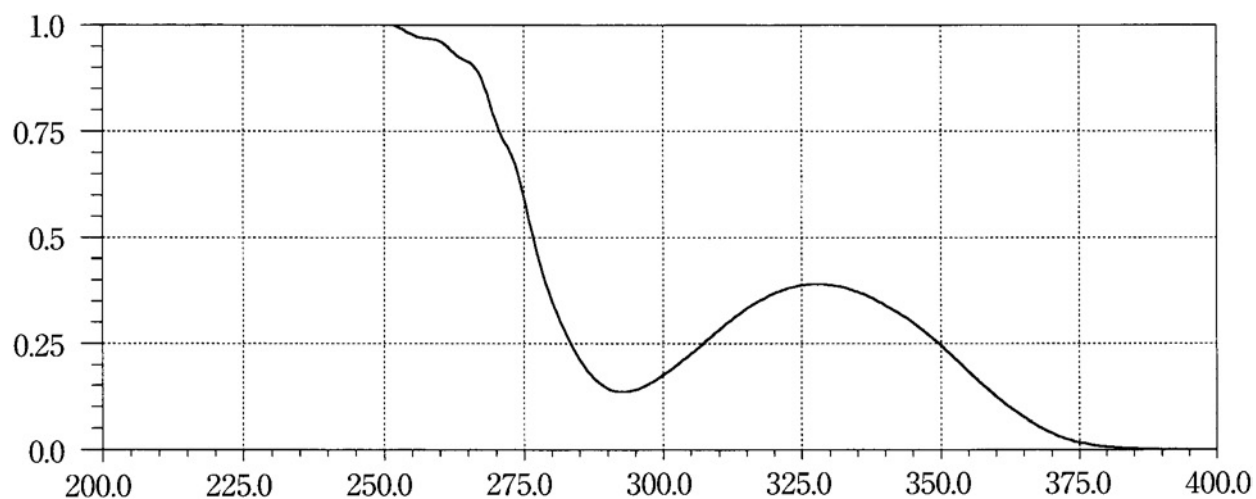
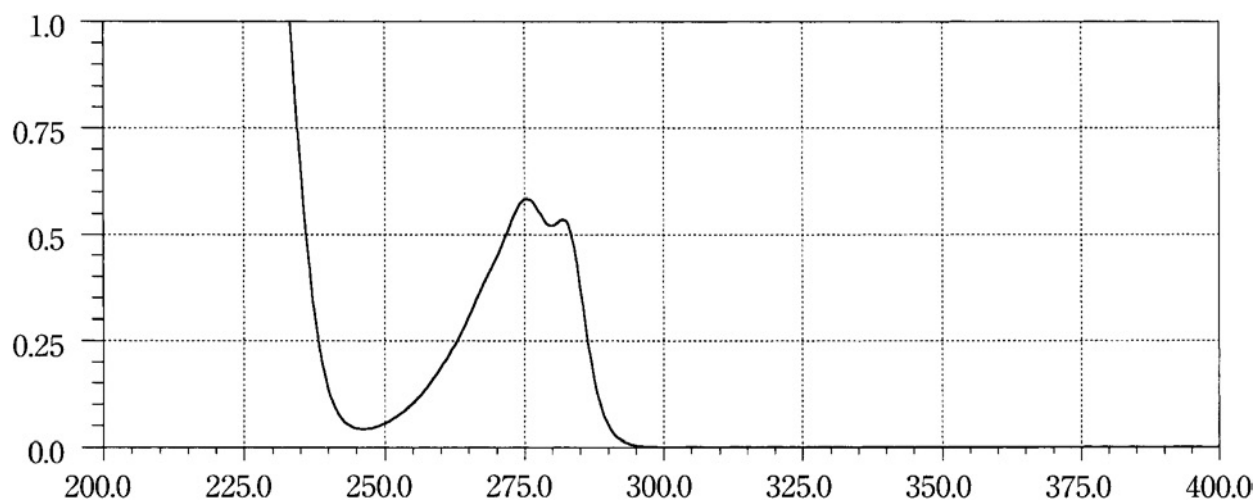
A solution in methanol (3 in 100,000)

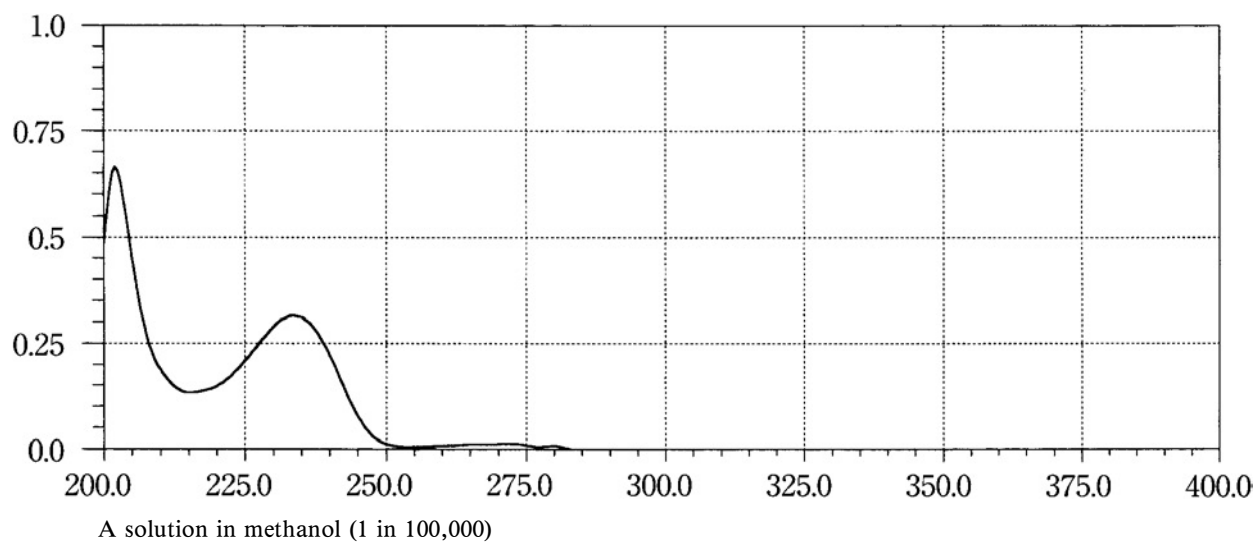
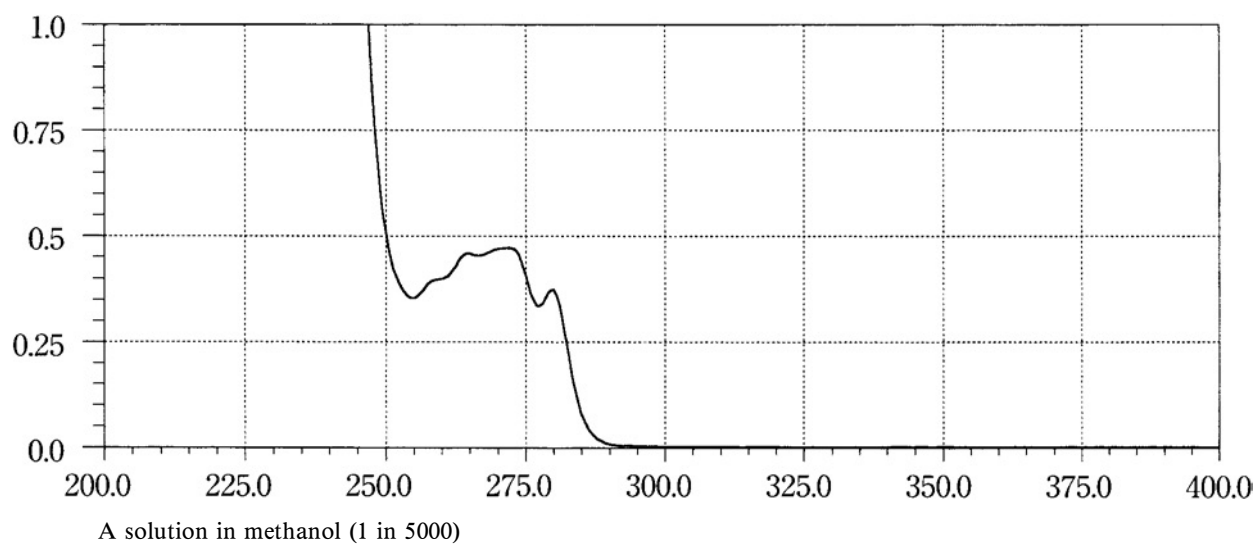
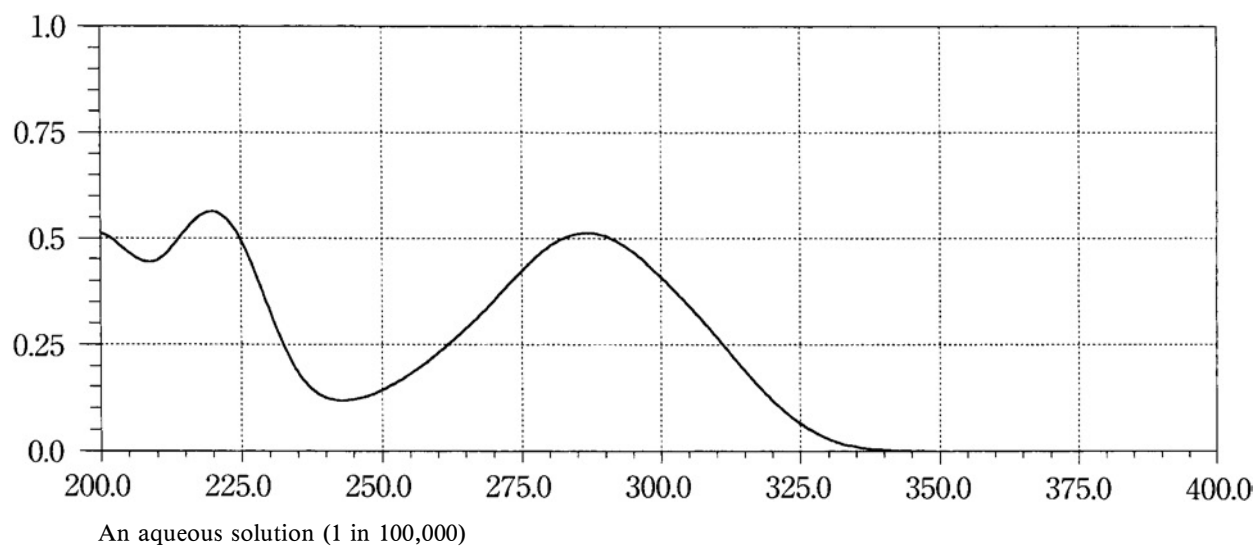
Bucumolol Hydrochloride

An aqueous solution (1 in 60,000)

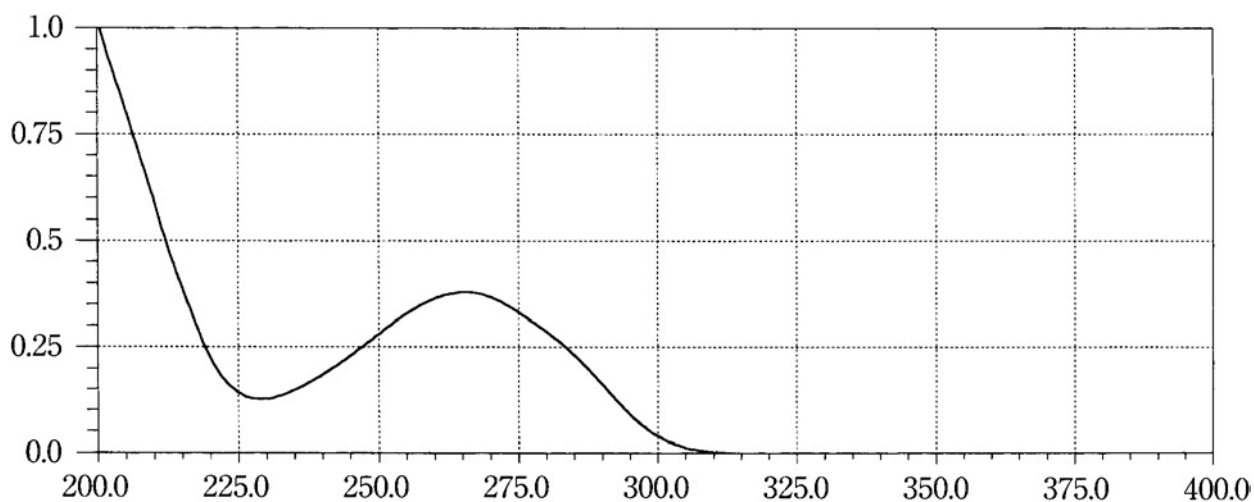
Bufetolol Hydrochloride

An aqueous solution (1 in 20,000)

Bufexamac**Bumetanide****Bupranolol Hydrochloride**

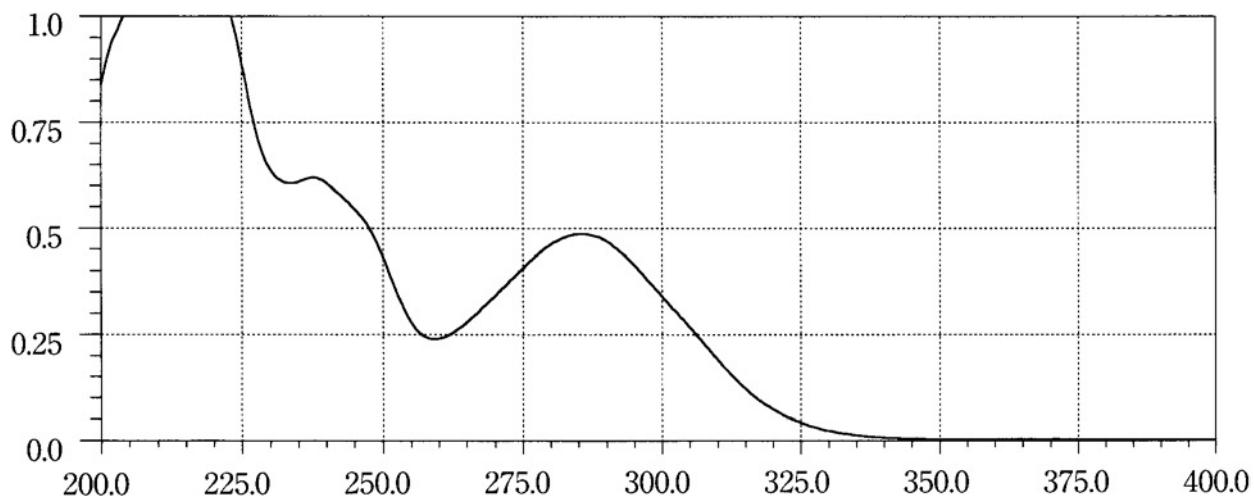
Butropium Bromide 1**Butropium Bromide 2****Calcium Folate**

Camostat Mesilate



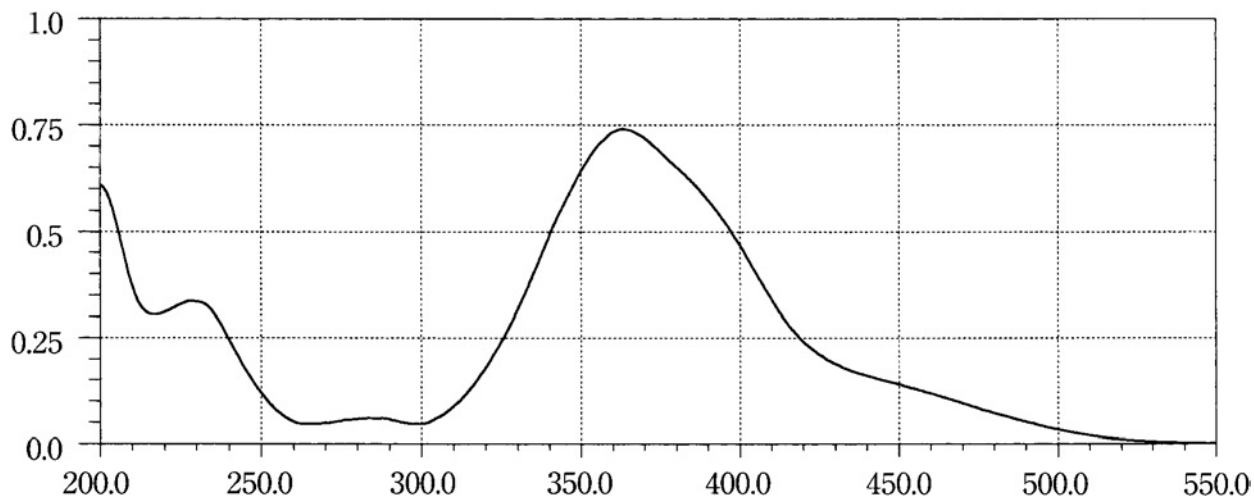
An aqueous solution (1 in 100,000)

Carbamazepine

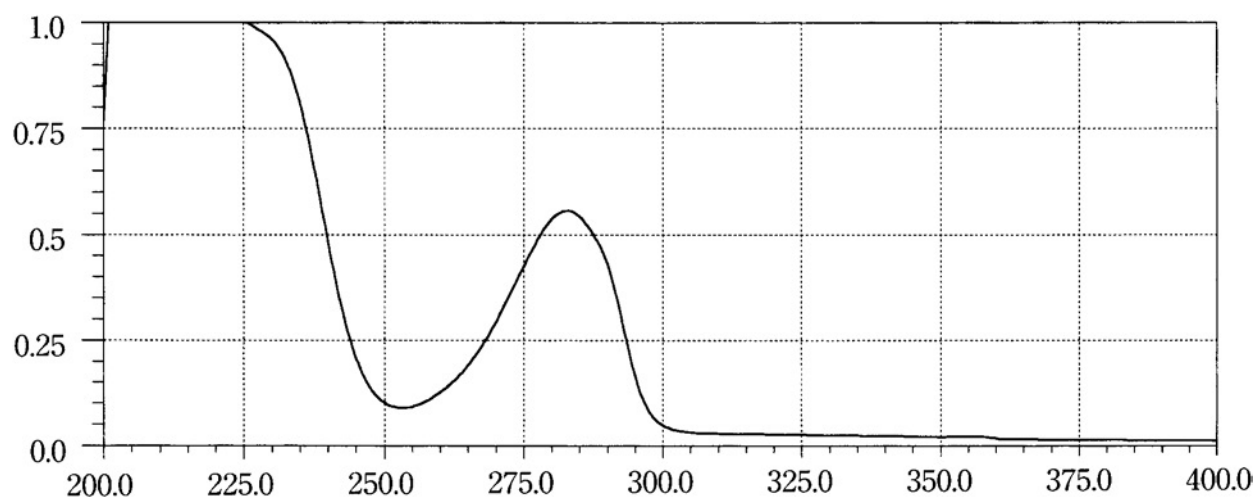


The sample solution obtained in the Assay

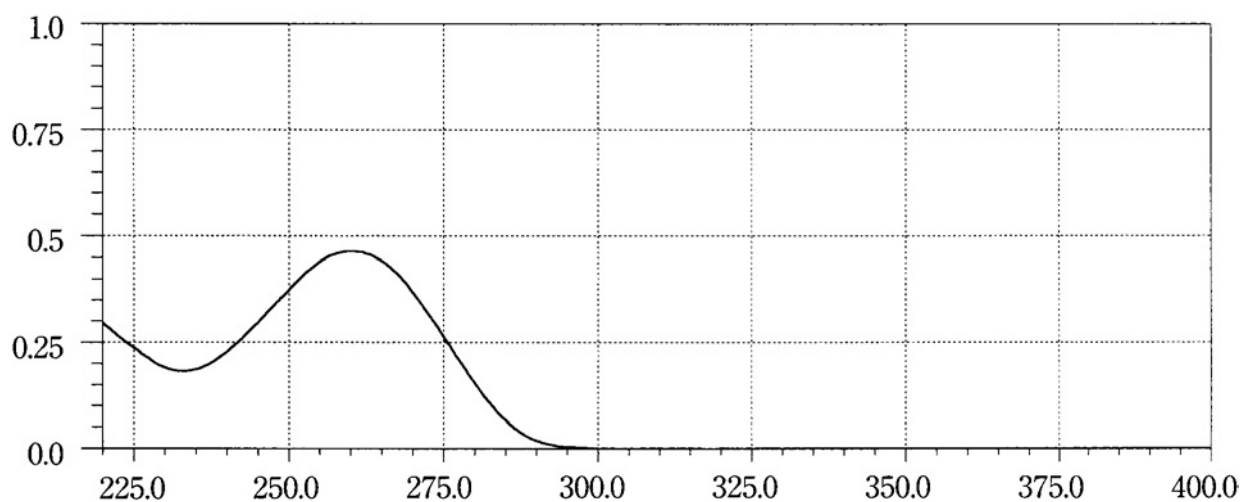
Carbazochrome Sodium Sulfonate Hydrate



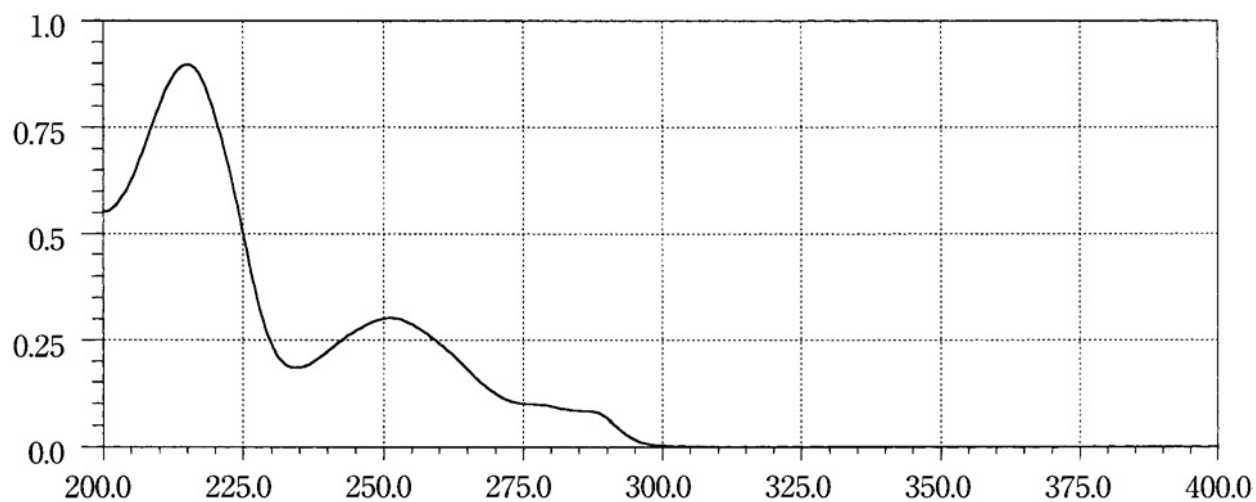
An aqueous solution (1 in 100,000)

Carbidopa Hydrate

A solution prepared as follows: Dissolve 0.01 g in 250 mL of a solution of hydrochloric acid in methanol (9 in 1000)

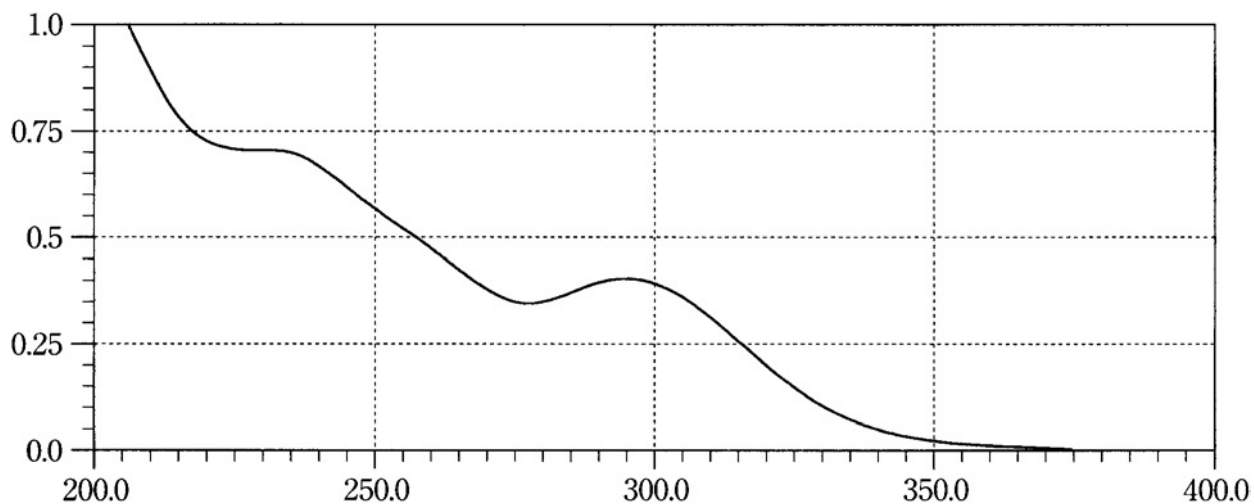
Carmofur

A solution in a mixture of methanol and phosphoric acid-acetic acid-boric acid buffer solution, pH 2.0 (9:1) (1 in 100,000)

Carteolol Hydrochloride

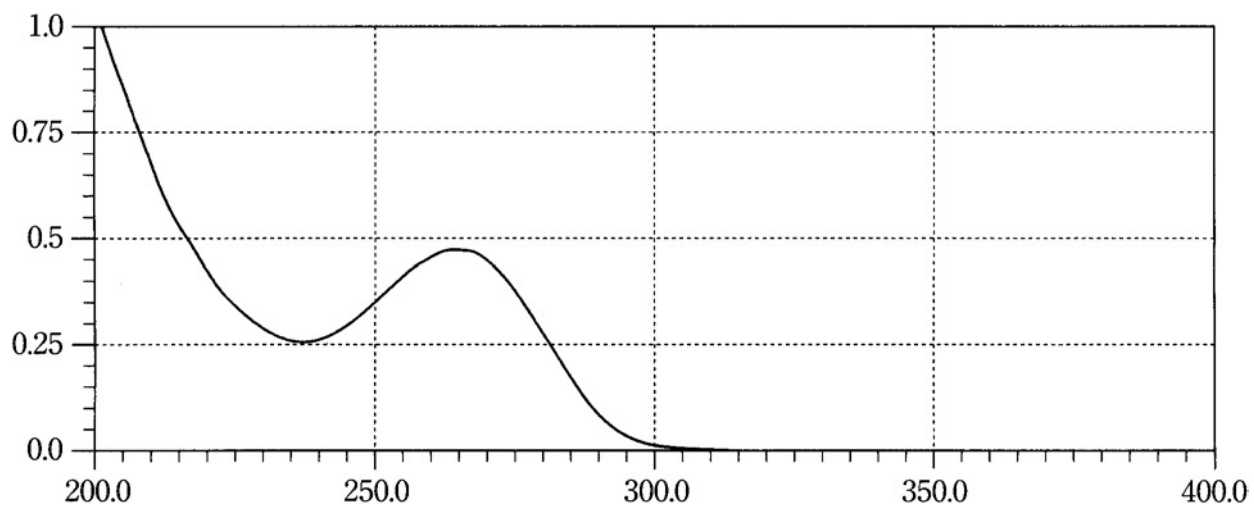
An aqueous solution (1 in 100,000)

Carumonam Sodium



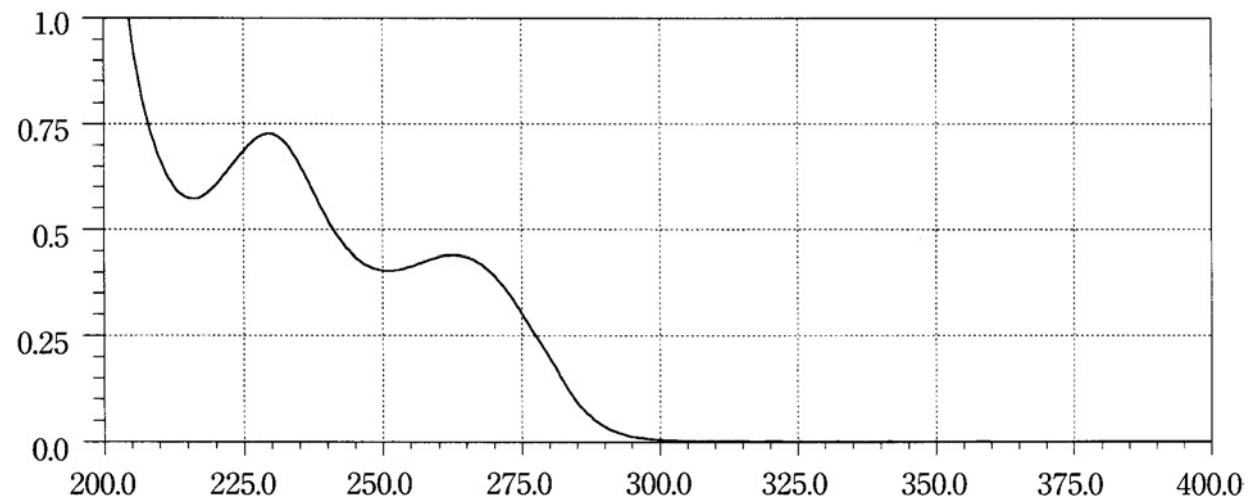
An aqueous solution (3 in 100,000)

Cefaclor

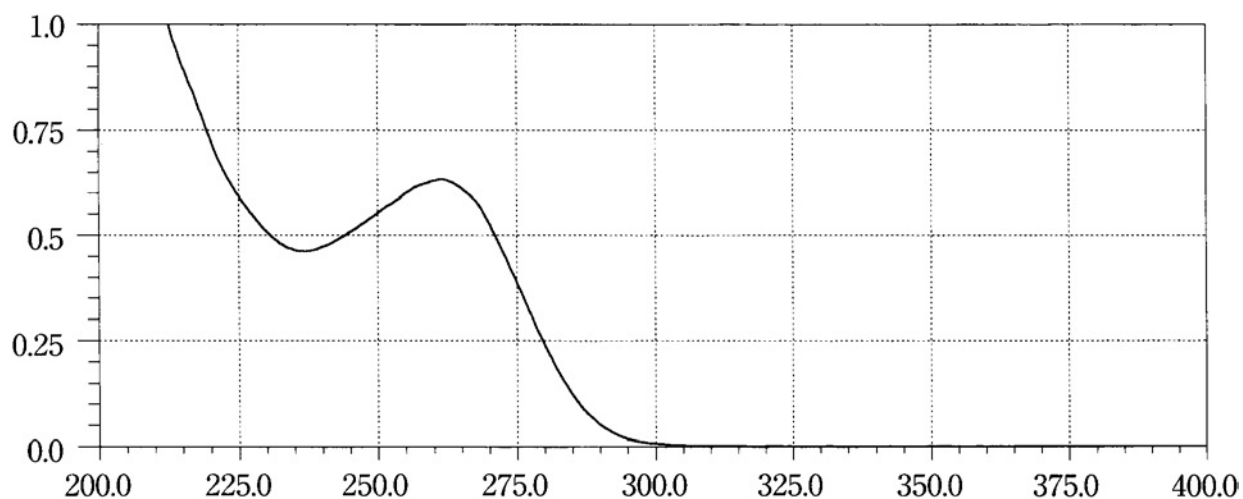


An aqueous solution (1 in 50,000)

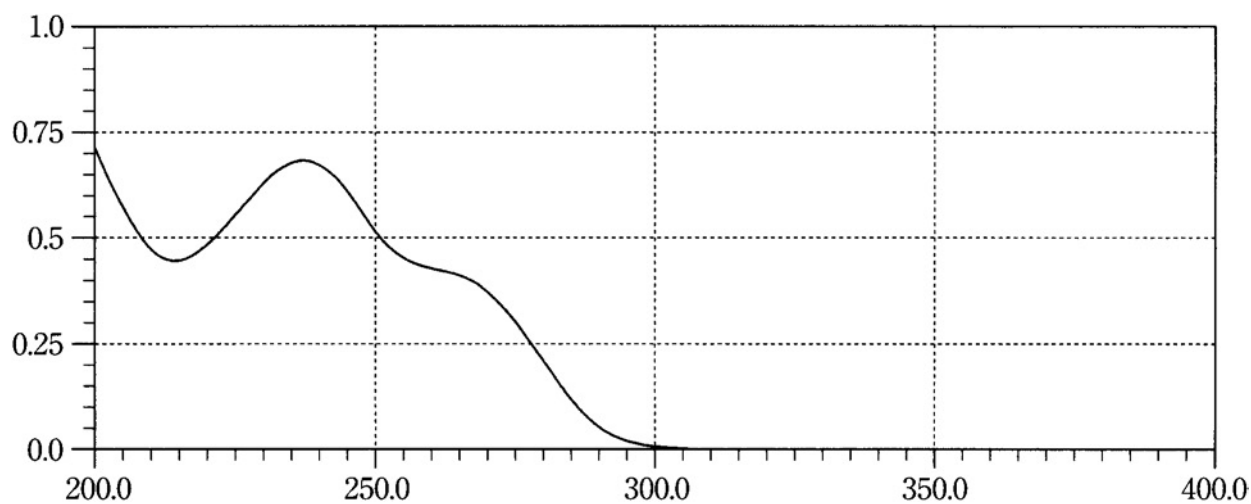
Cefadroxil



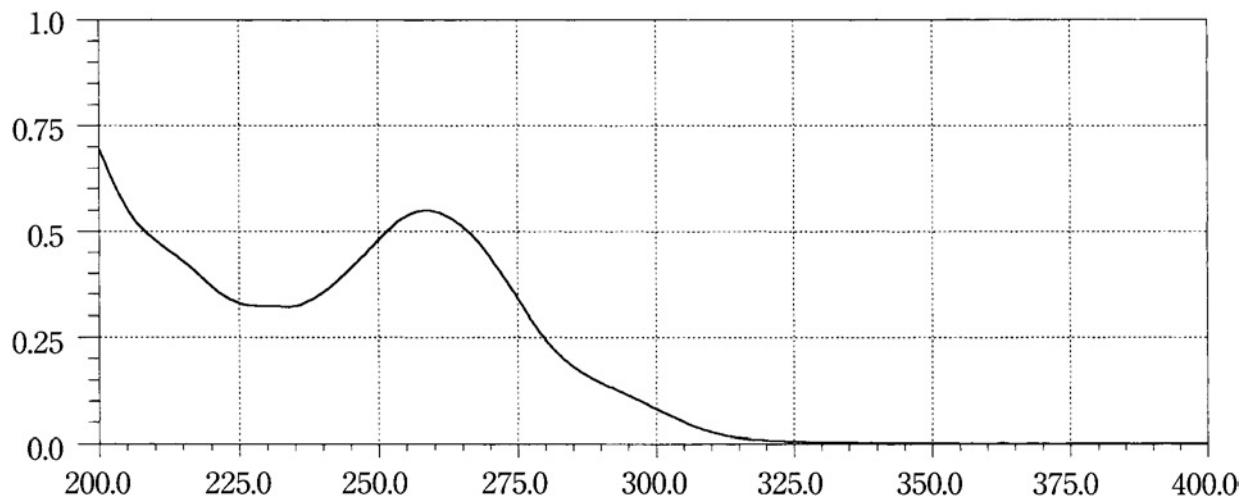
An aqueous solution (1 in 50,000)

Cefalexin

An aqueous solution (3 in 100,000)

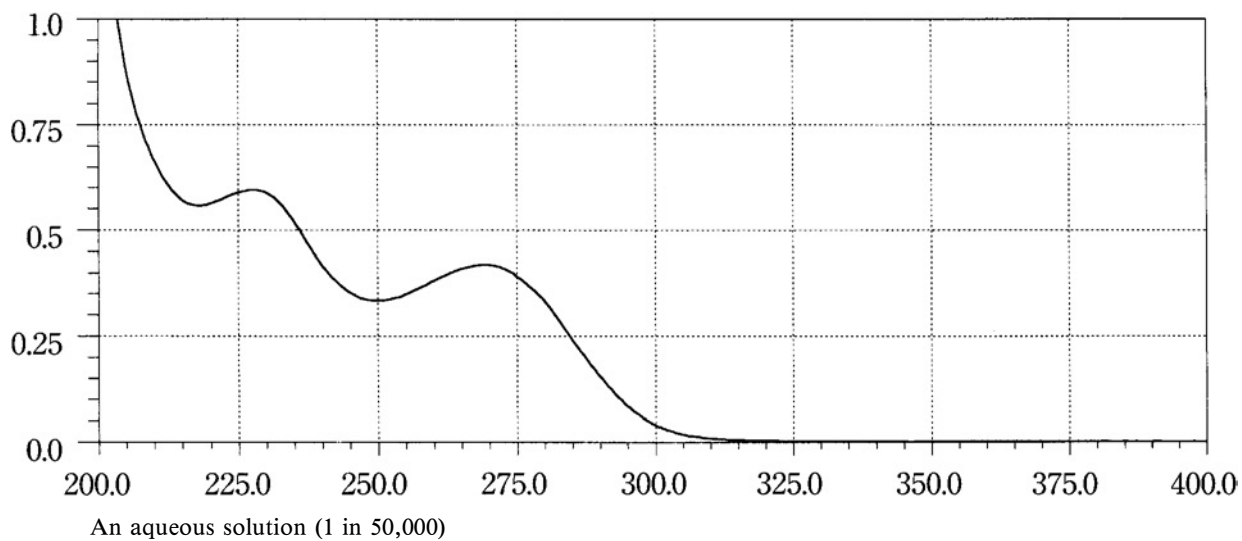
Cefalotin Sodium

An aqueous solution (1 in 50,000)

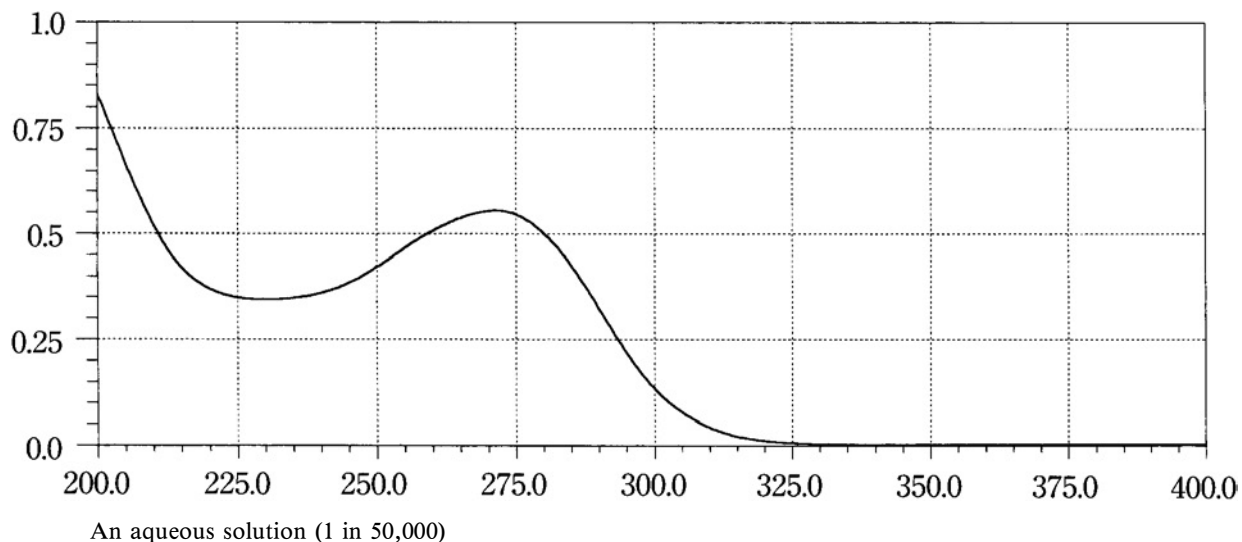
Cefapirin Sodium

An aqueous solution (3 in 200,000)

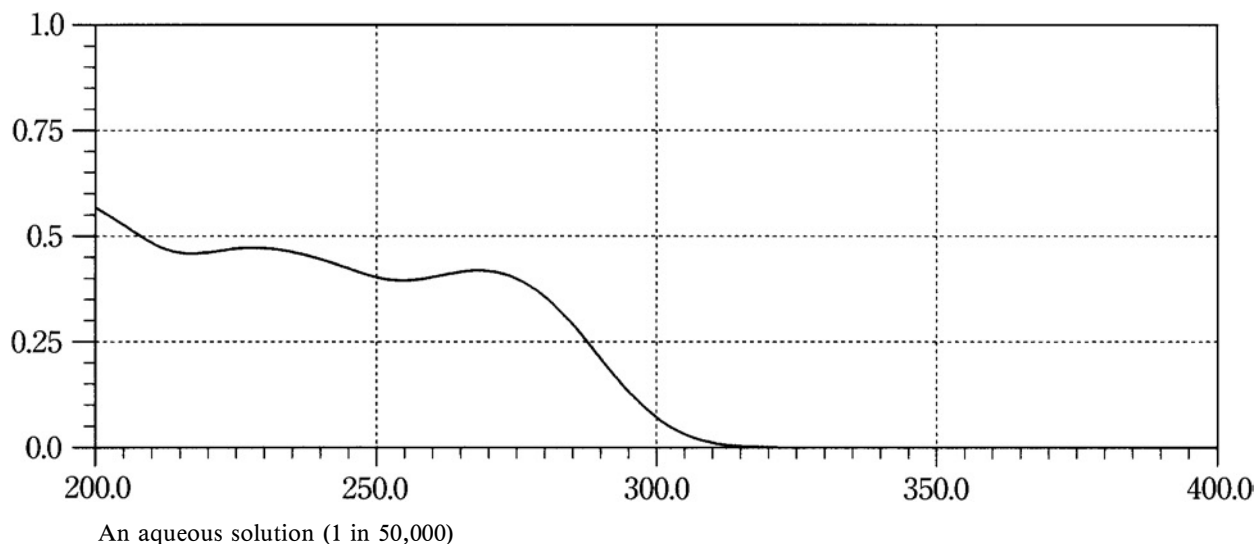
Cefatrizine Propylene Glycolate

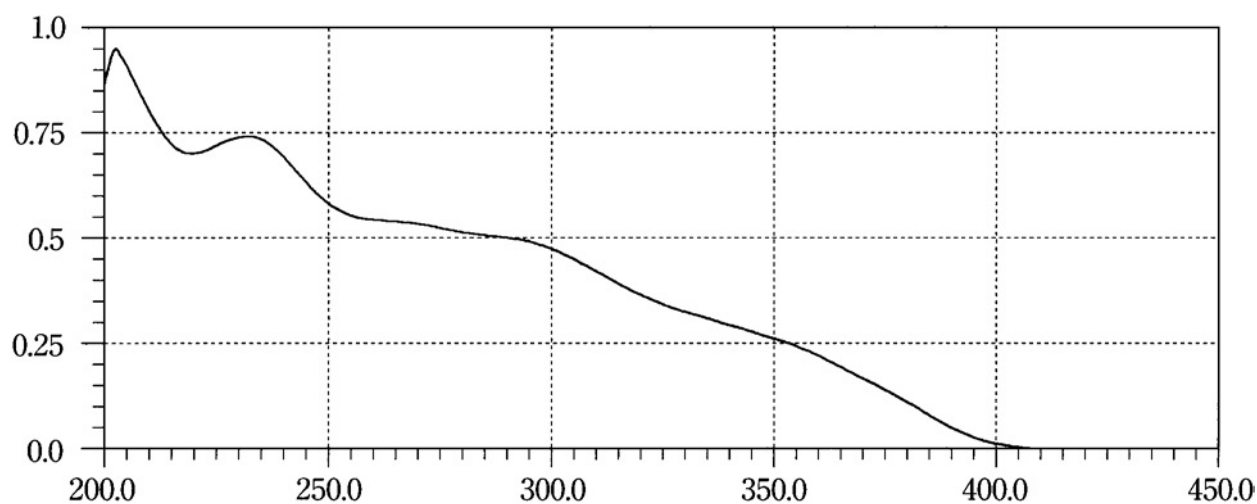
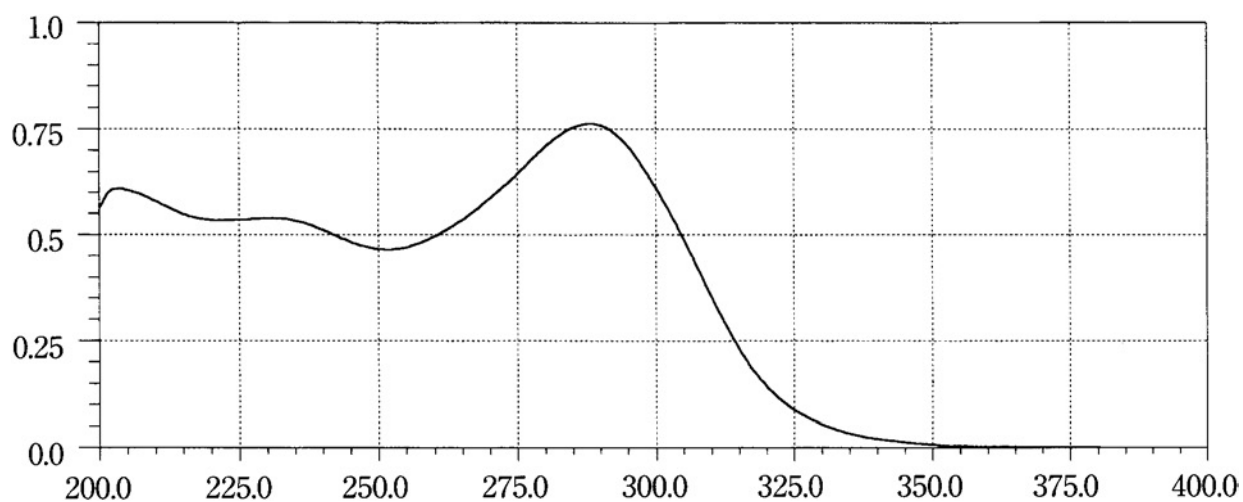
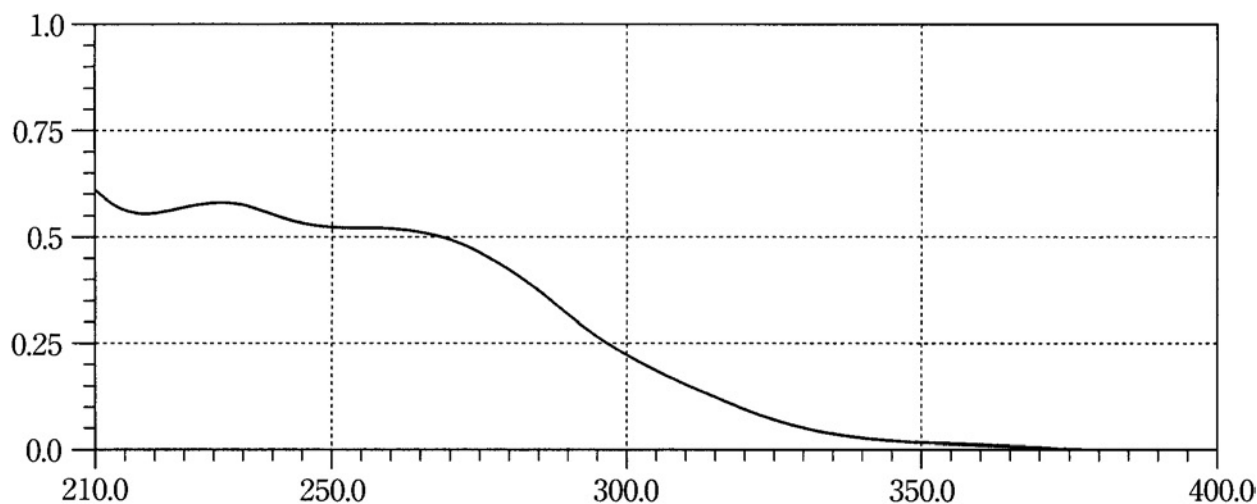


Cefazolin Sodium

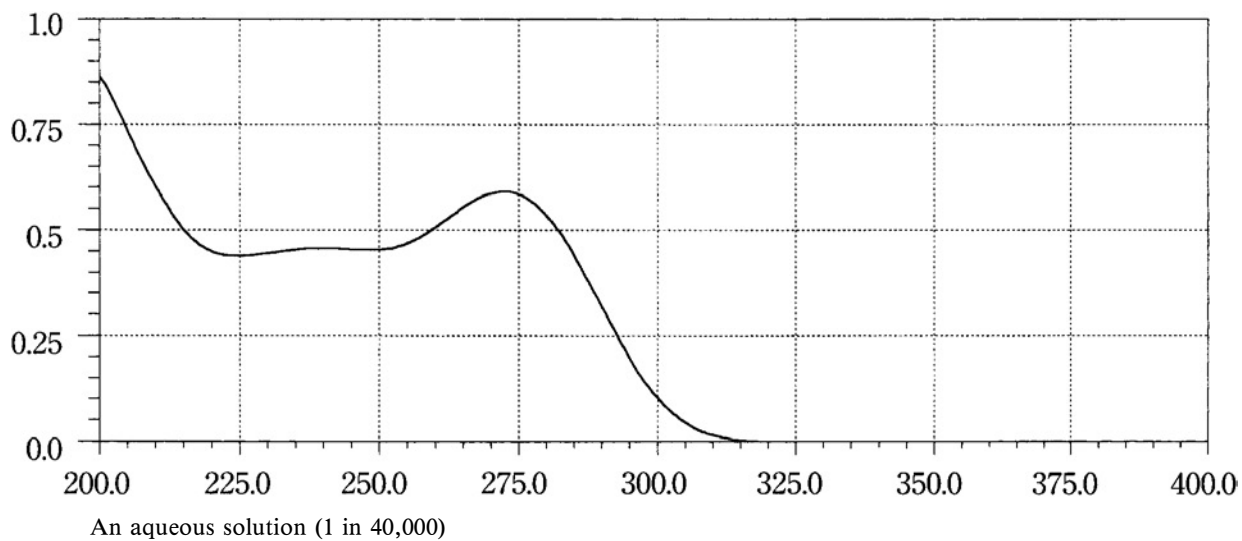


Cefbuperazone Sodium

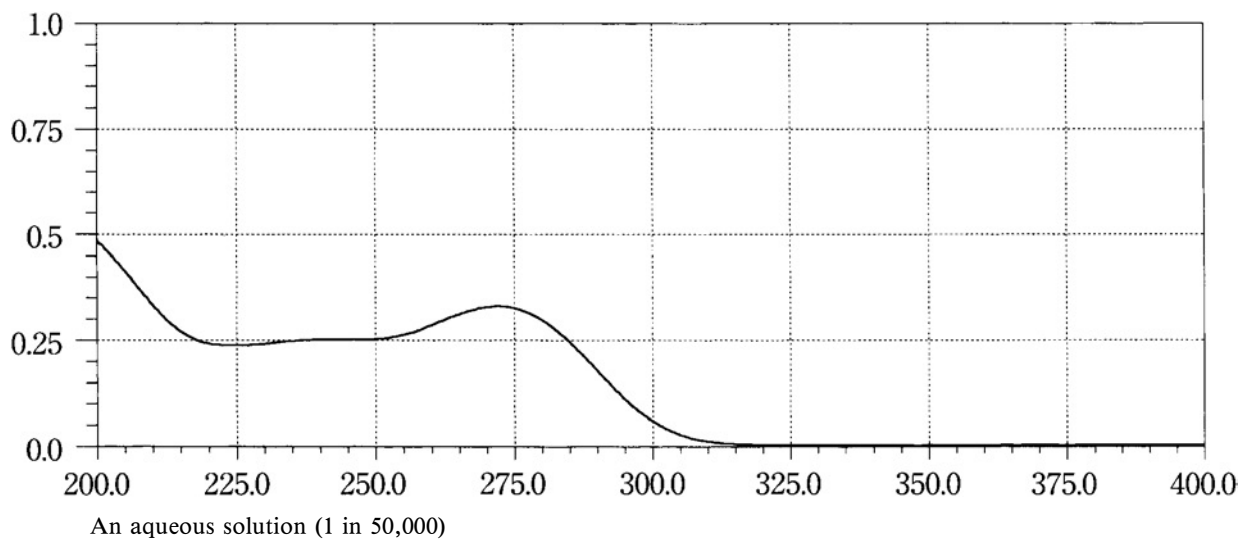


Cefditoren Pivoxil**Cefixime****Cefmenoxime Hydrochloride**

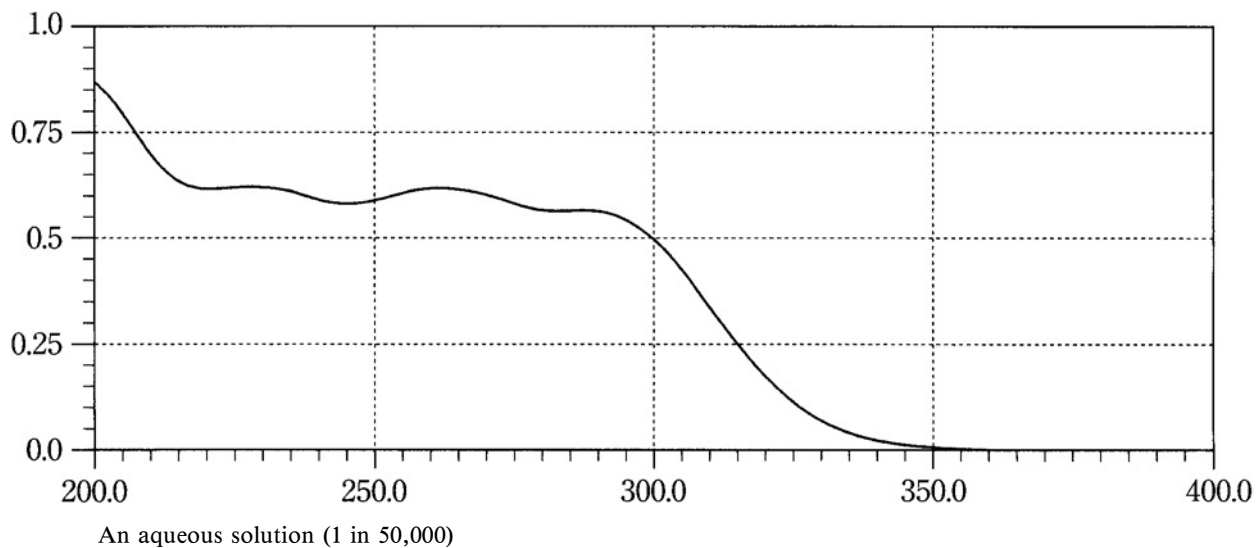
Cefmetazole Sodium

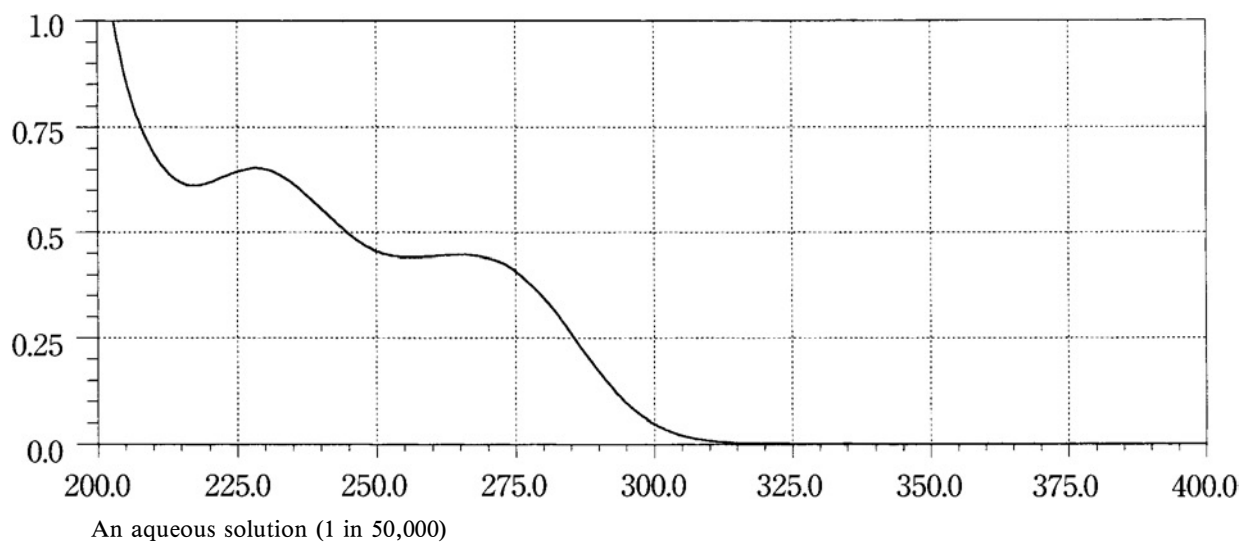
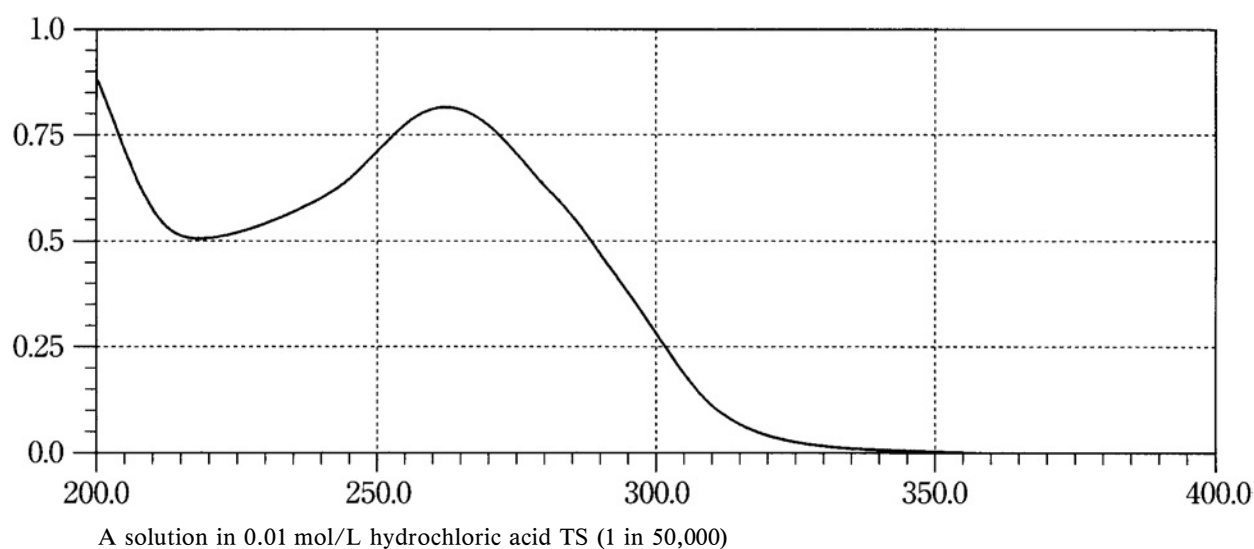
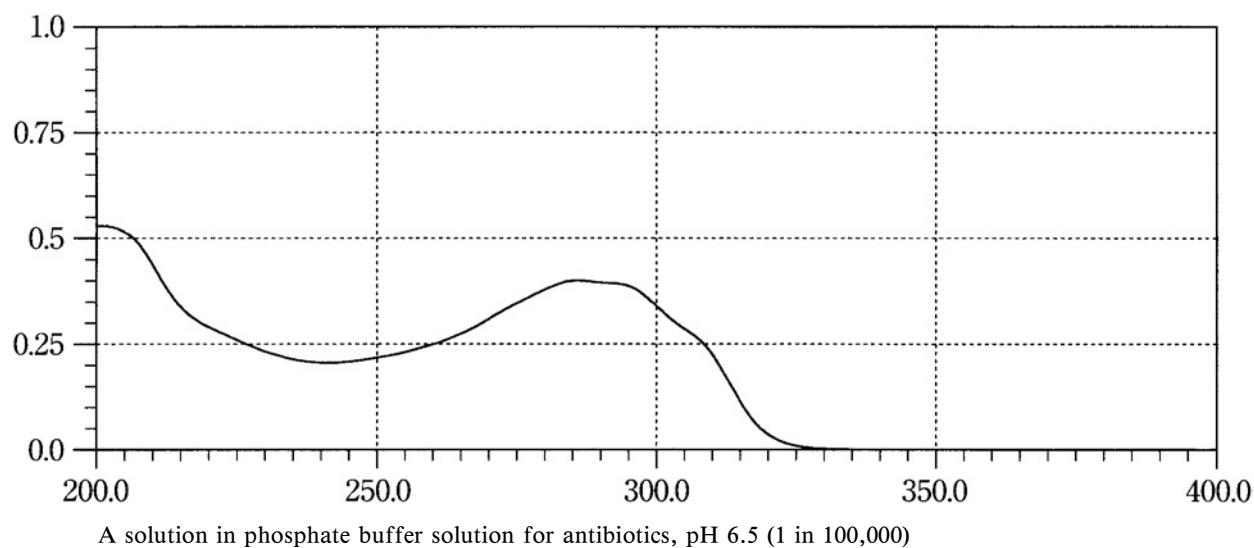


Cefminox Sodium Hydrate

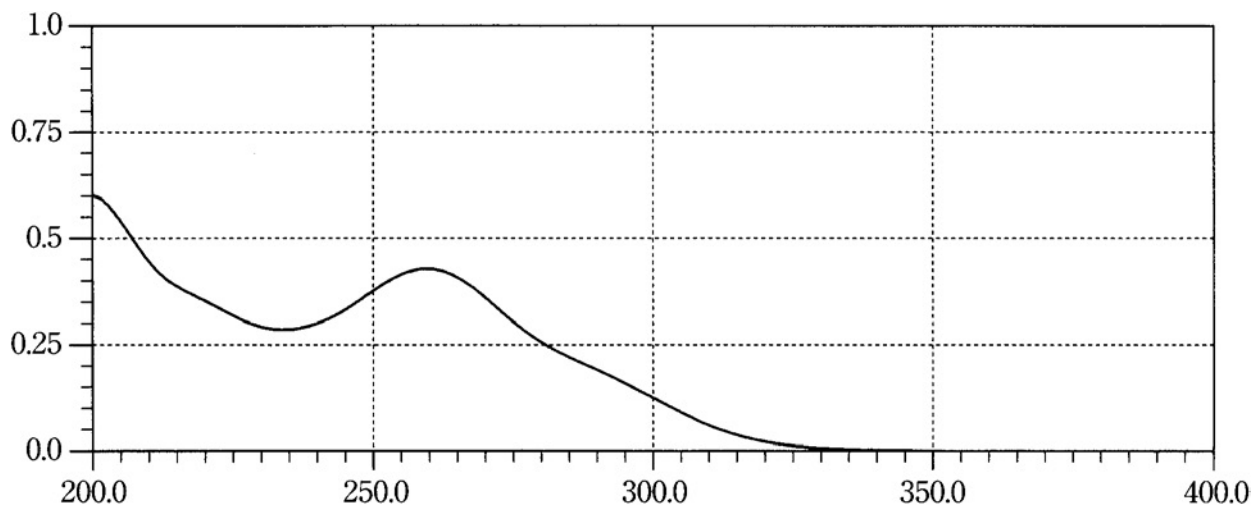


Cefodizime Sodium



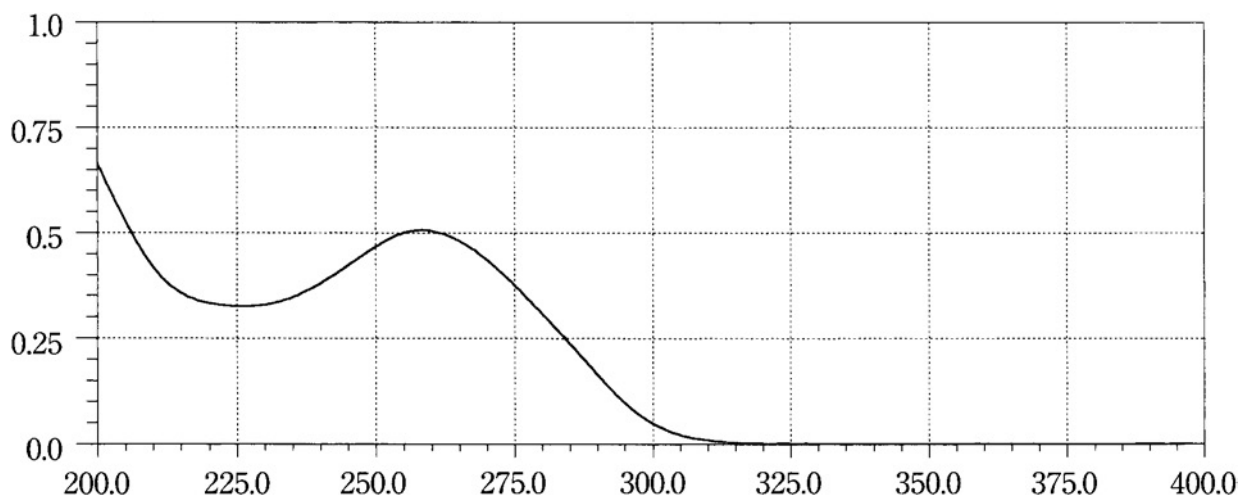
Cefoperazone Sodium**Cefotaxime Sodium****Cefotetan**

Cefotiam Hexetil Hydrochloride



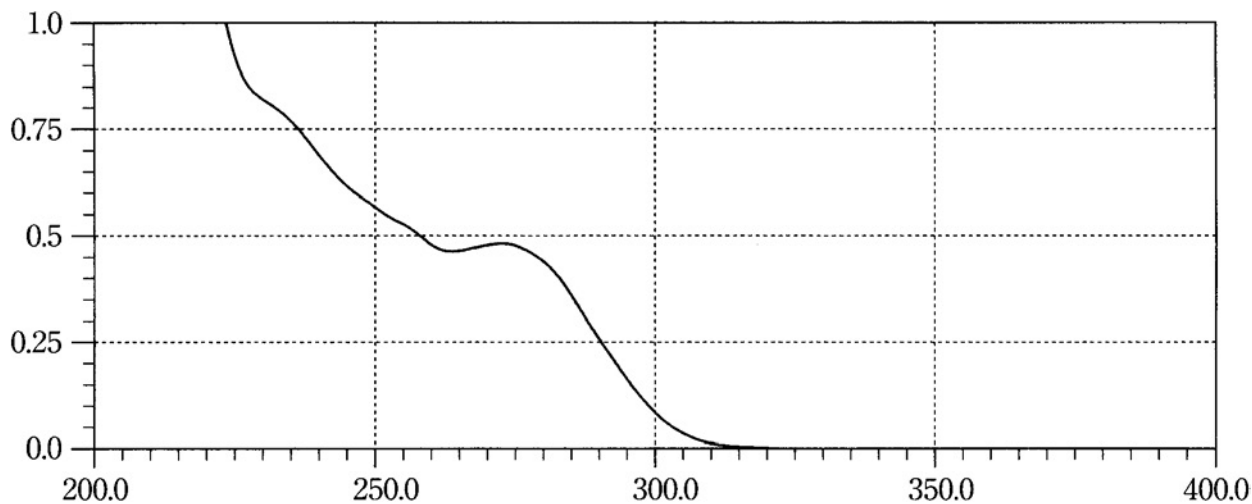
A solution in 0.1 mol/L hydrochloric acid TS (3 in 125,000)

Cefotiam Hydrochloride

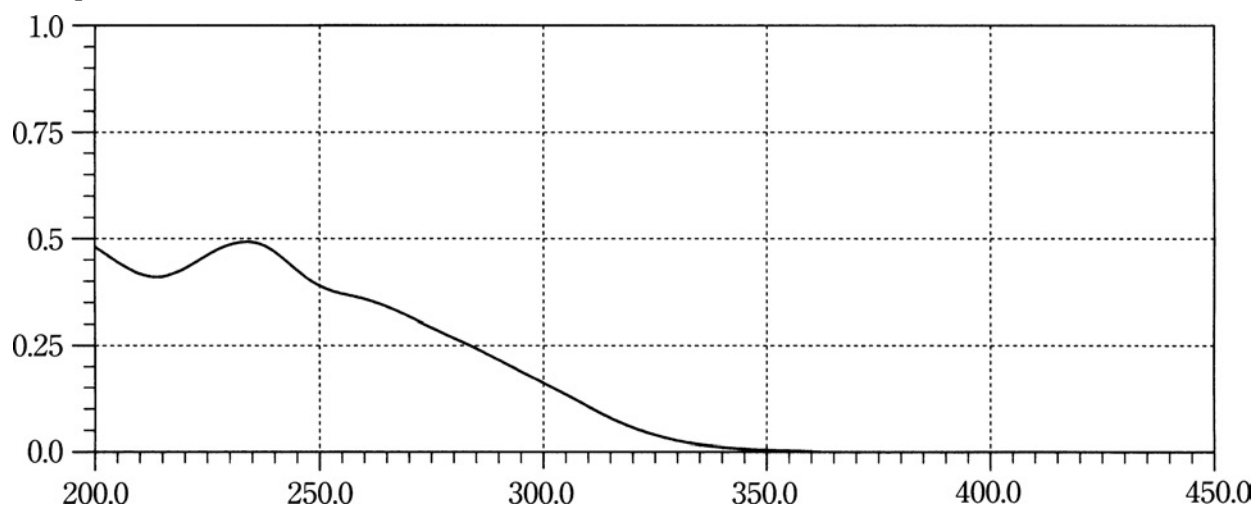
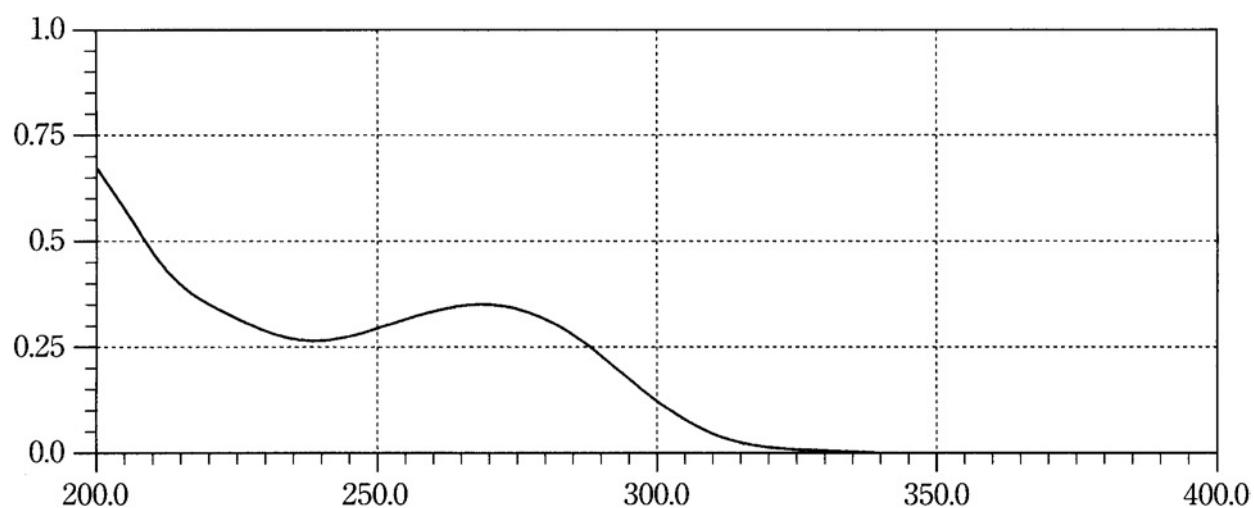
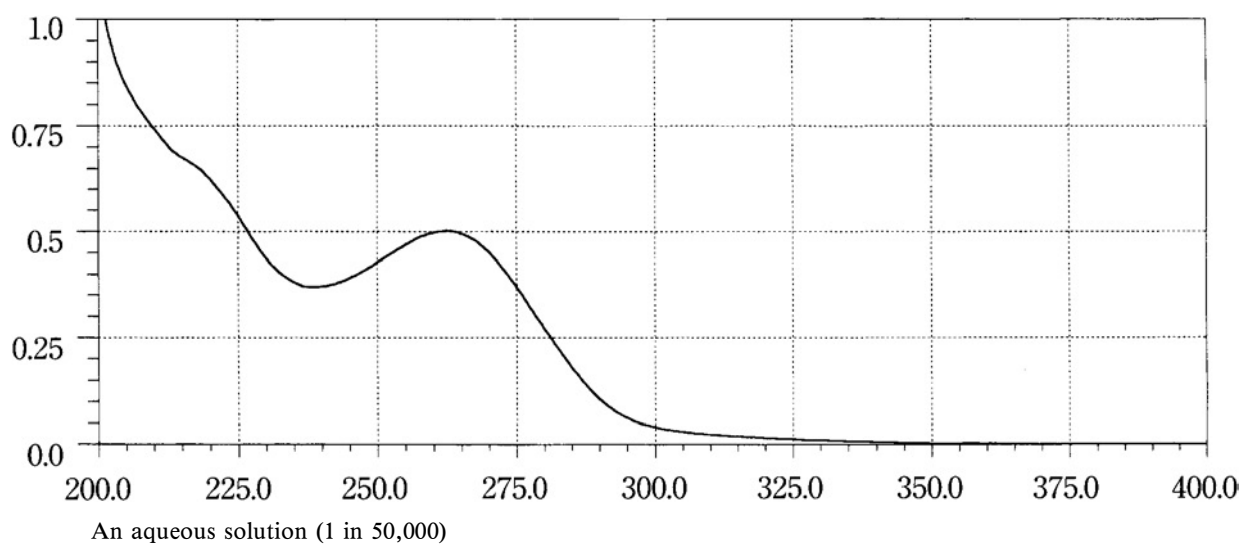


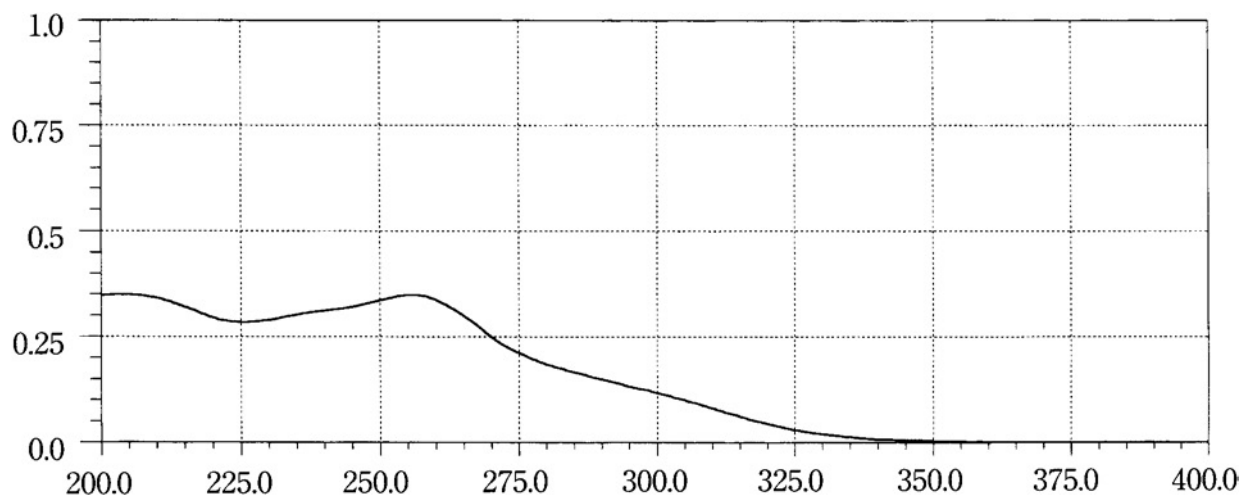
An aqueous solution (1 in 50,000)

Cefpiramide Sodium

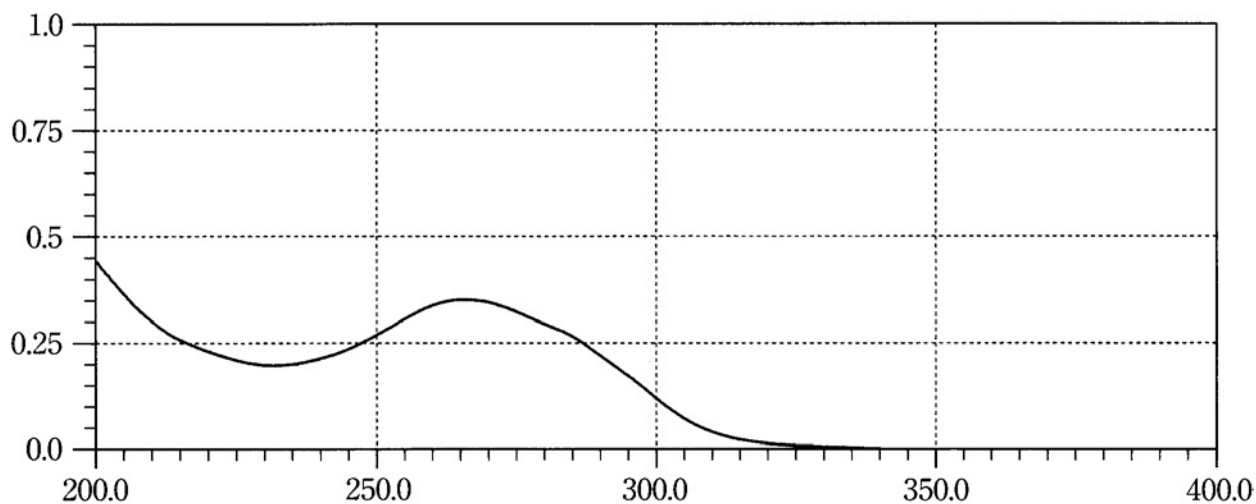


A solution in 0.05 mol/L phosphate buffer solution, pH 7.0 (1 in 50,000)

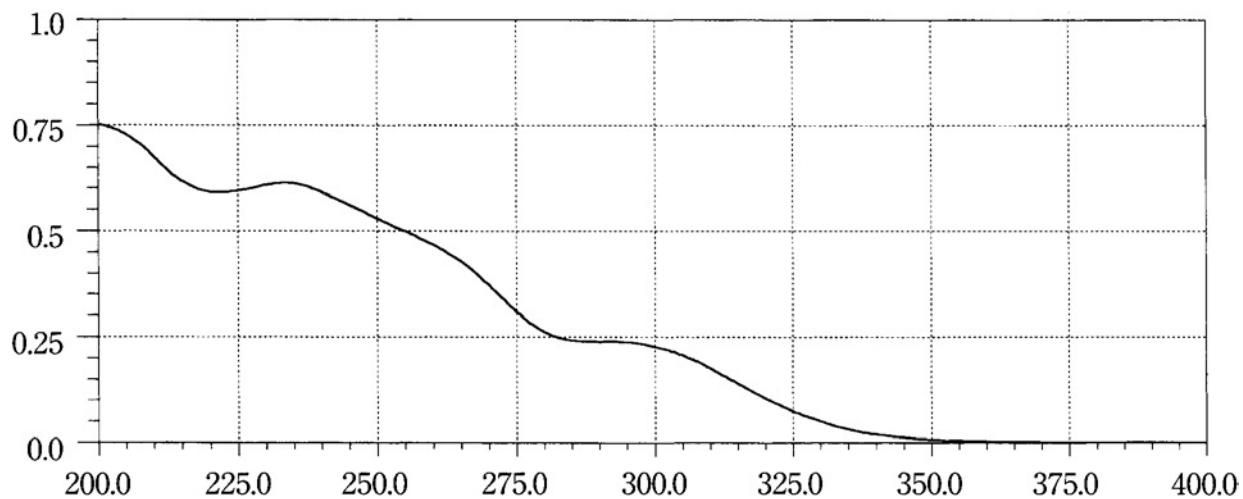
Cefpodoxime Proxetil**Cefroxadine Hydrate****Cefsulodin Sodium**

Ceftazidime Hydrate

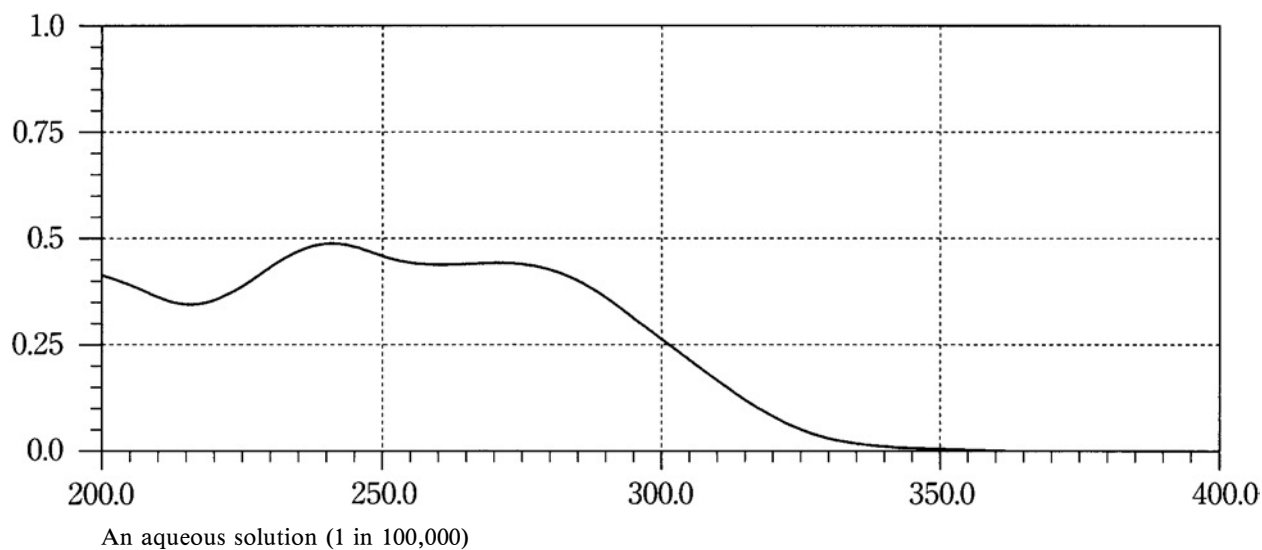
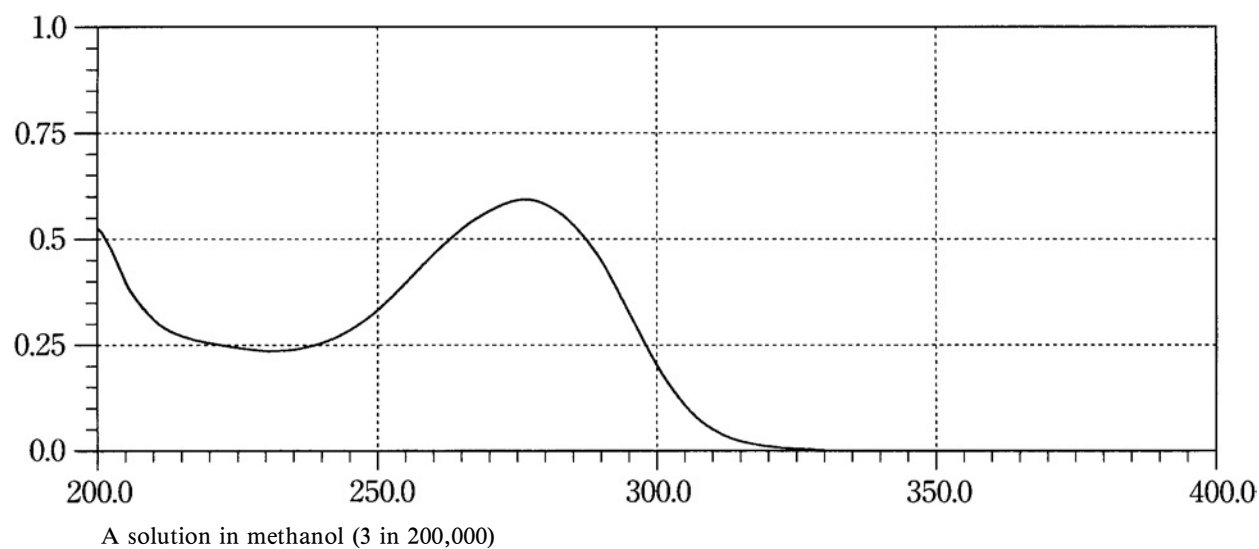
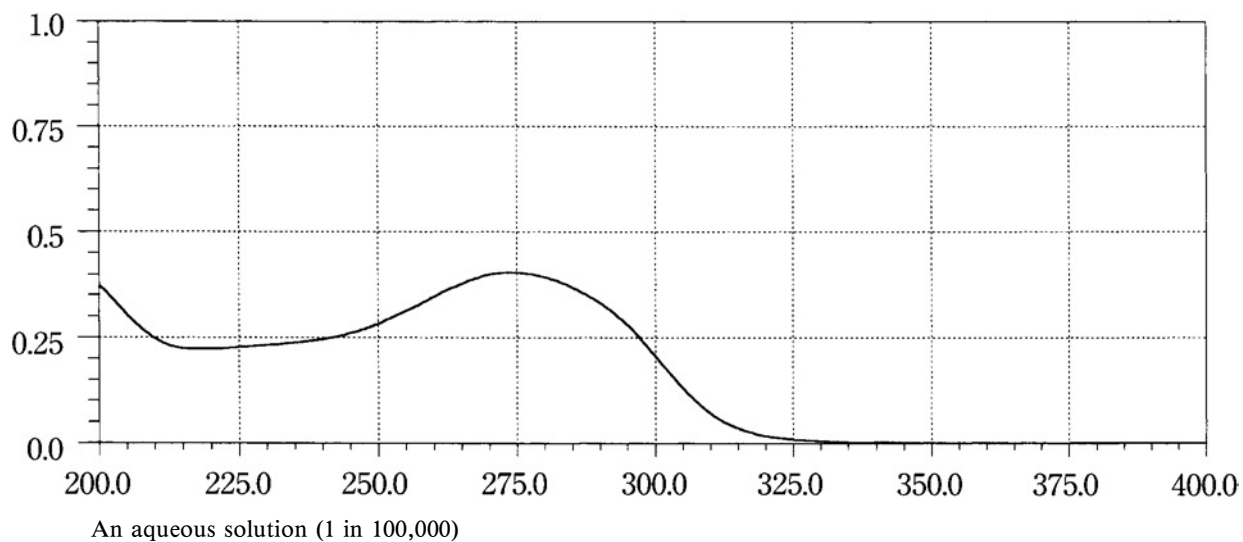
A solution in phosphate buffer solution, pH 6.0 (1 in 100,000)

Cefteram Pivoxil

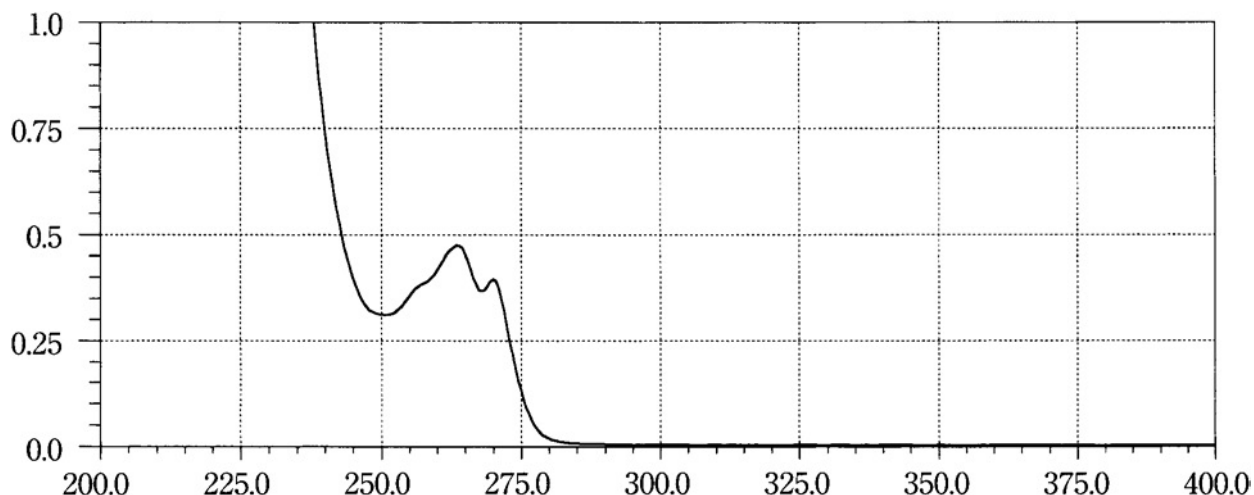
A solution in 0.05 mol/L hydrochloric acid-methanol TS (1 in 100,000)

Ceftizoxime Sodium

An aqueous solution (1 in 63,000)

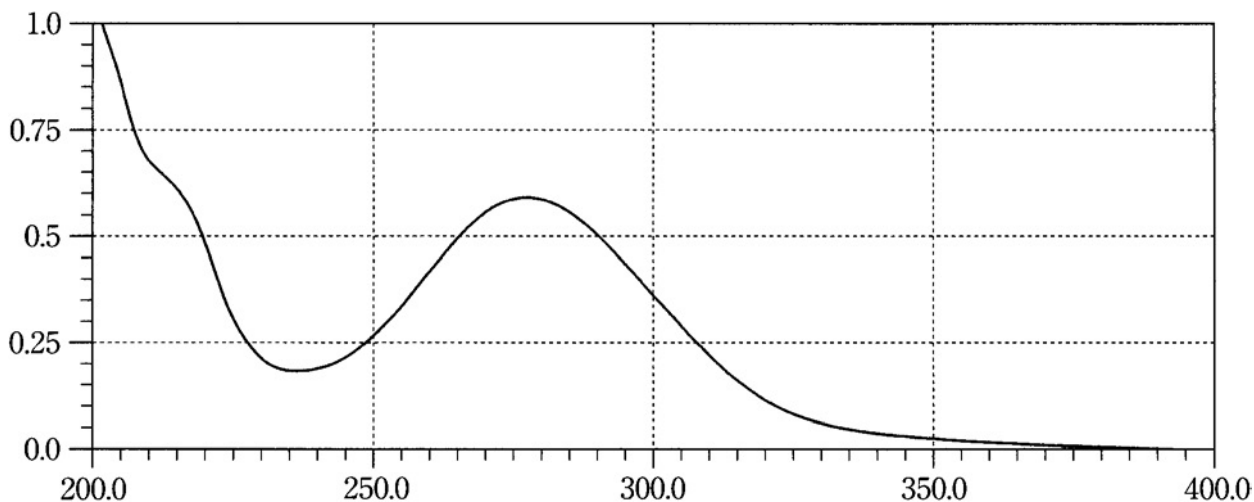
Ceftriaxone Sodium Hydrate**Cefuroxime Axetil****Cefuroxime Sodium**

Cetraxate Hydrochloride



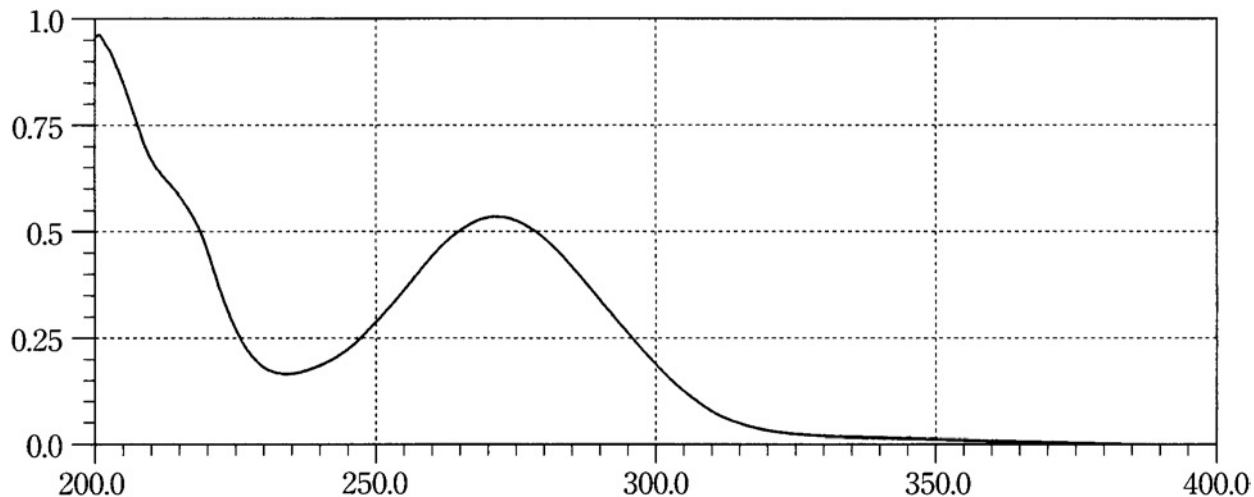
A solution in methanol (1 in 2500)

Chloramphenicol

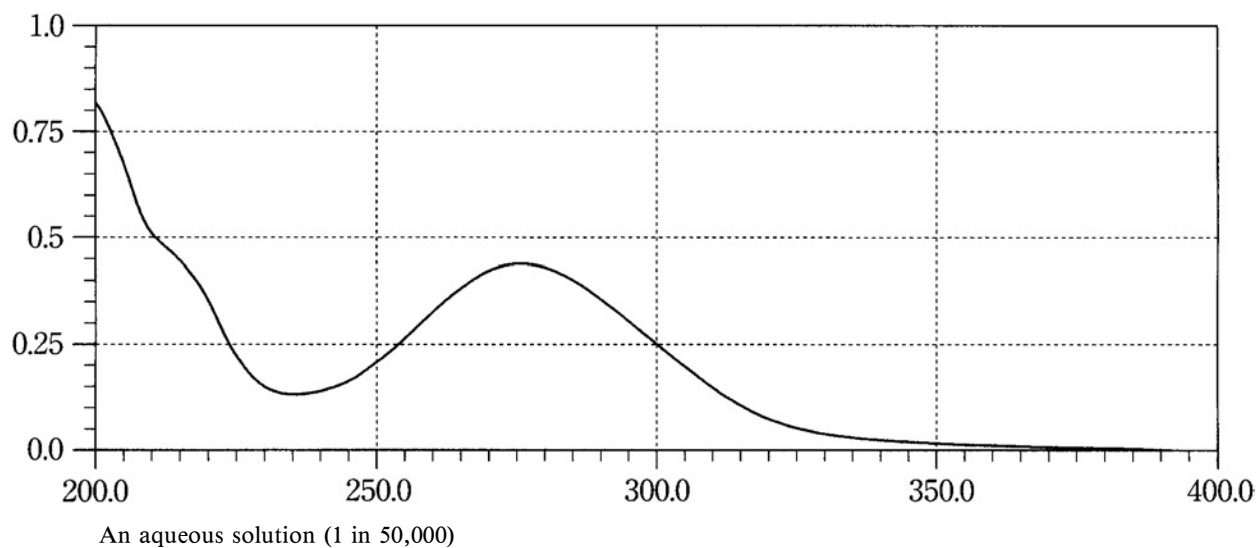
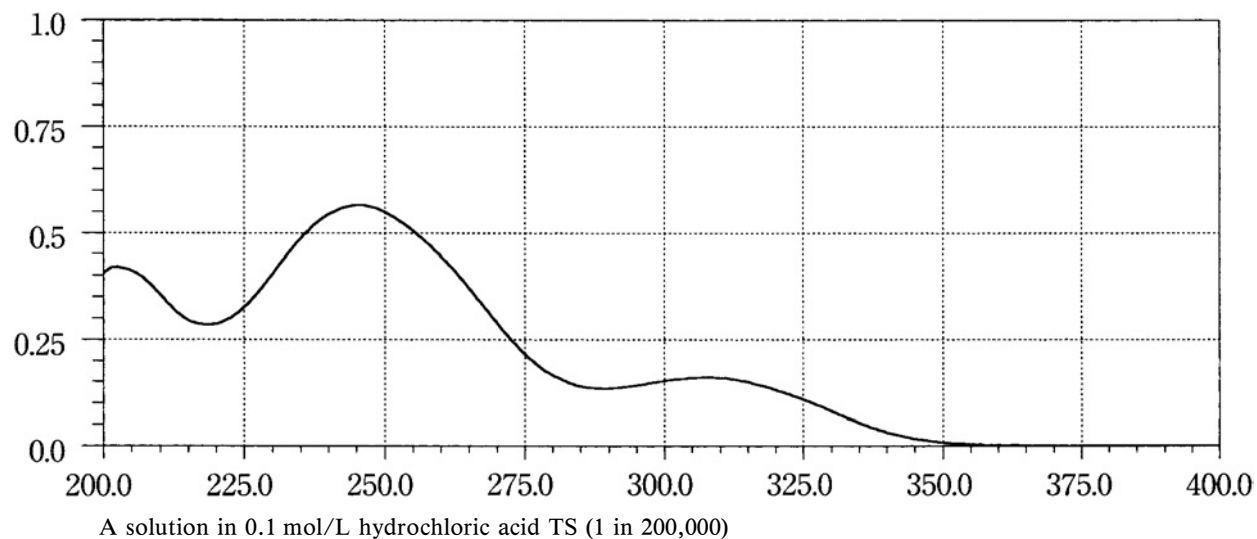
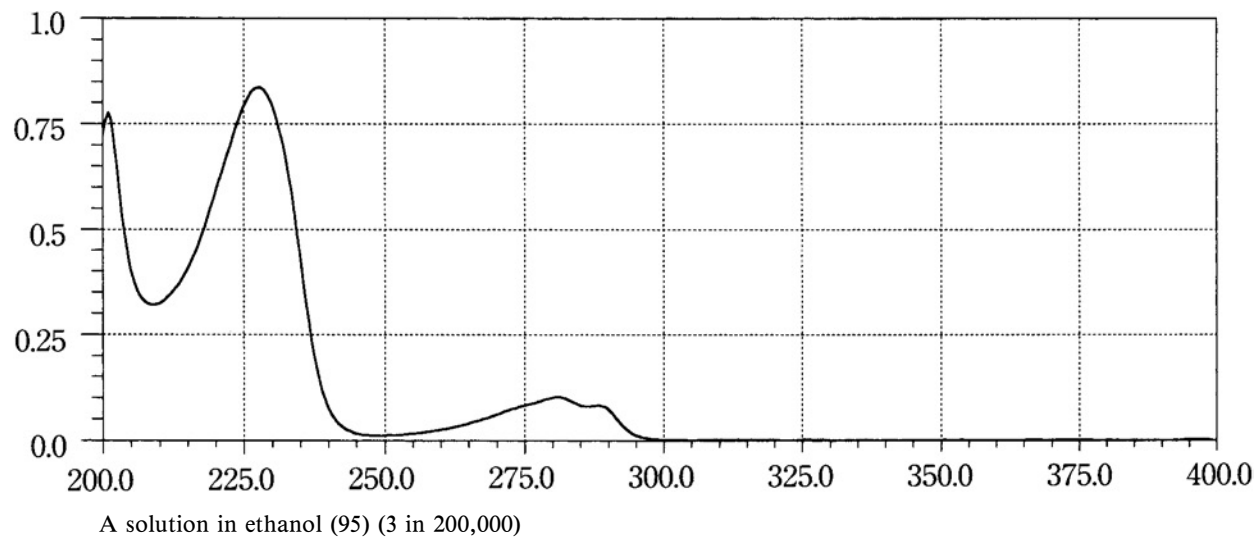


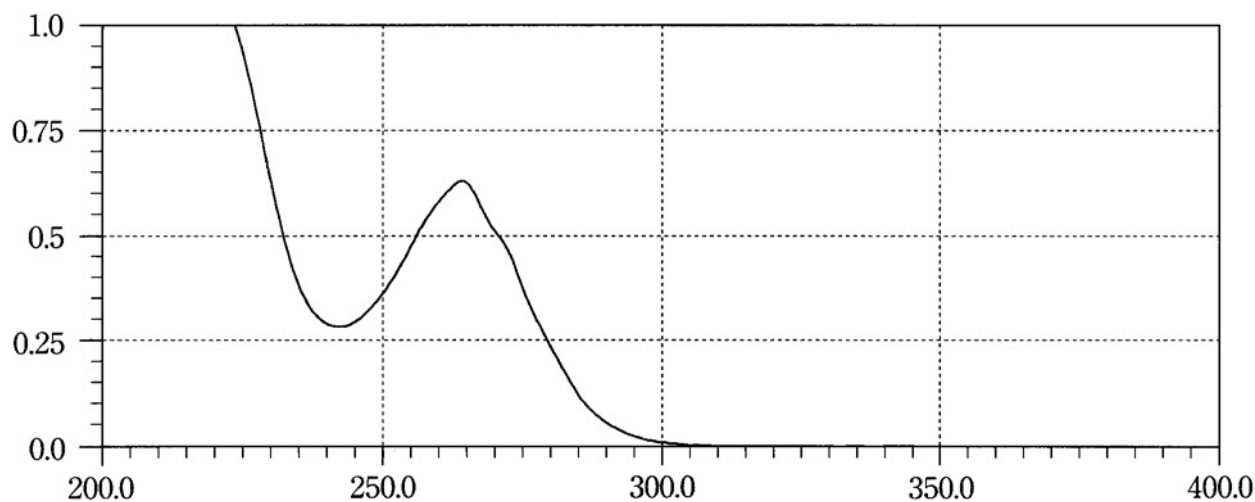
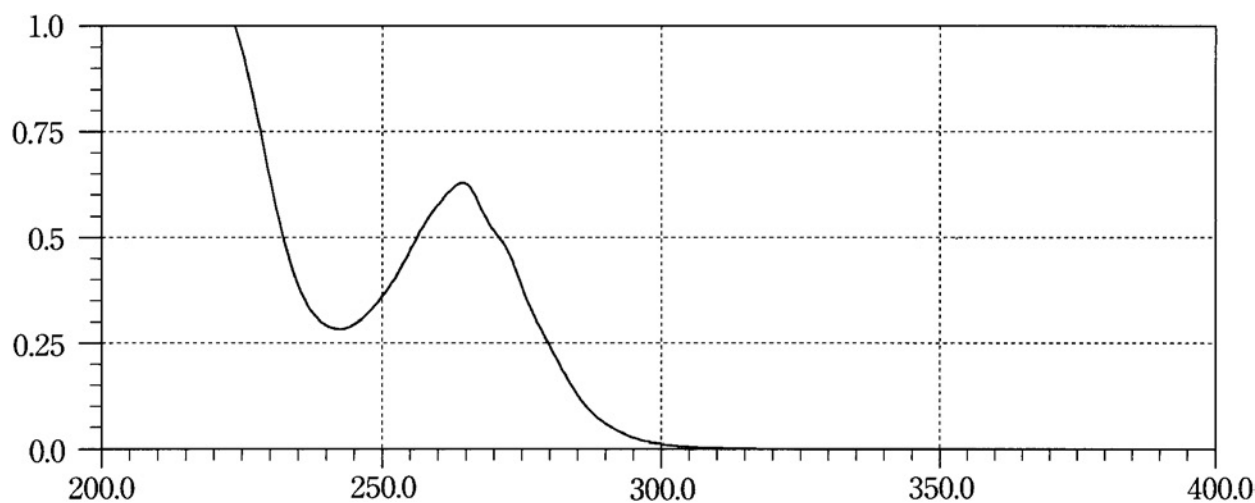
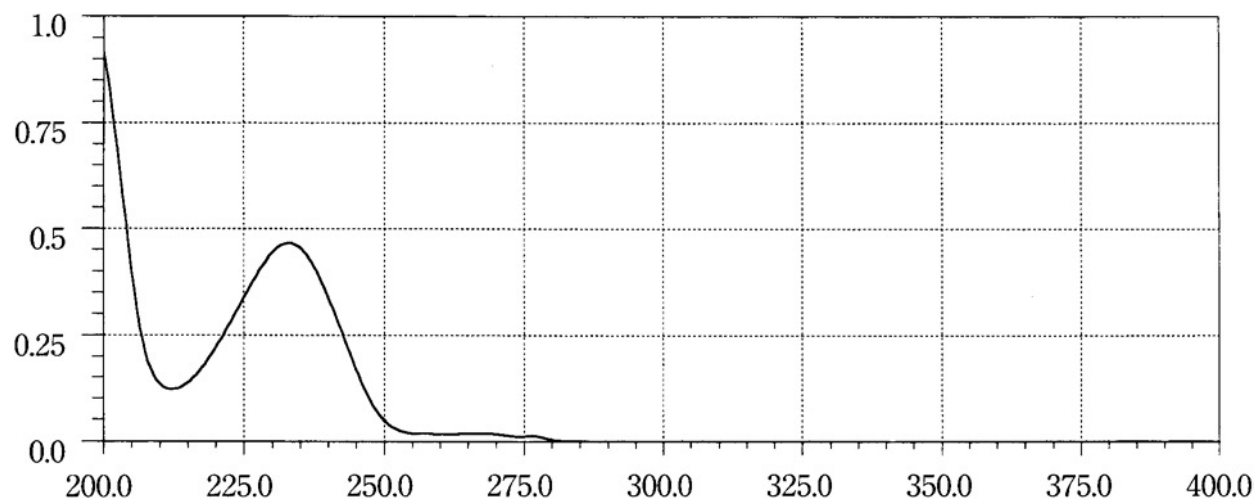
The sample solution obtained in the Assay

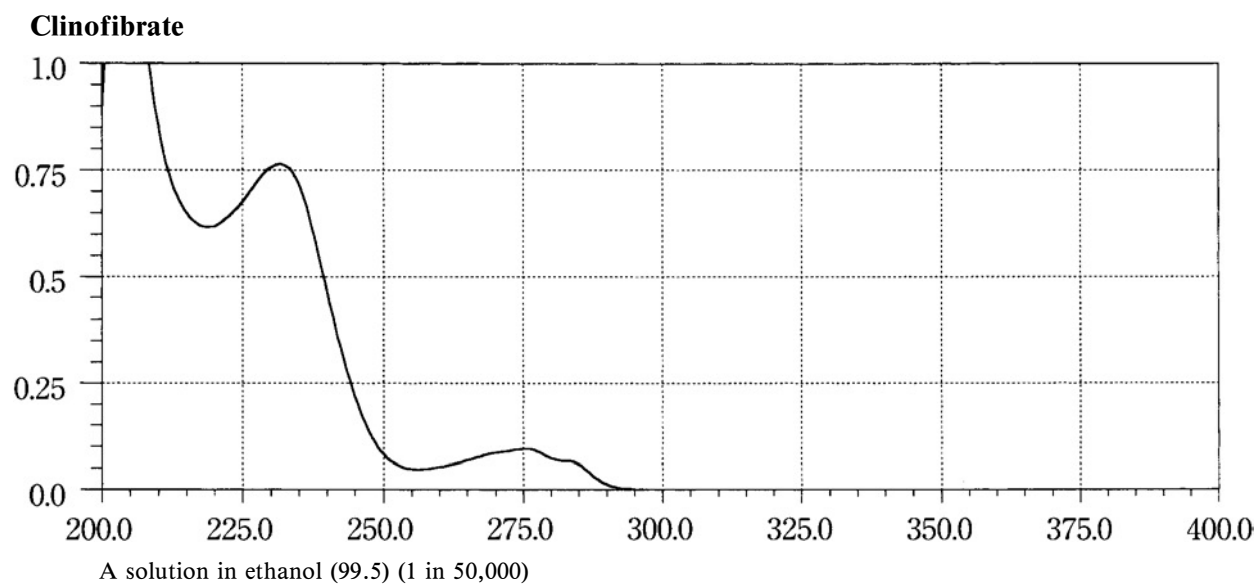
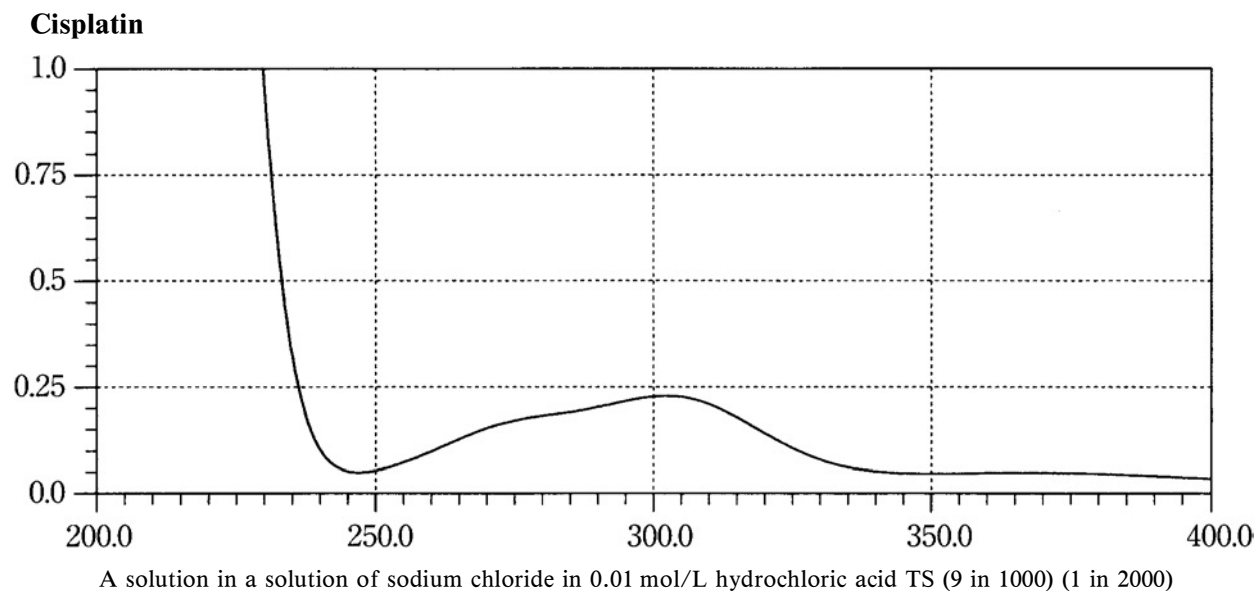
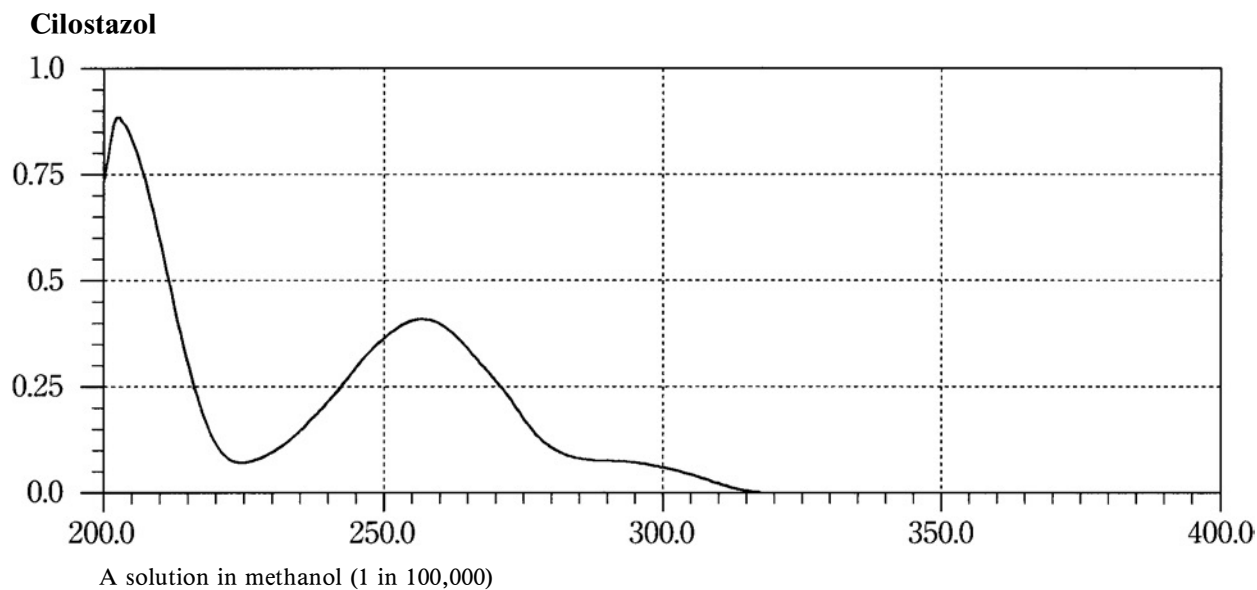
Chloramphenicol Palmitate

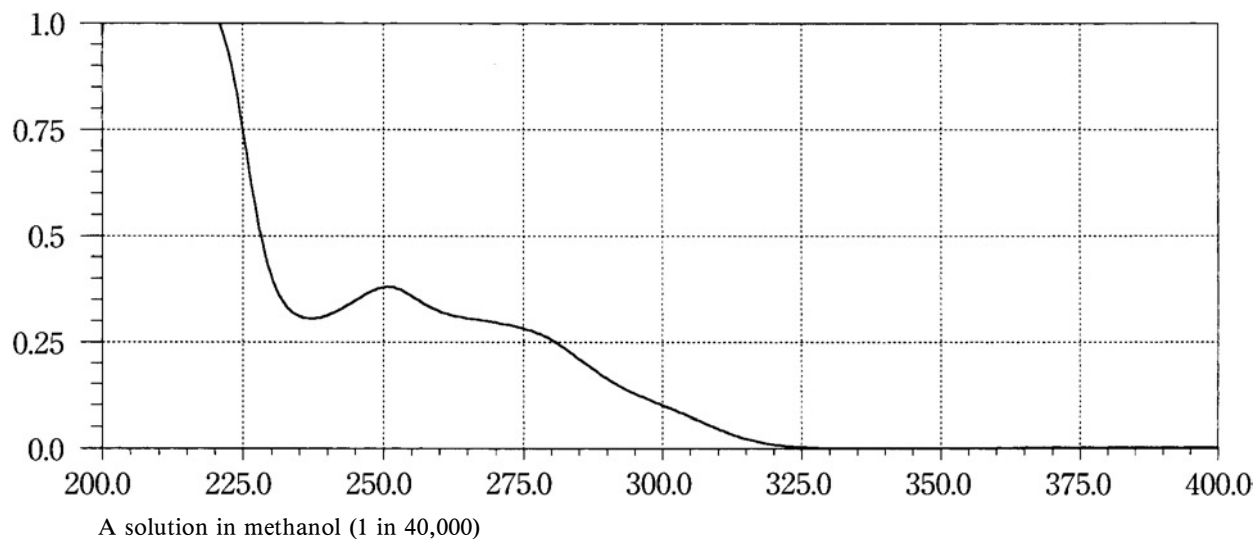
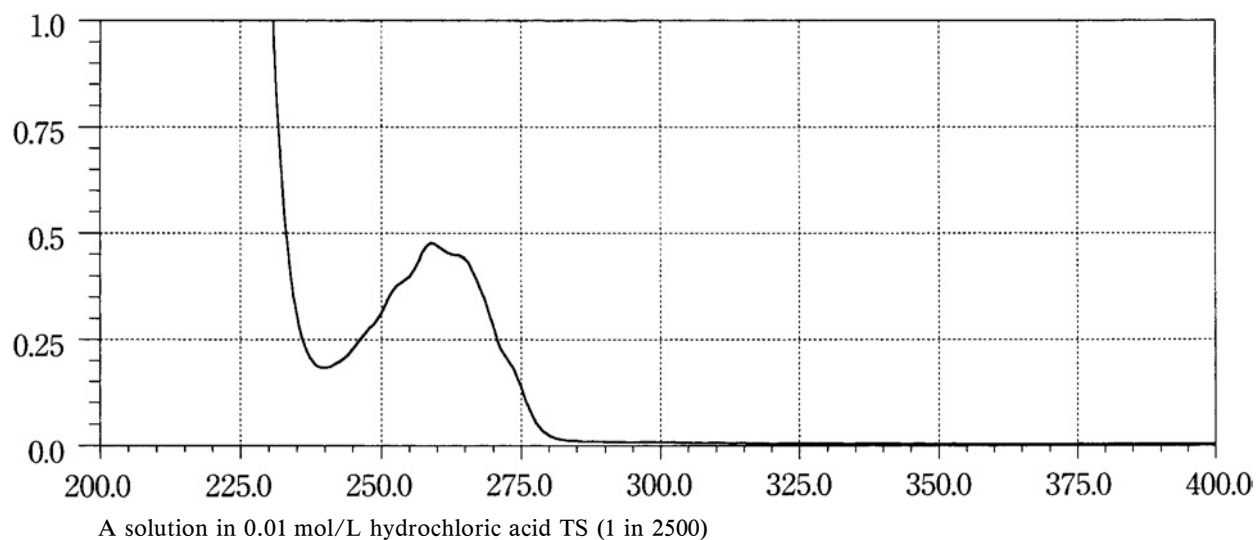
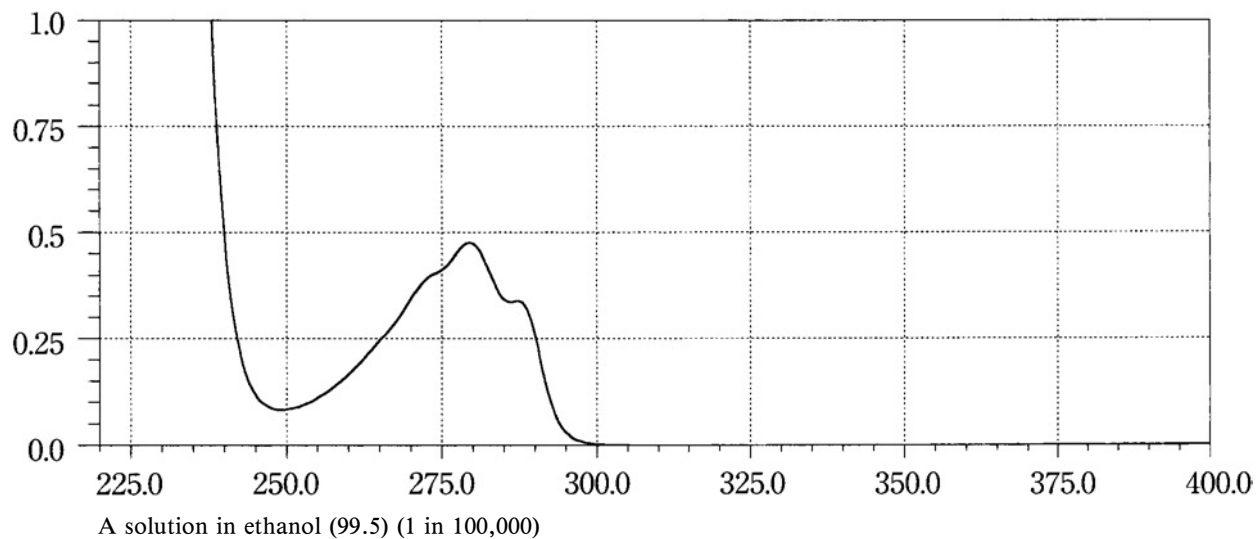


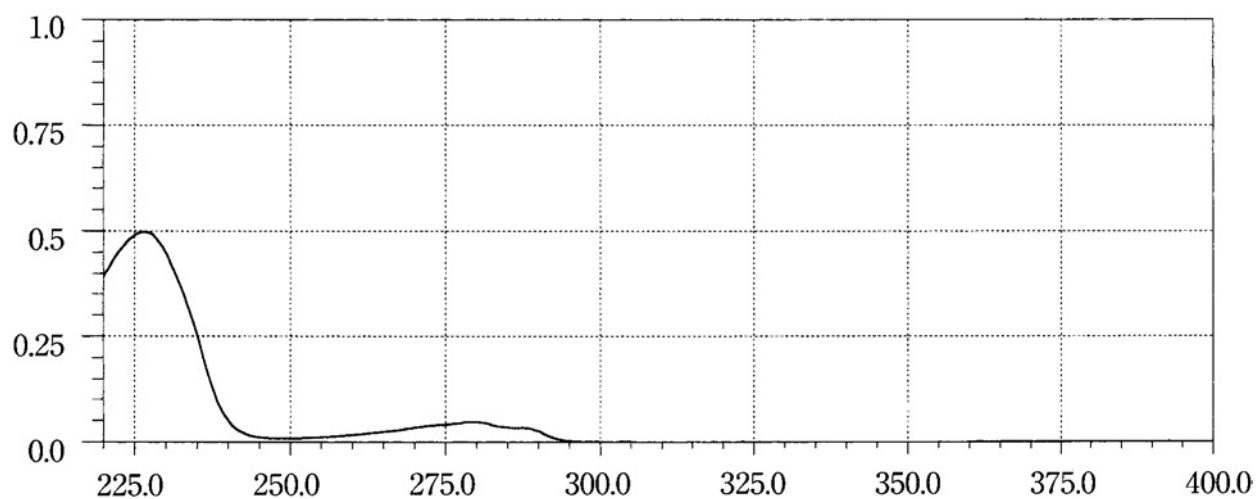
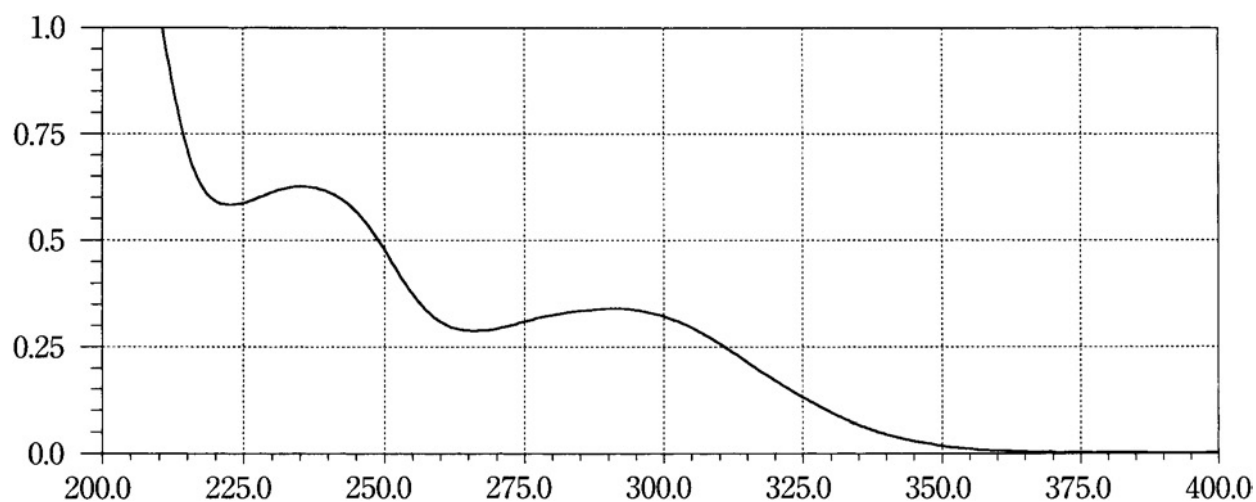
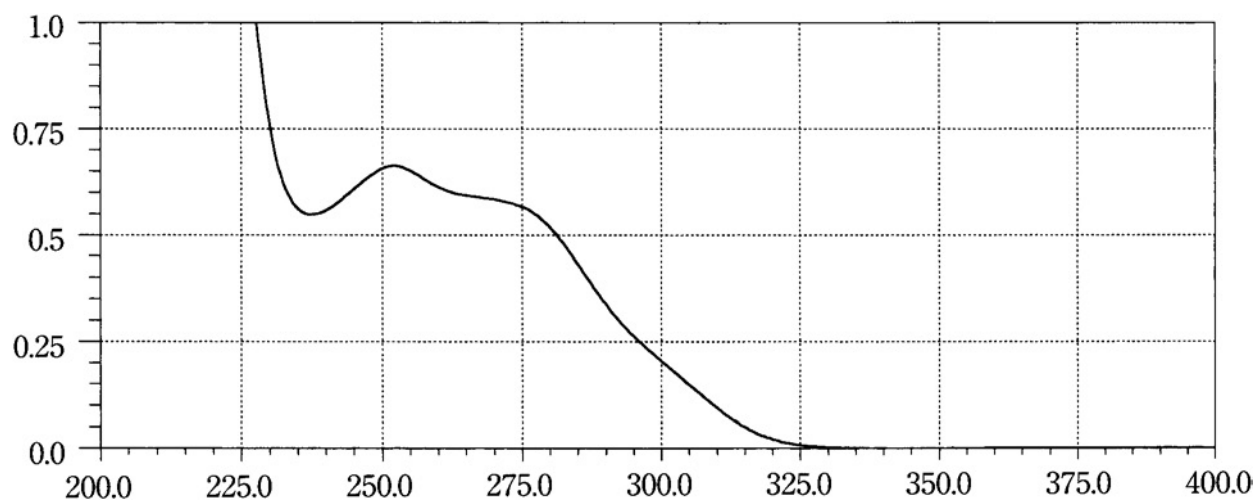
A solution in ethanol (99.5) (1 in 33,000)

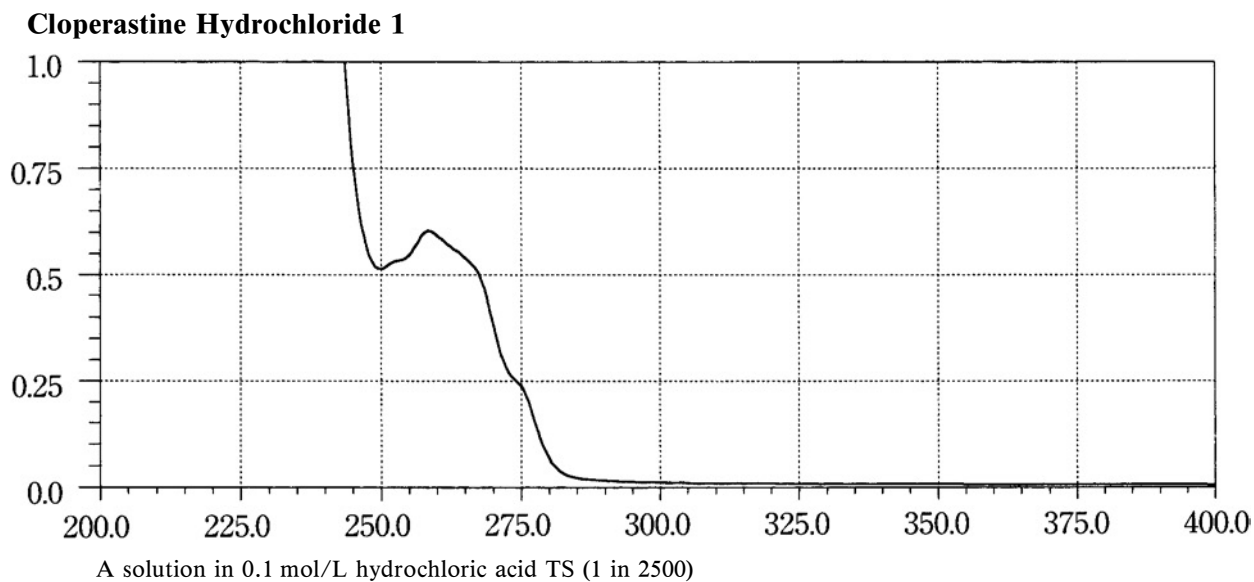
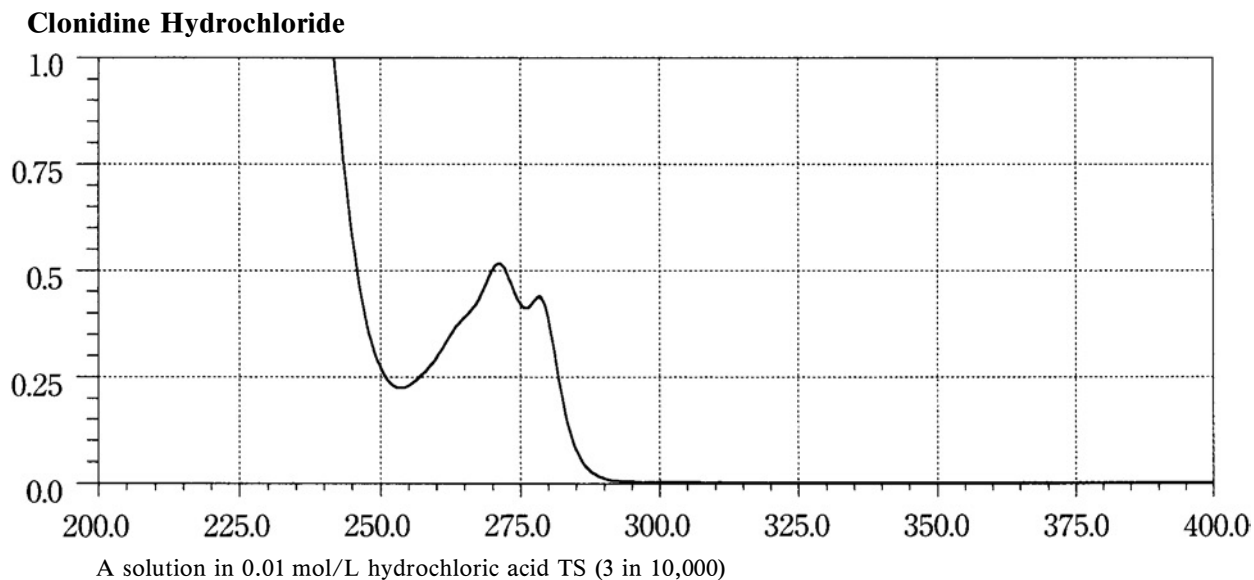
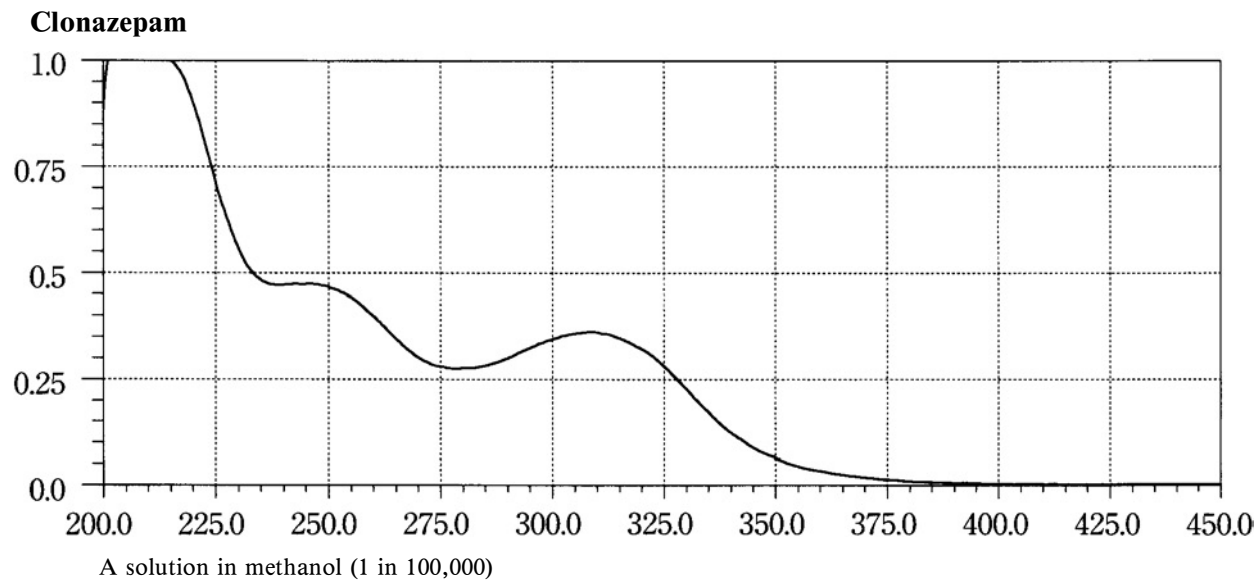
Chloramphenicol Sodium Succinate**Chlordiazepoxide****Chlorphenesin Carbamate**

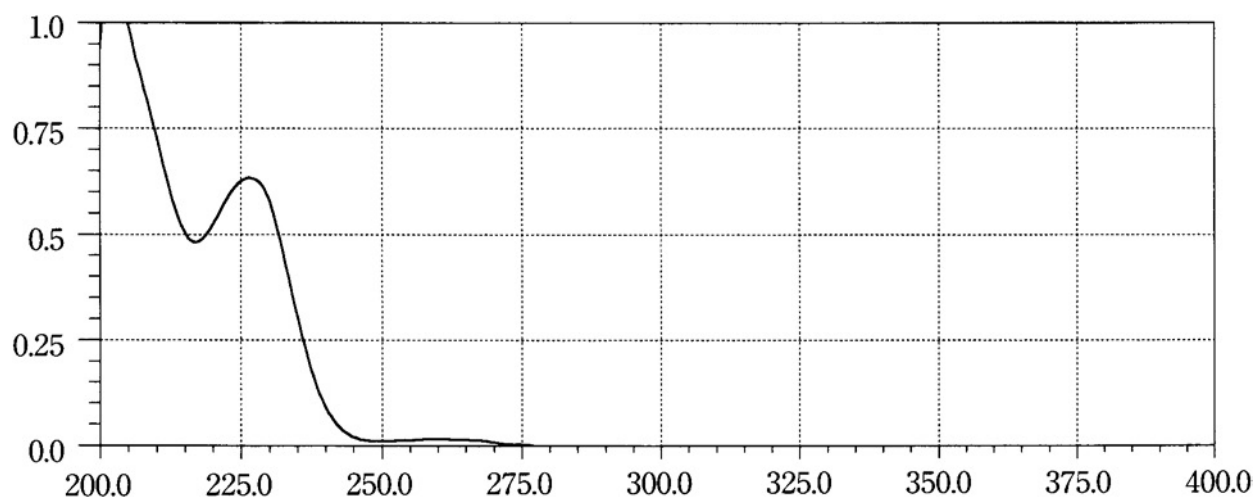
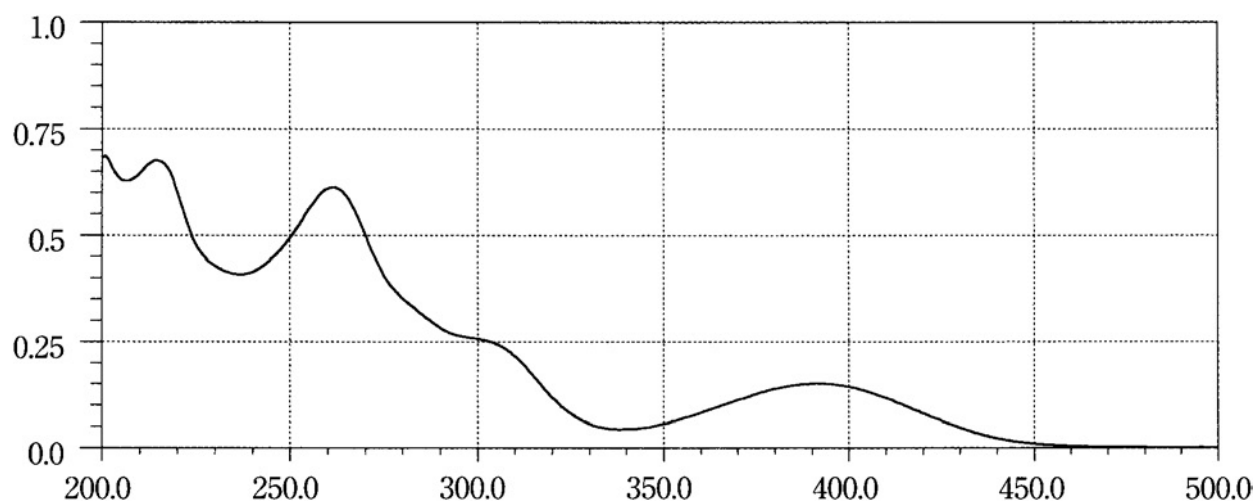
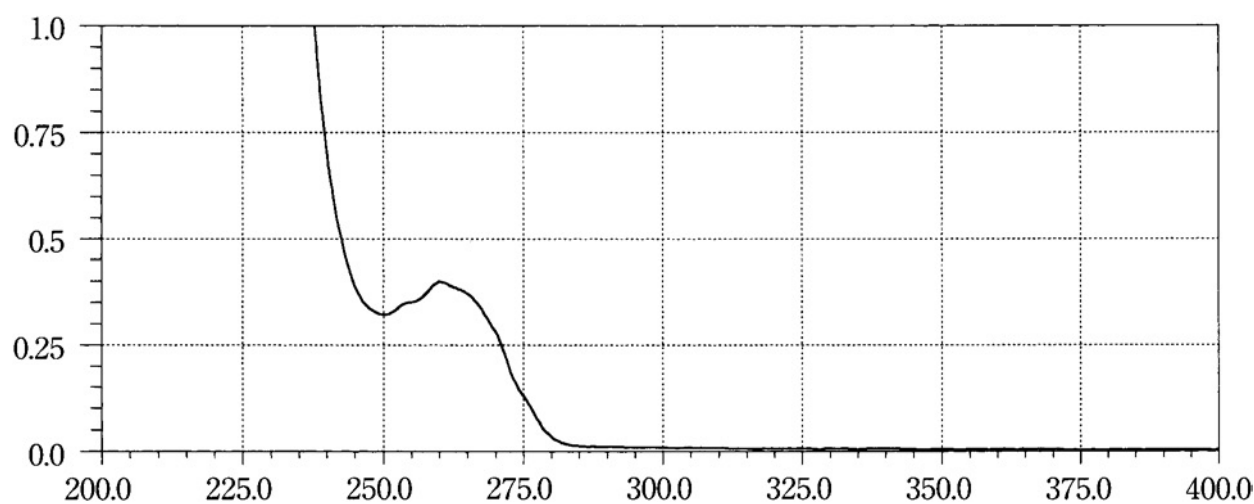
Chlorpheniramine Maleate***d*-Chlorpheniramine Maleate****Chlorpropamide**

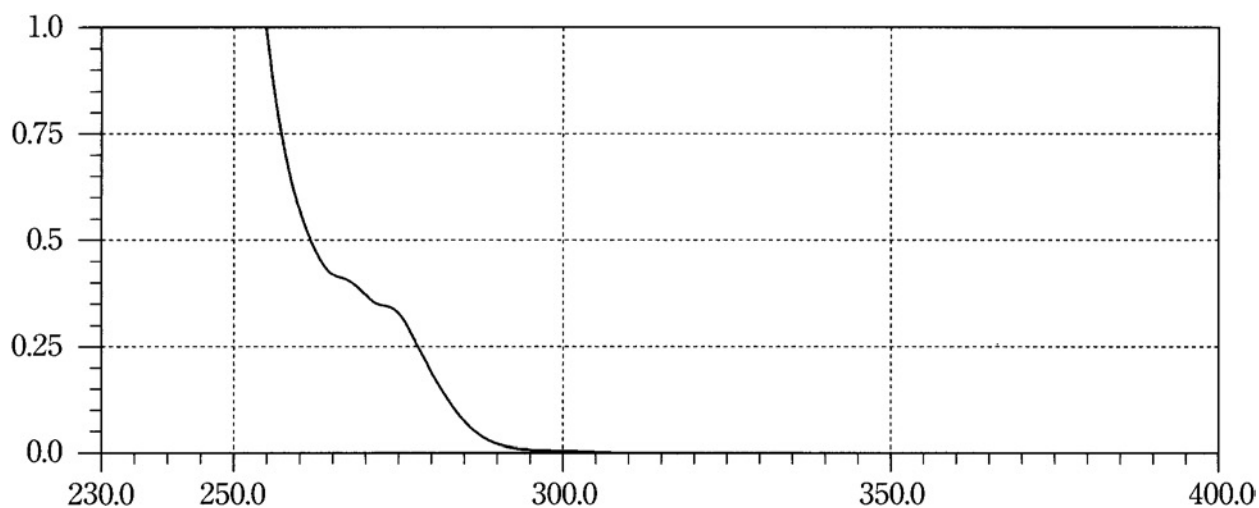


Clocapramine Hydrochloride Hydrate**Clofedanol Hydrochloride****Clofibrate 1**

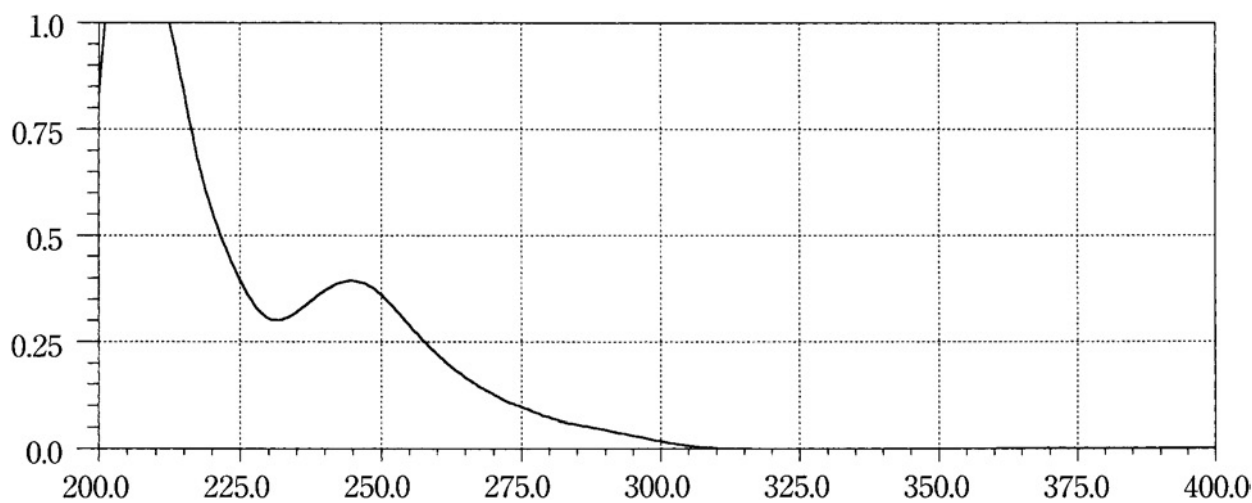
Clofibrate 2**Clomifene Citrate****Clomipramine Hydrochloride**



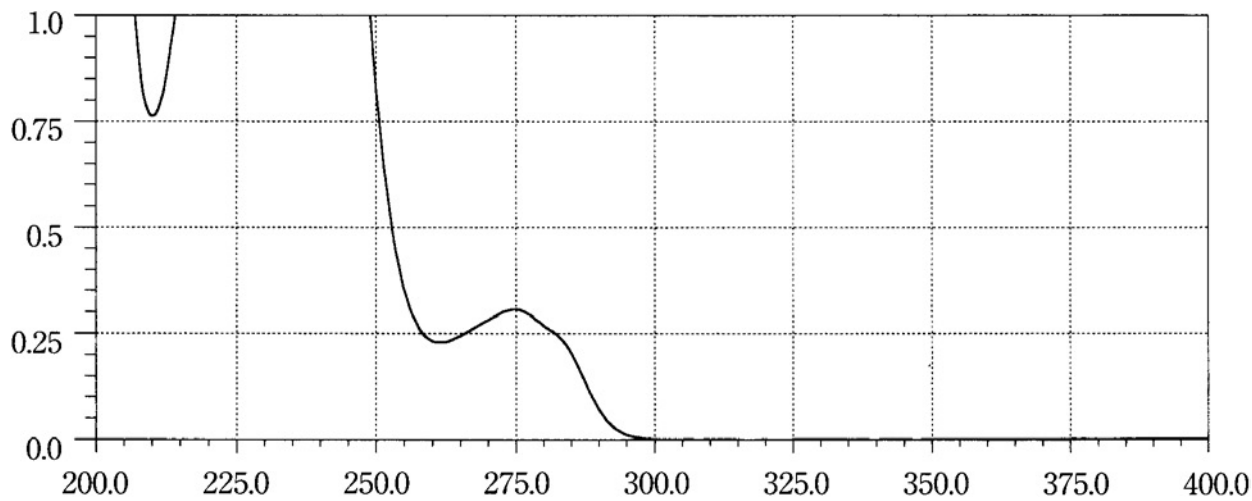
Cloperastine Hydrochloride 2**Clotiazepam****Clotrimazole**

Cloxacillin Sodium Hydrate

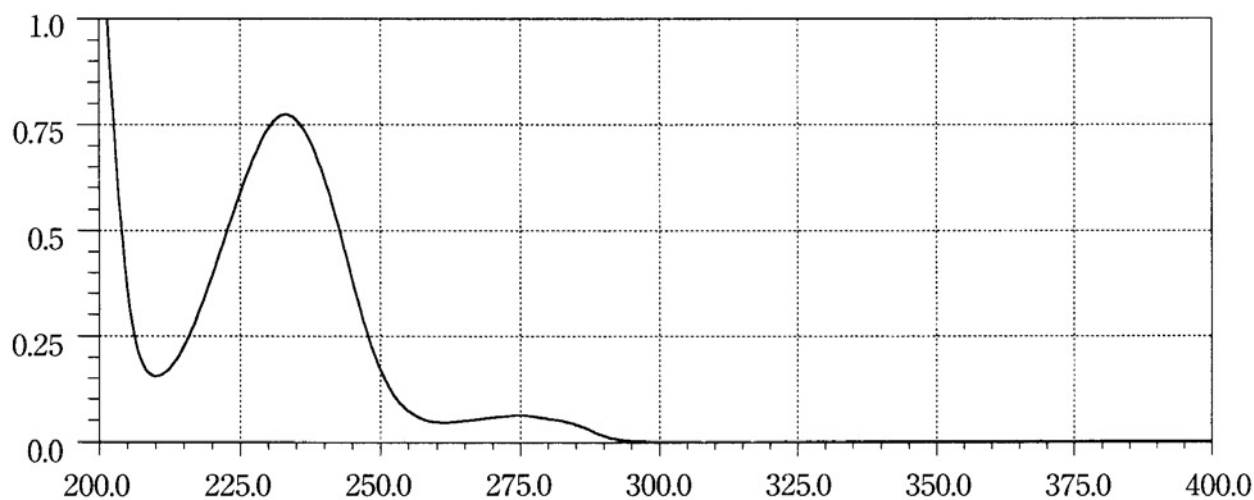
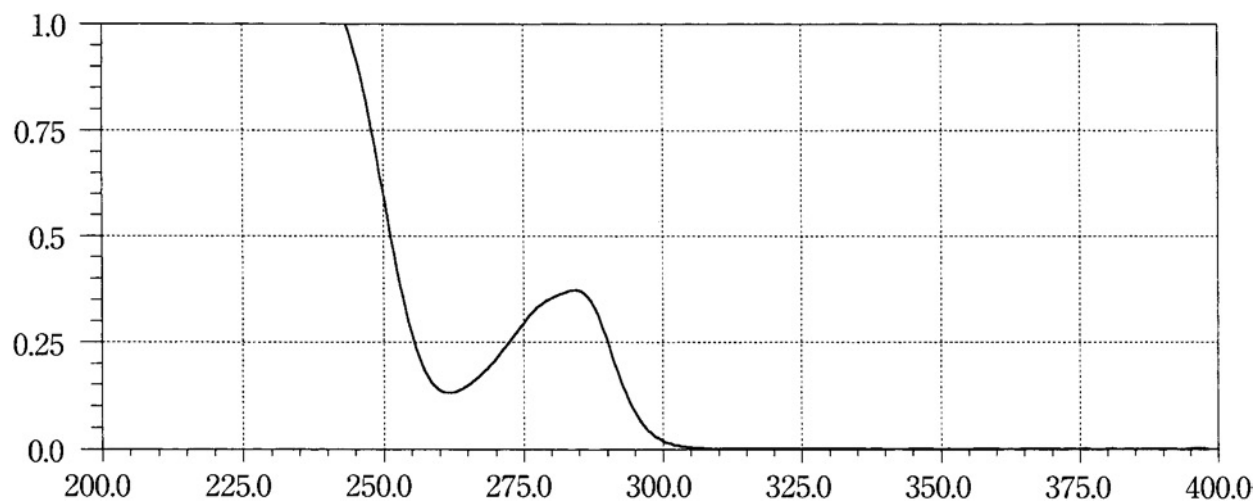
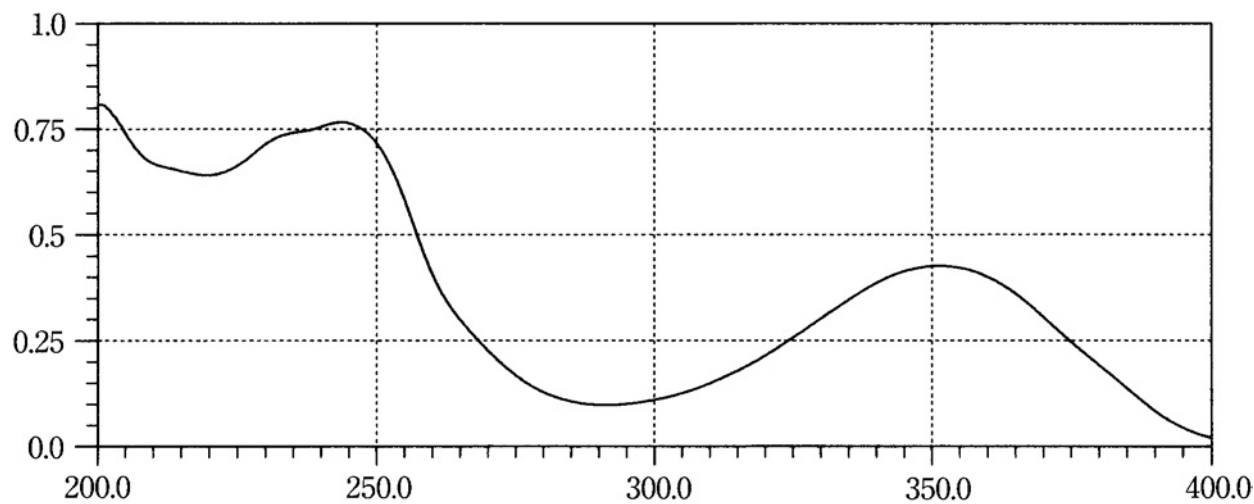
A solution in methanol (1 in 2500)

Cloxazolam

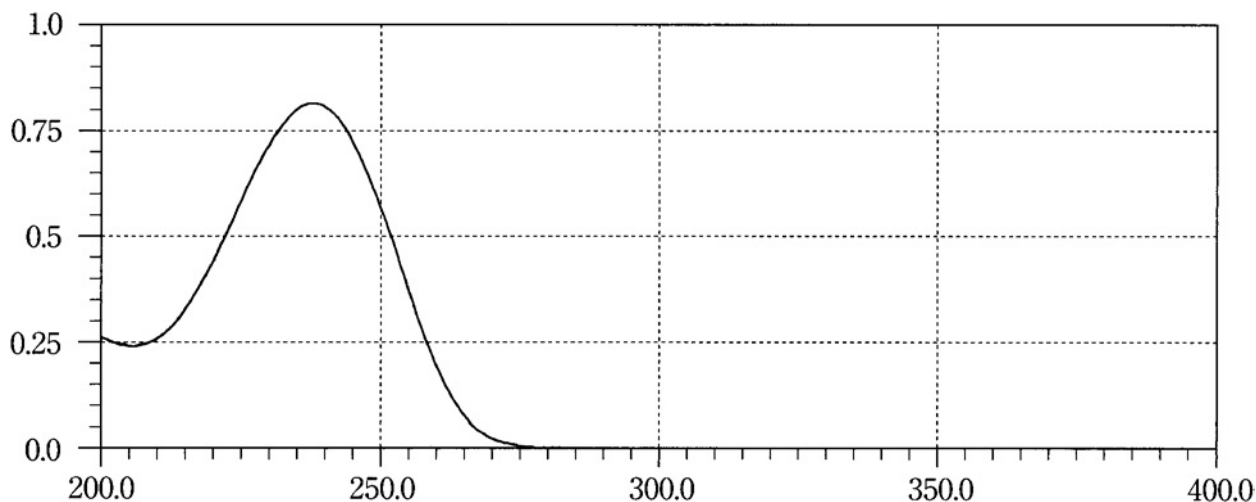
A solution in ethanol (99.5) (1 in 100,000)

Cocaine Hydrochloride 1

A solution in 0.01 mol/L hydrochloric acid TS (1 in 10,000)

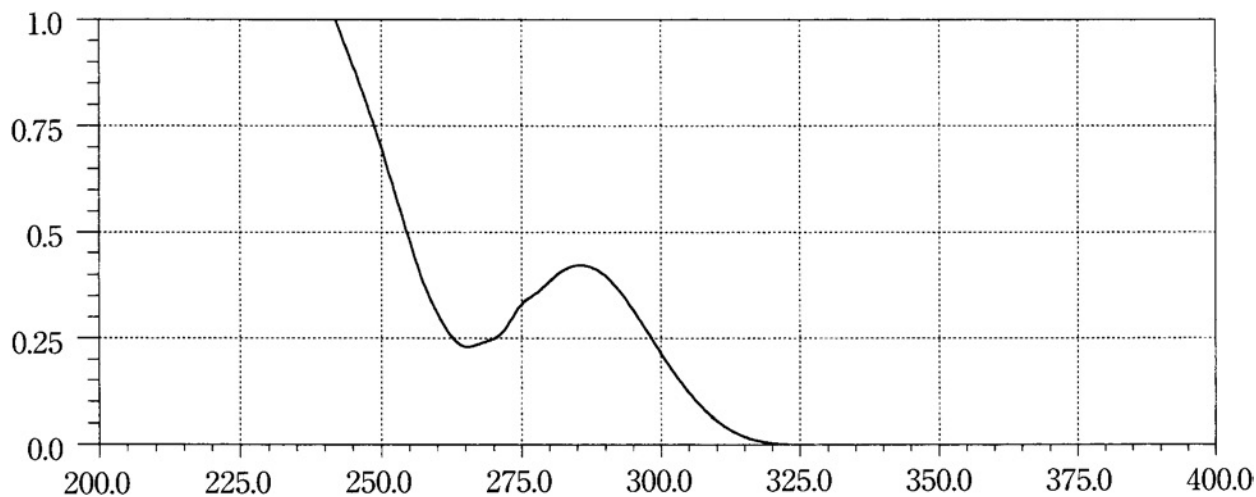
Cocaine Hydrochloride 2**Codeine Phosphate Hydrate****Colchicine**

Cortisone Acetate



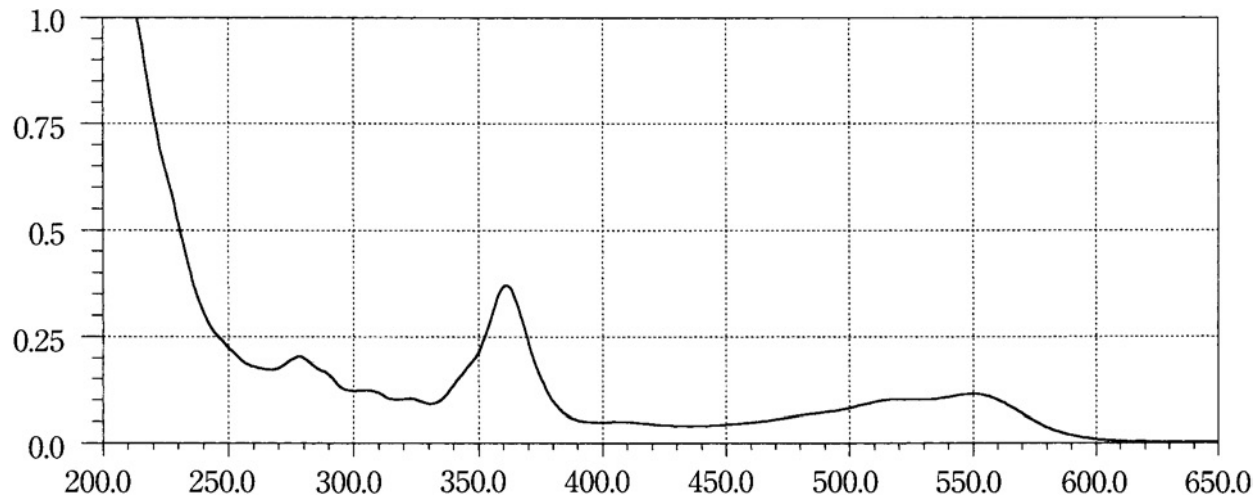
A solution in methanol (1 in 50,000)

Croconazole Hydrochloride

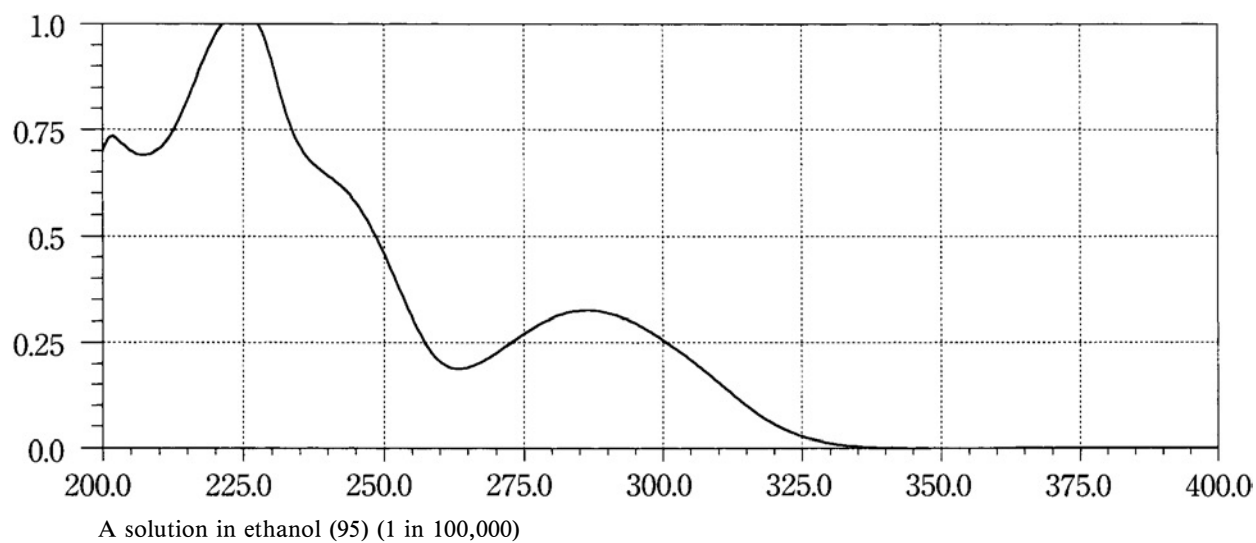
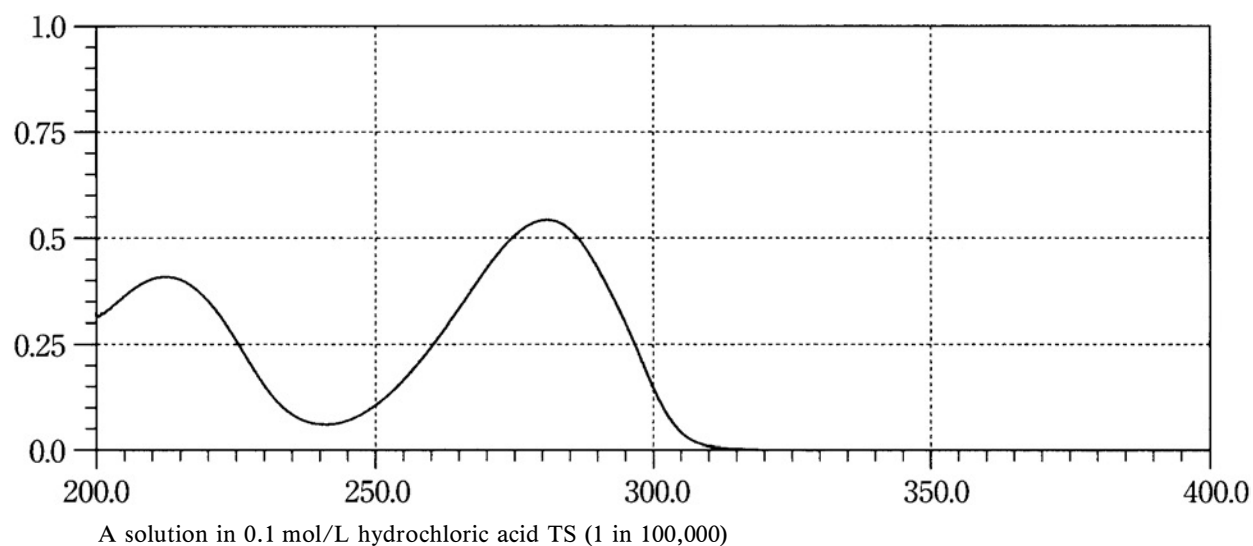
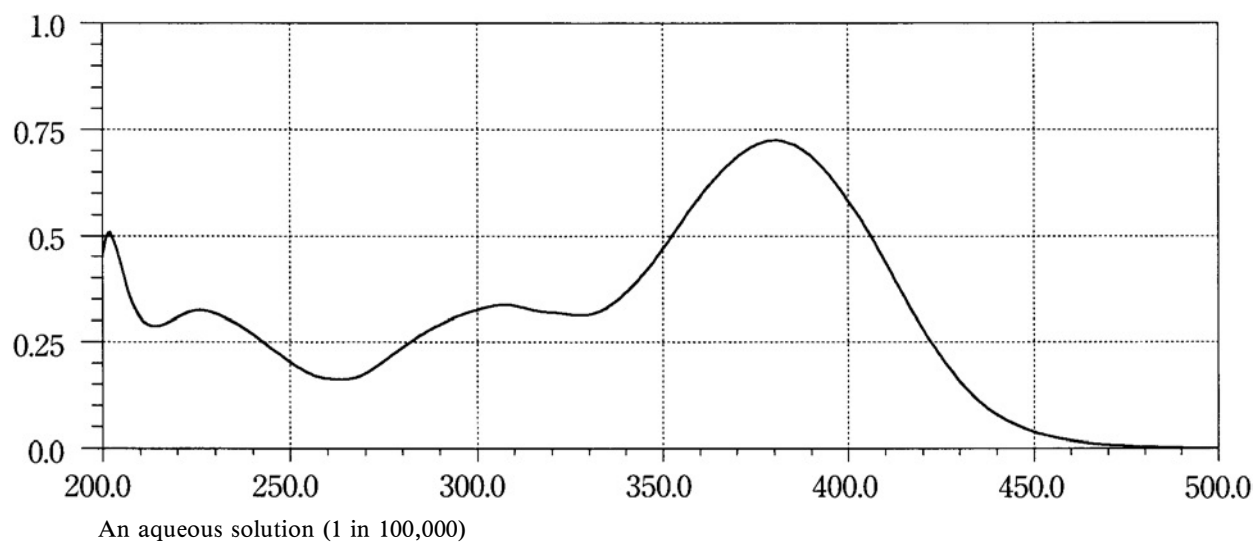


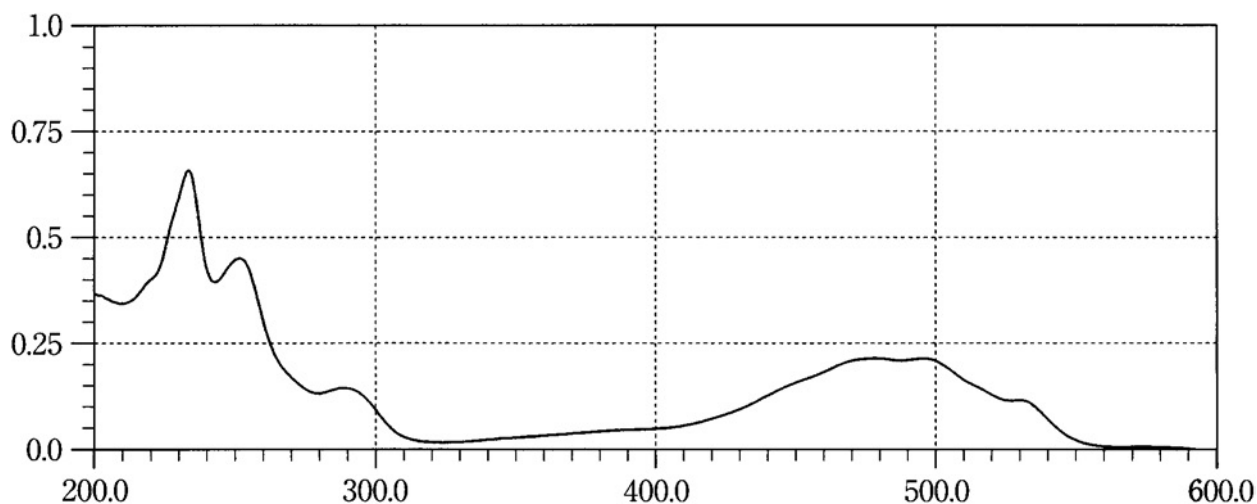
A solution in methanol (1 in 20,000)

Cyanocobalamin

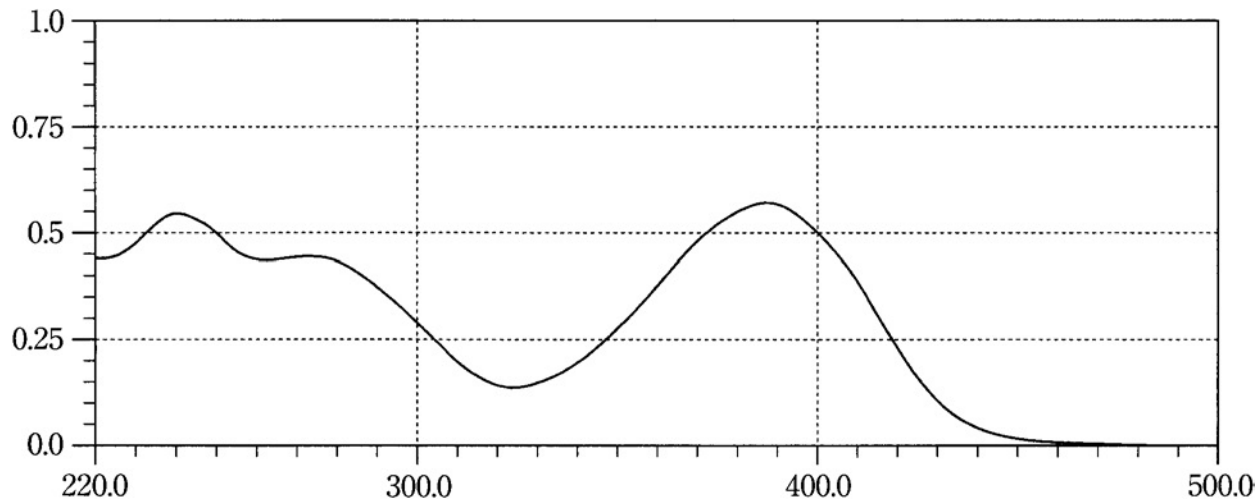


The sample solution obtained in the Assay

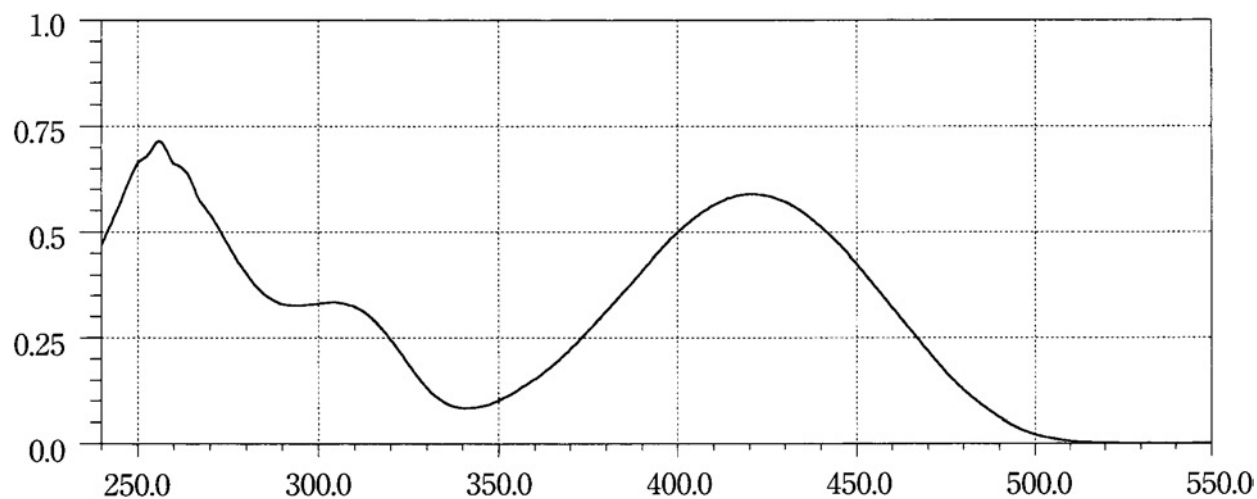
Cyproheptadine Hydrochloride Hydrate**Cytarabine****Dantrolene Sodium Hydrate**

Daunorubicin Hydrochloride

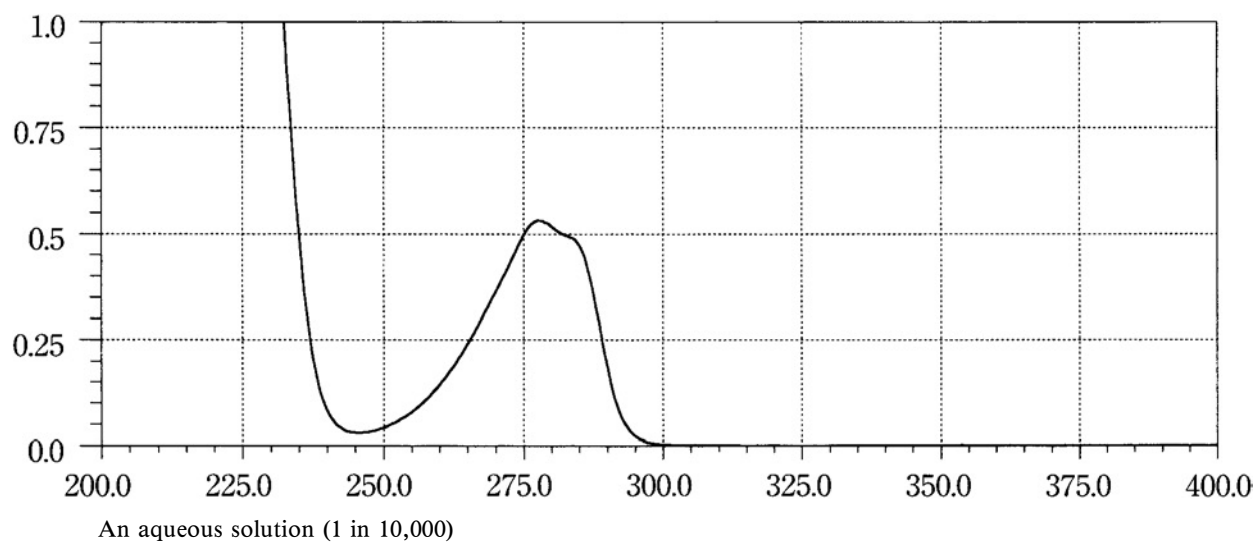
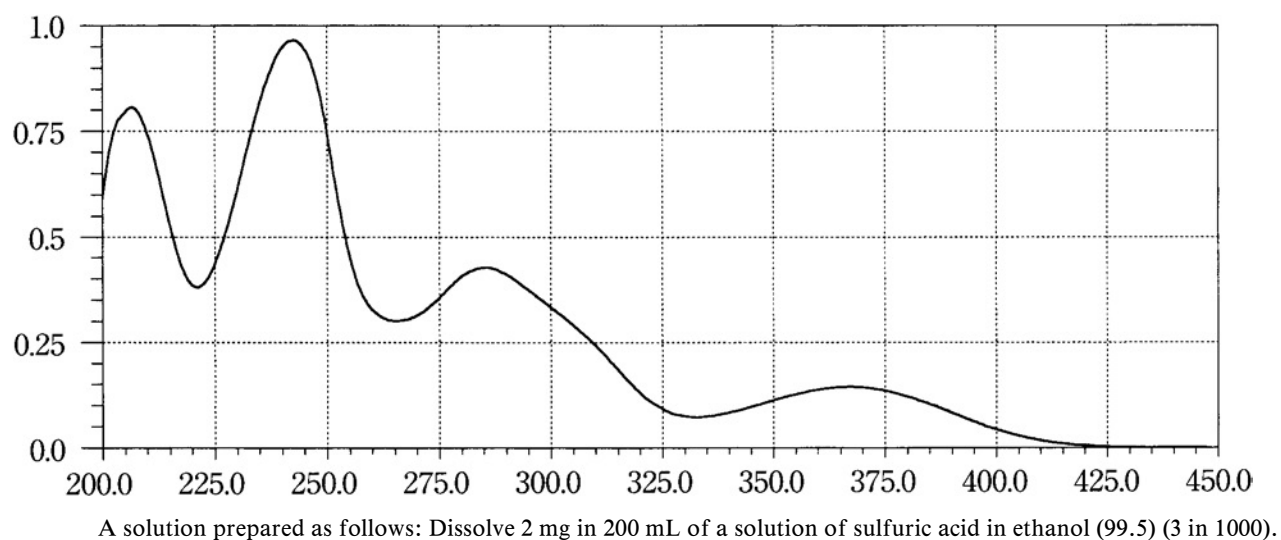
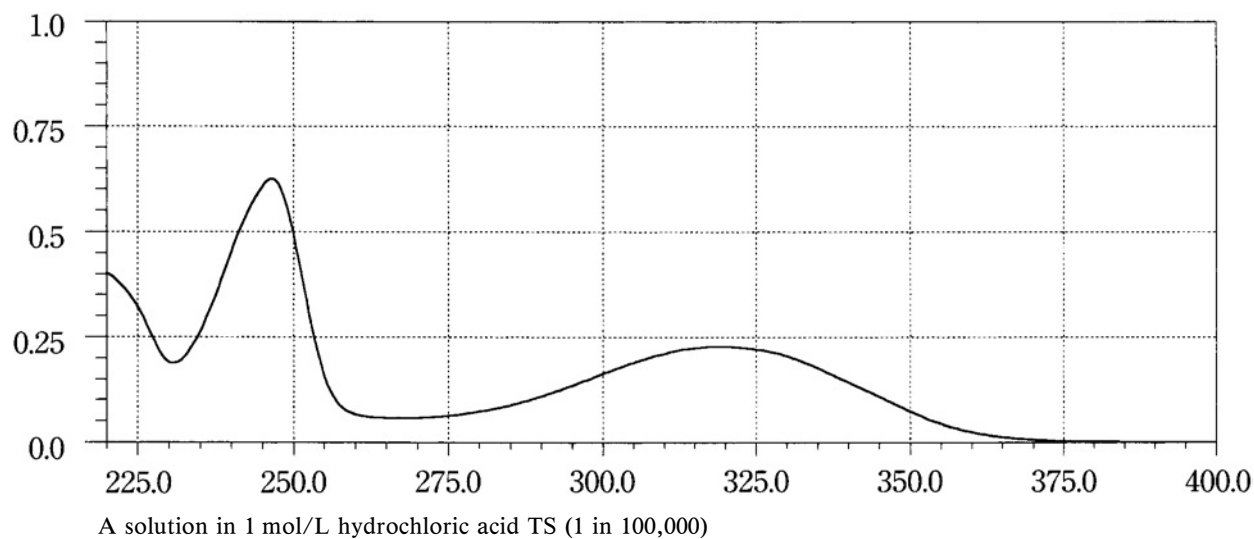
A solution in methanol (1 in 100,000)

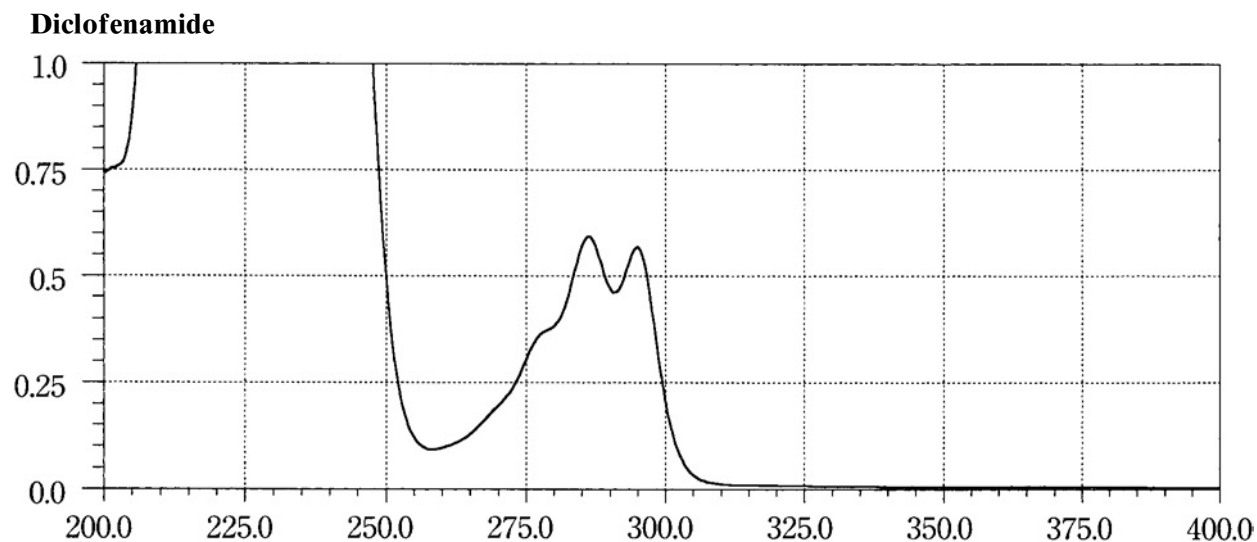
Demethylchlortetracycline Hydrochloride

A solution prepared as follows: Dissolve 40 mg in 250 mL of water. To 10 mL of this solution add 85 mL of water and 5 mL of a solution of sodium hydroxide (1 in 5).

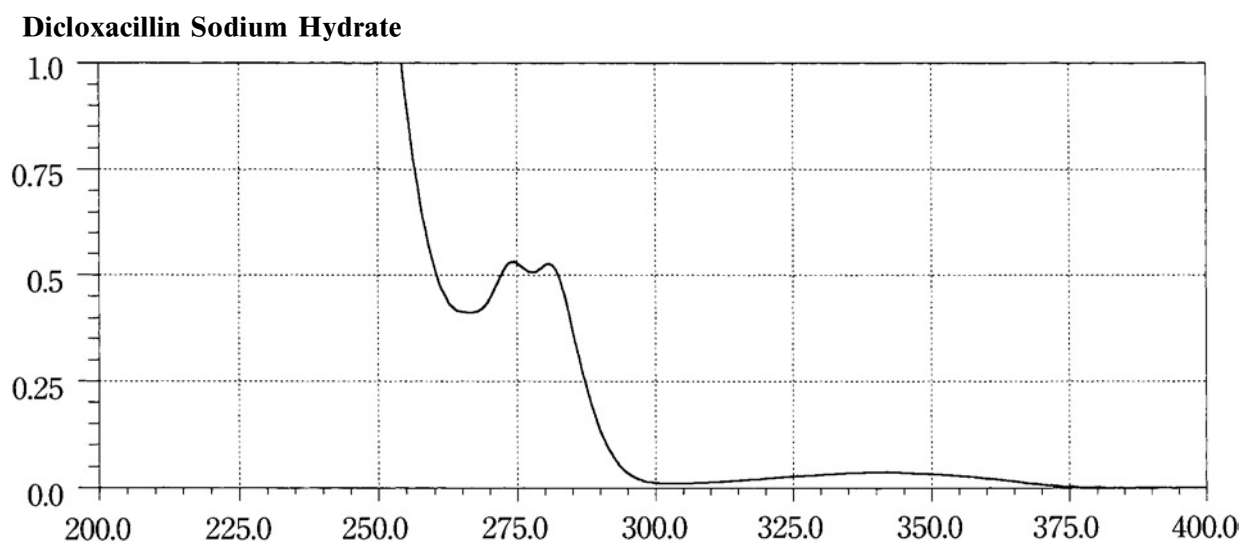
Dexamethasone

A solution prepared as follows: Dissolve 1 mg in 10 mL of ethanol (95). To 2 mL of this solution add 10 mL of phenylhydrazine hydrochloride TS, shake, warm in a water bath at 60°C for 20 minutes, and allow to cool.
Blank: A solution obtained by proceeding in the same manner with 2.0 mL of ethanol (95).

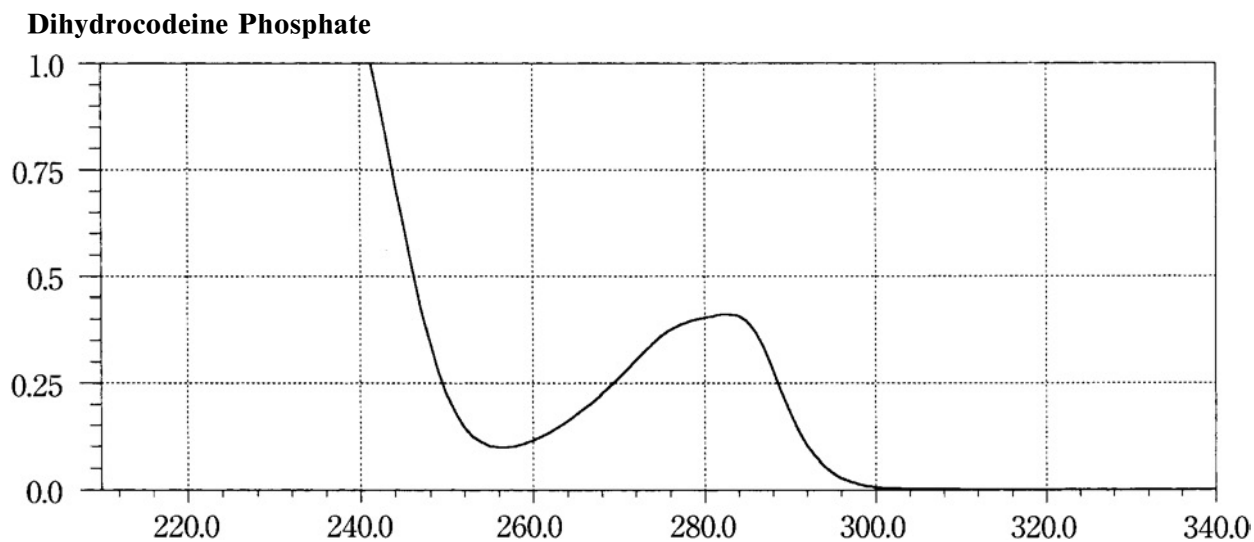
Dextromethorphan Hydrobromide Hydrate**Diazepam****Dibucaine Hydrochloride**



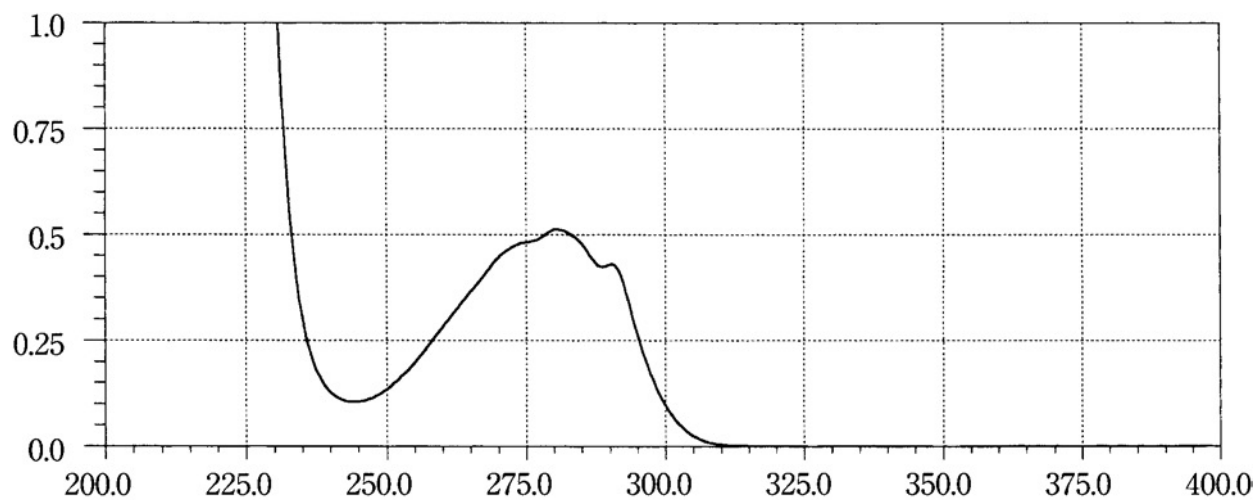
A solution prepared as follows: Dissolve 0.01 g in 100 mL of 0.01 mol/L sodium hydroxide TS. To 10 mL of this solution add 0.1 mL of hydrochloric acid.



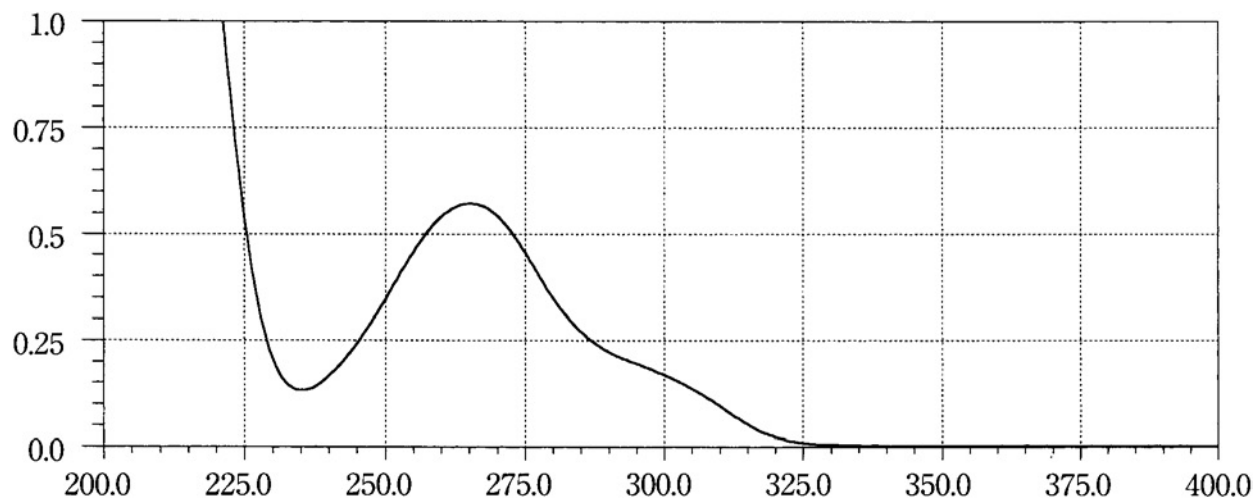
An aqueous solution (1 in 2500)



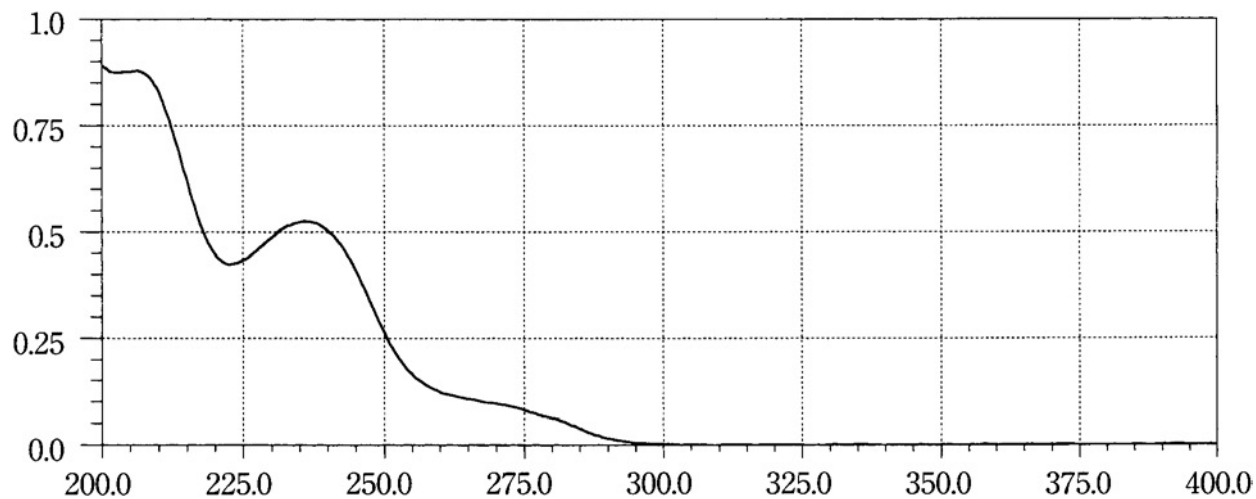
An aqueous solution (1 in 10,000)

Dihydroergotamine Mesilate

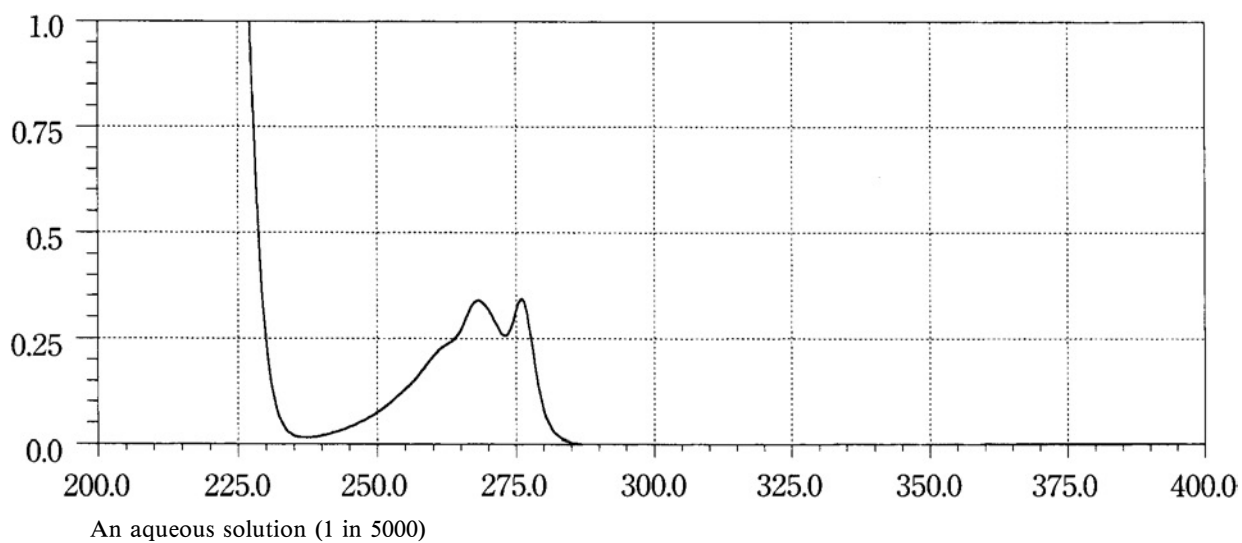
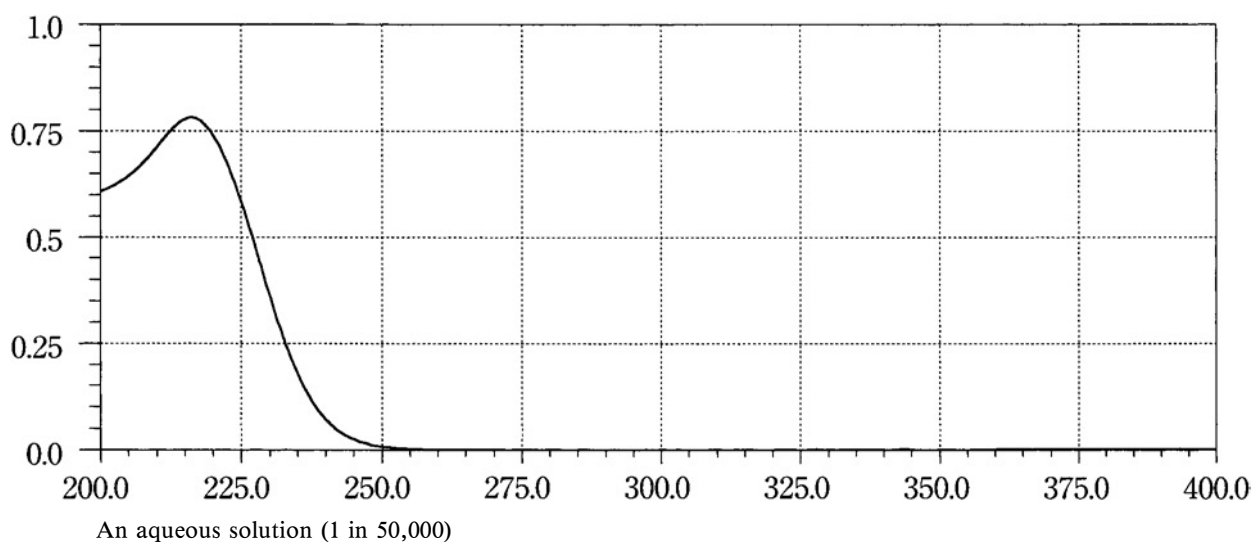
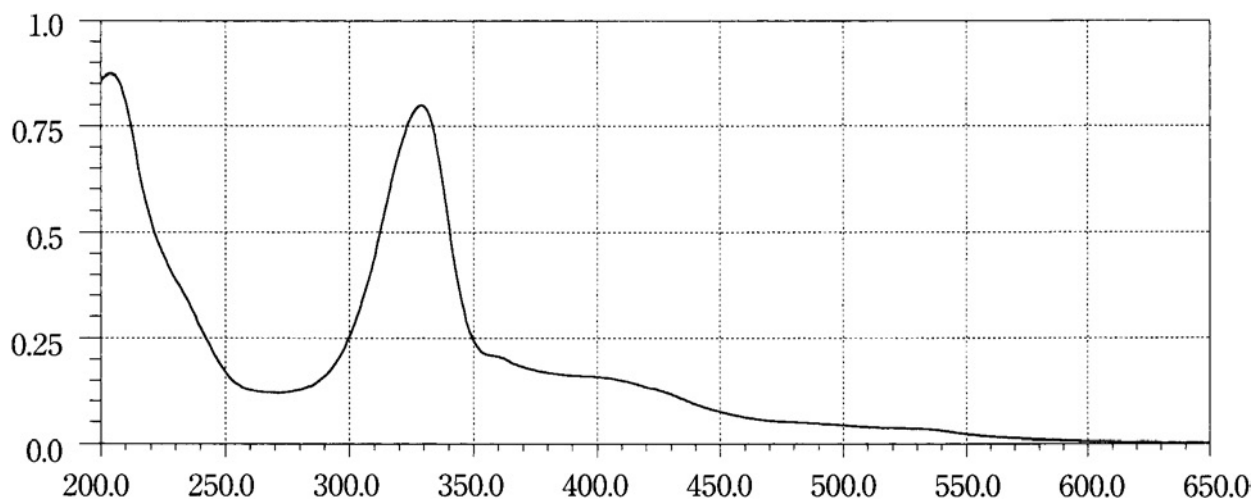
A solution in methanol (1 in 20,000)

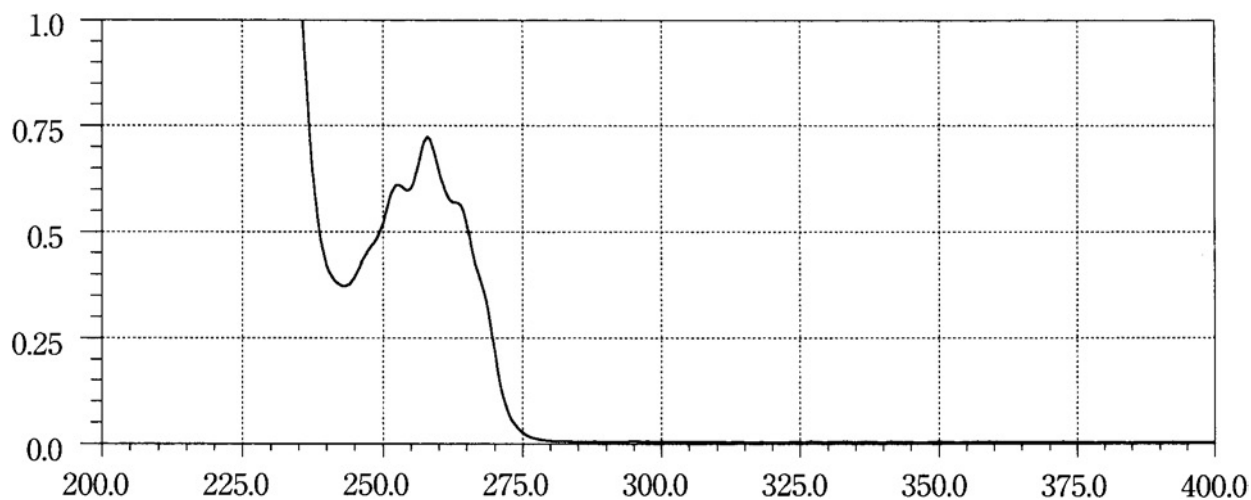
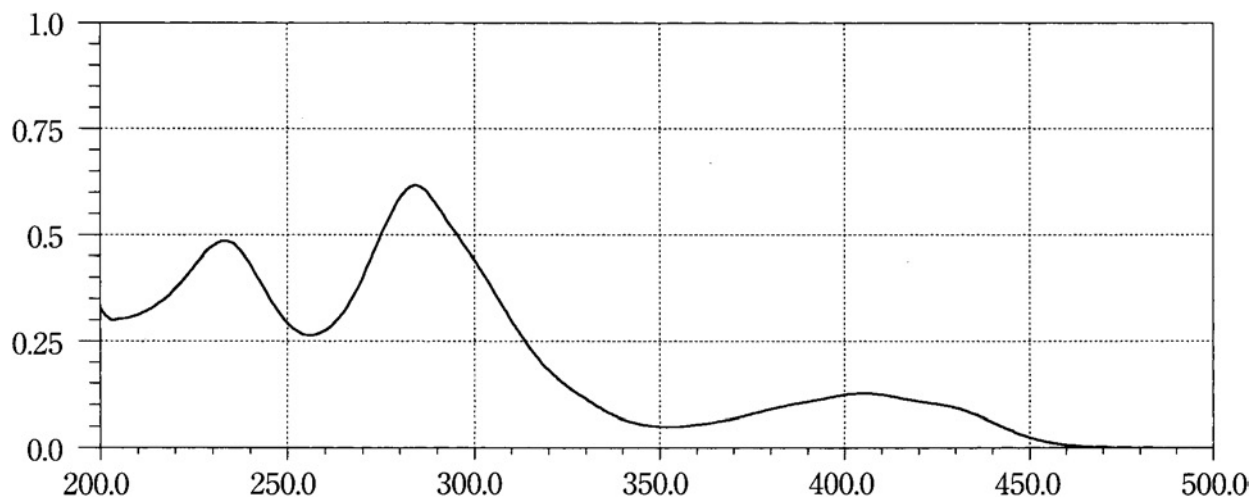
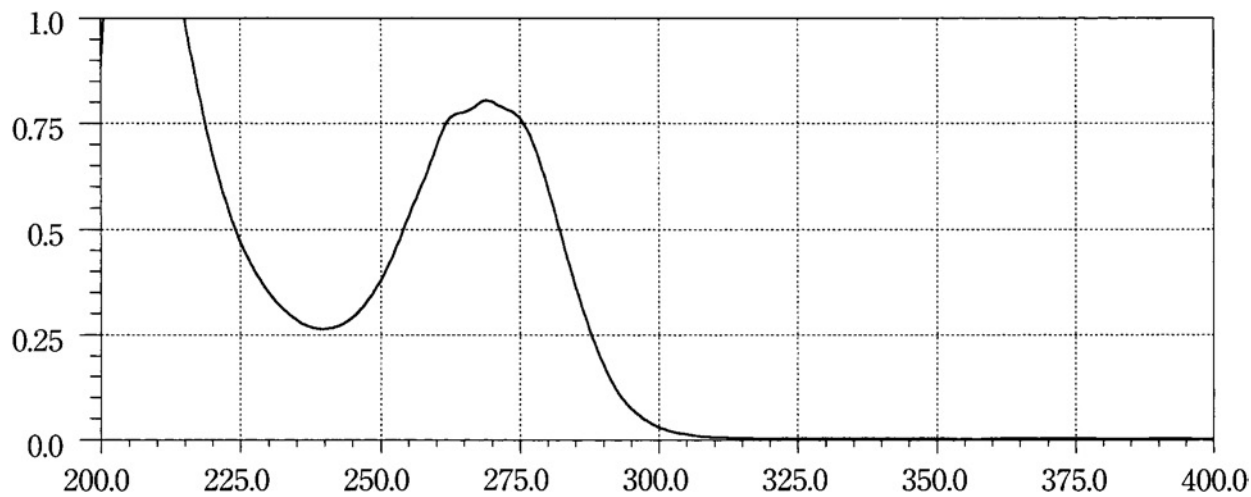
Dilazep Hydrochloride Hydrate

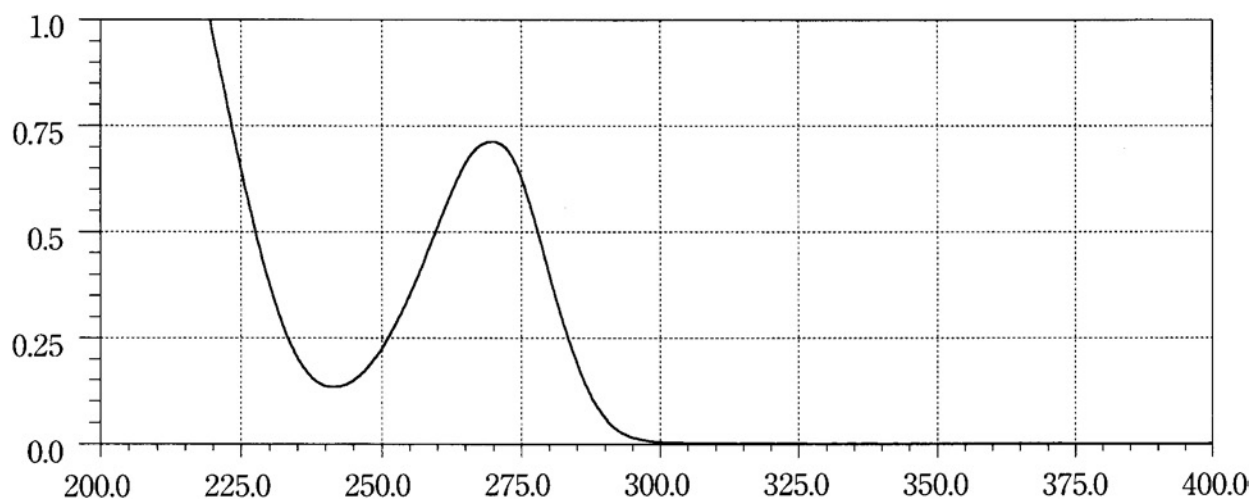
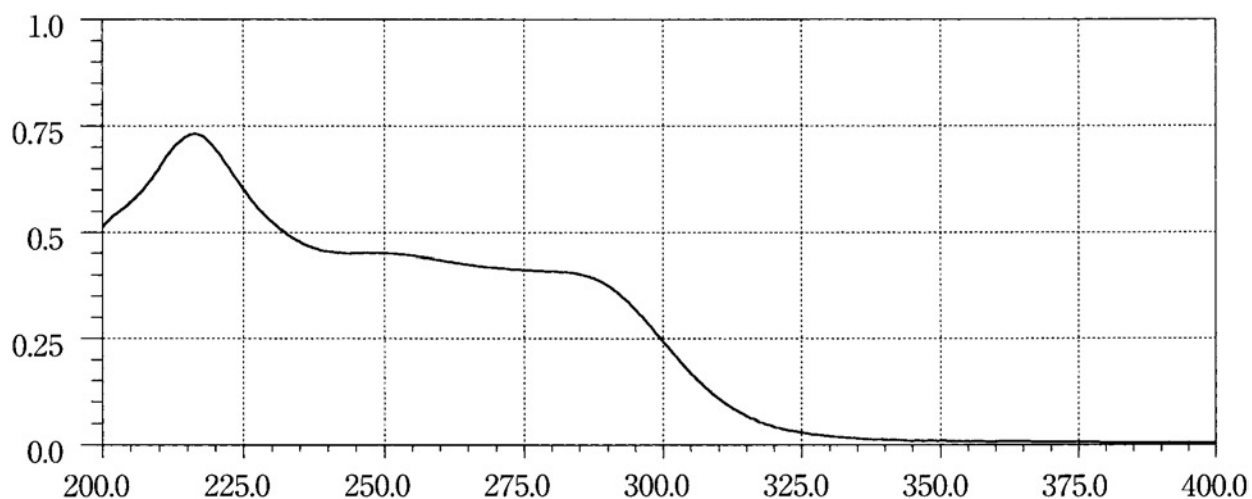
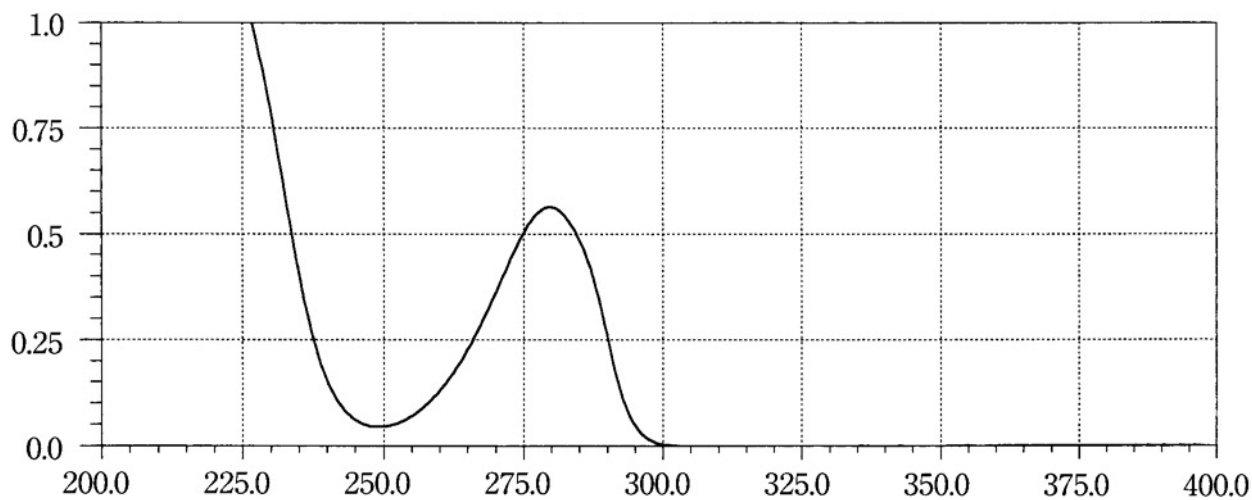
An aqueous solution (1 in 50,000)

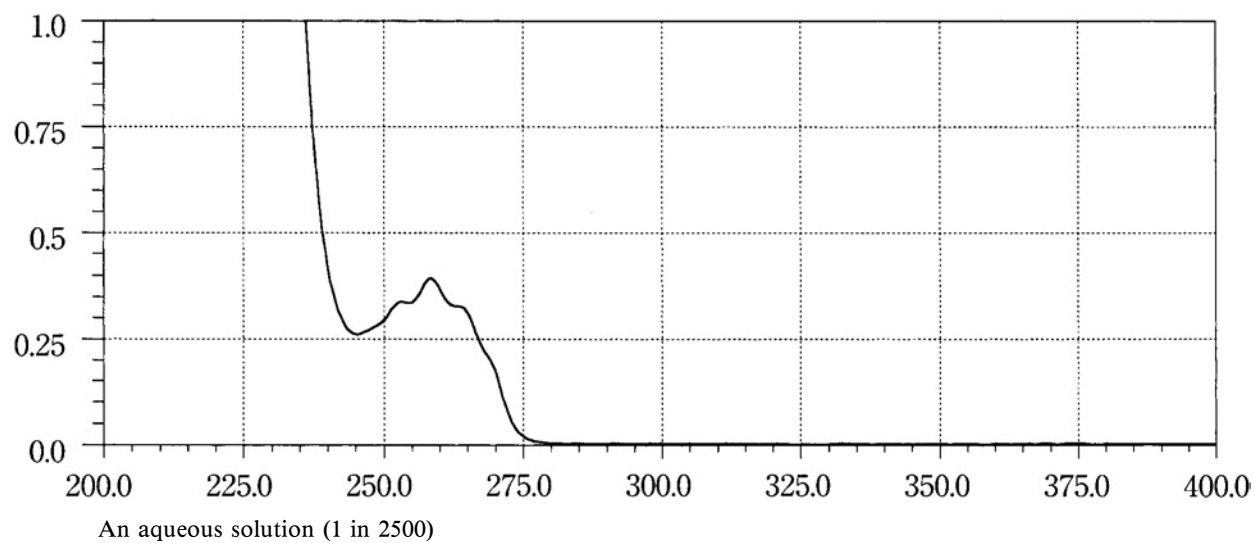
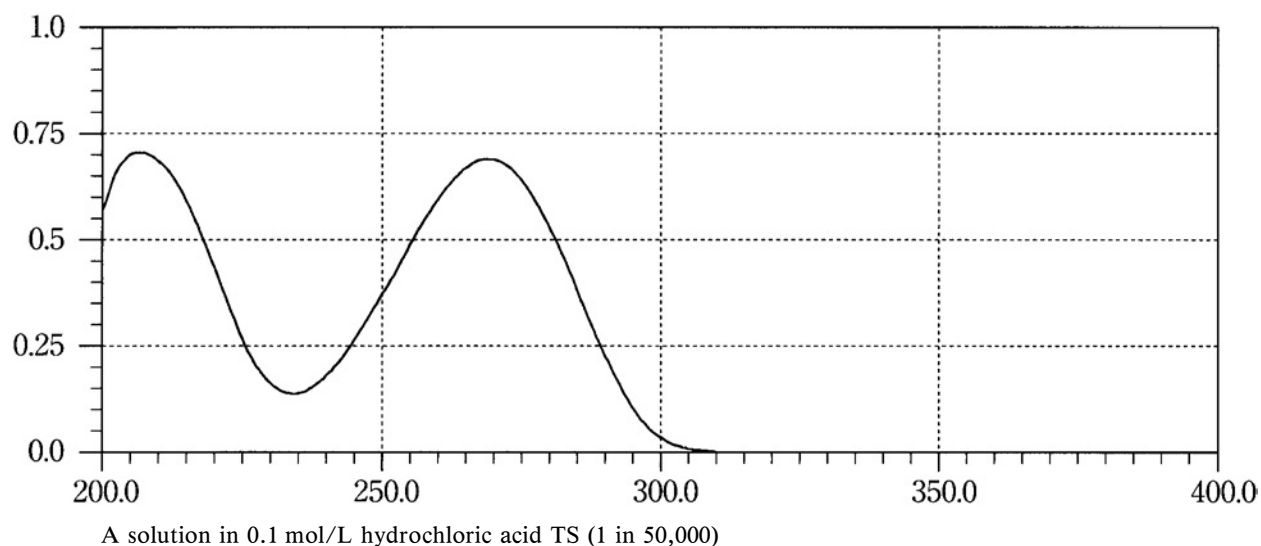
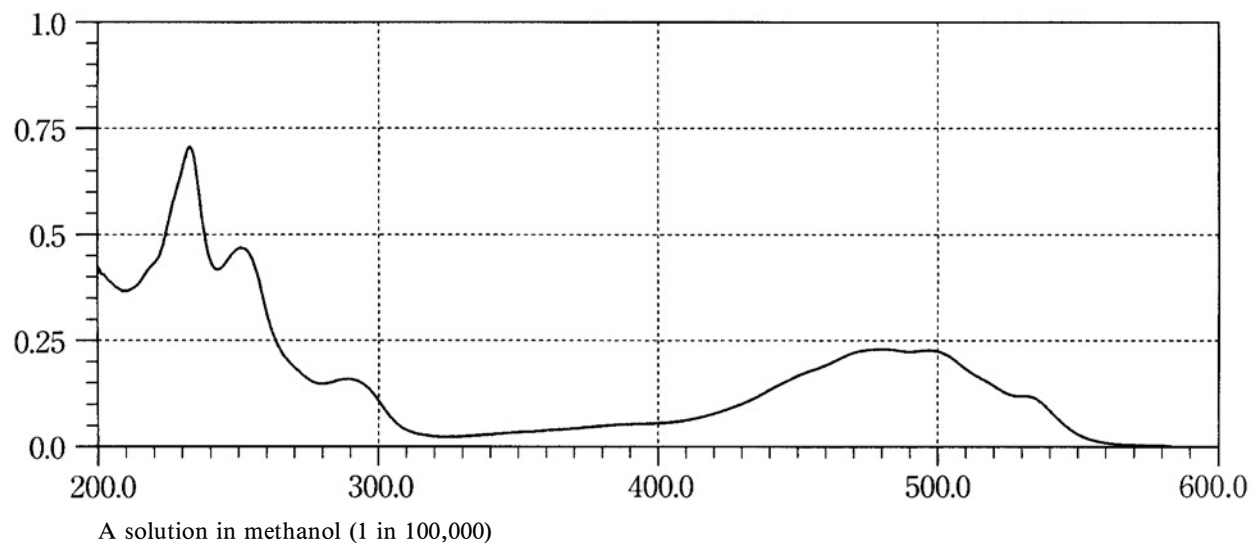
Diltiazem Hydrochloride

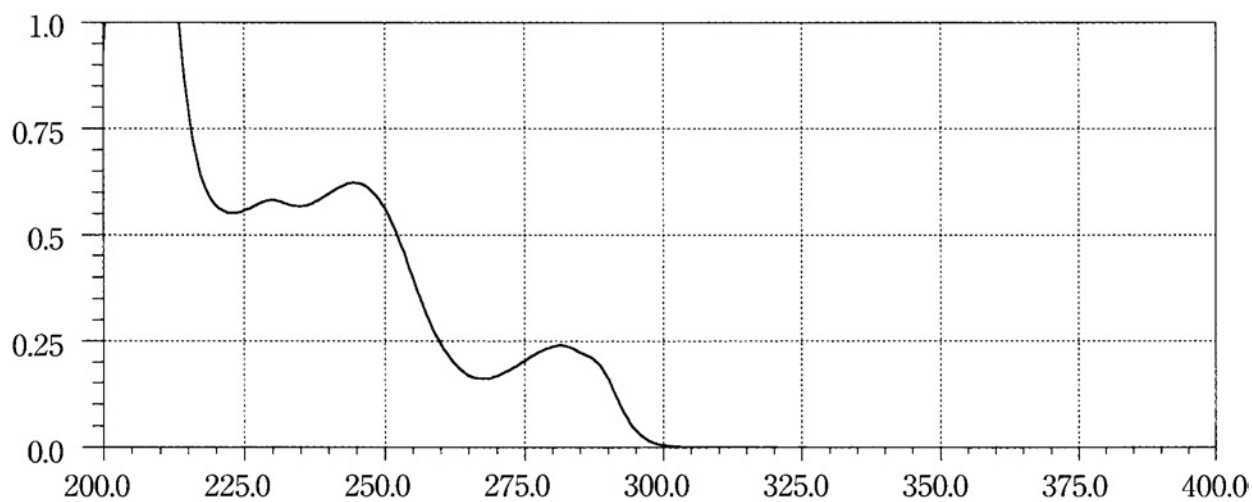
A solution in 0.01 mol/L hydrochloric acid TS (1 in 100,000)

Dimemorfan Phosphate**Dimorpholamine****Dinoprost**

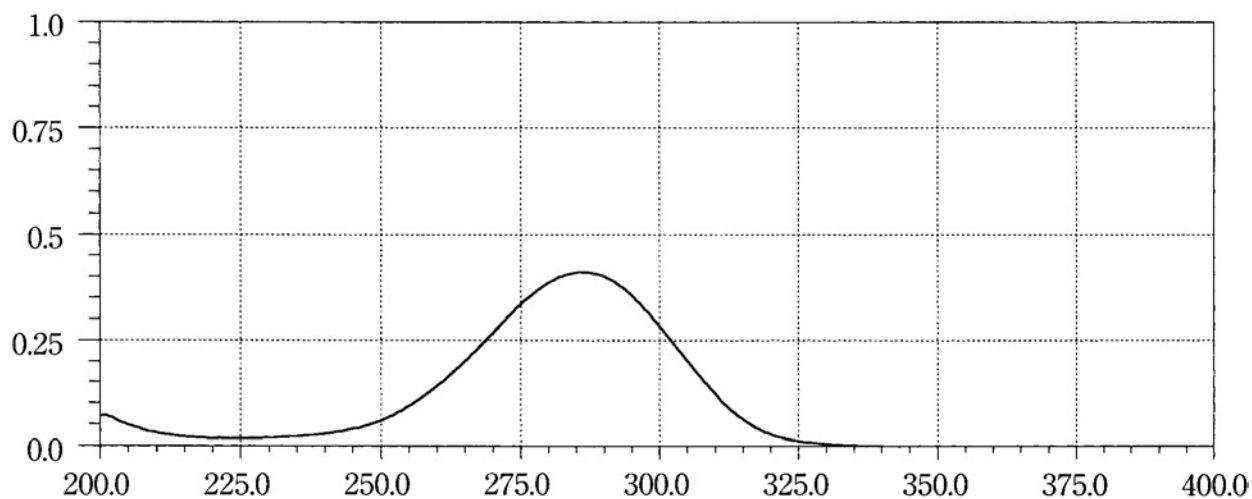
Diphenhydramine Hydrochloride**Dipyridamole****Disopyramide**

Distigmine Bromide**Disulfiram****Dopamine Hydrochloride**

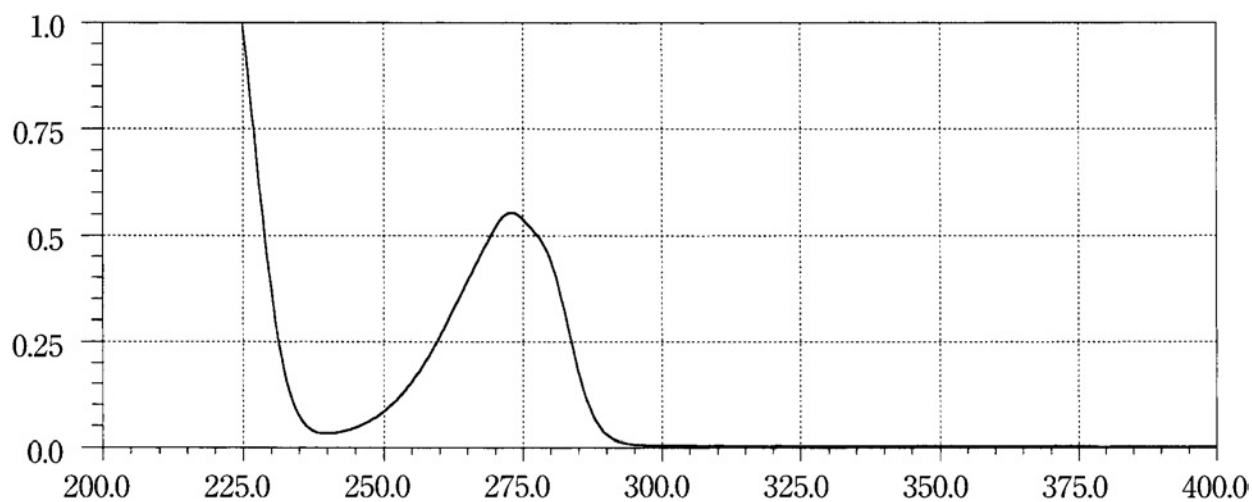
Doxapram Hydrochloride Hydrate**Doxifluridine****Doxorubicin Hydrochloride**

Droperidol

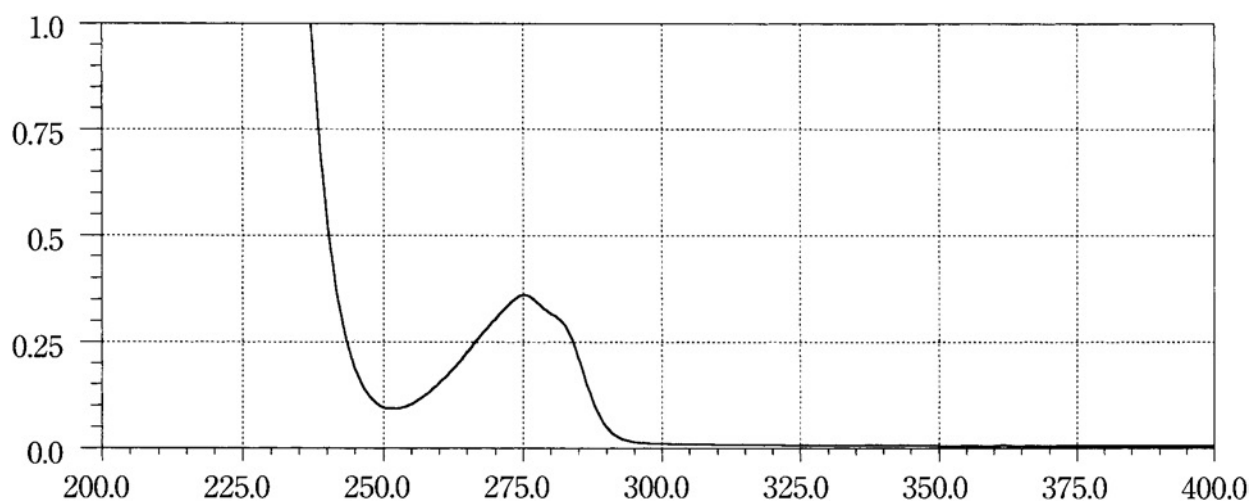
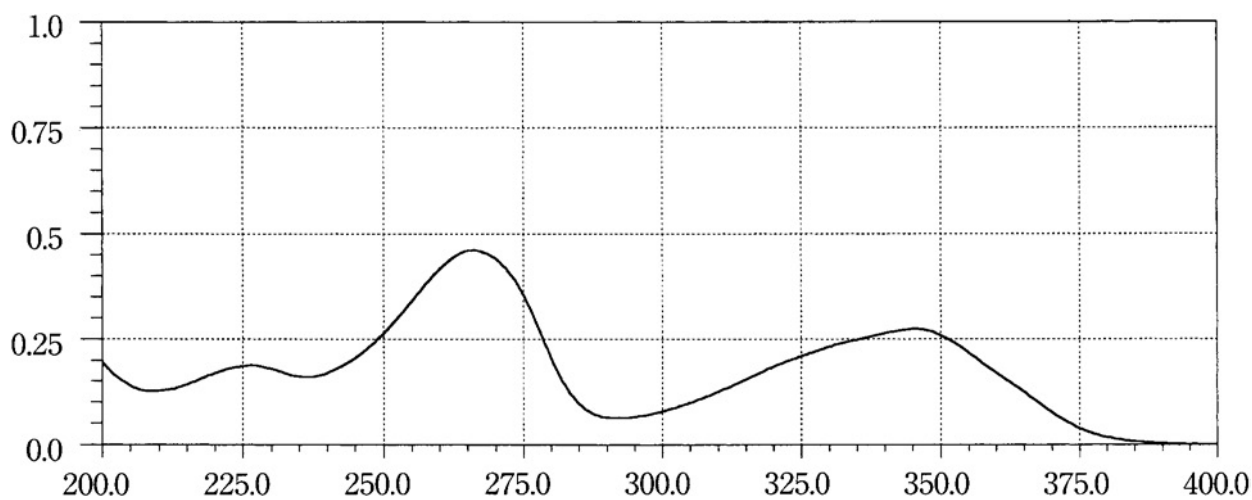
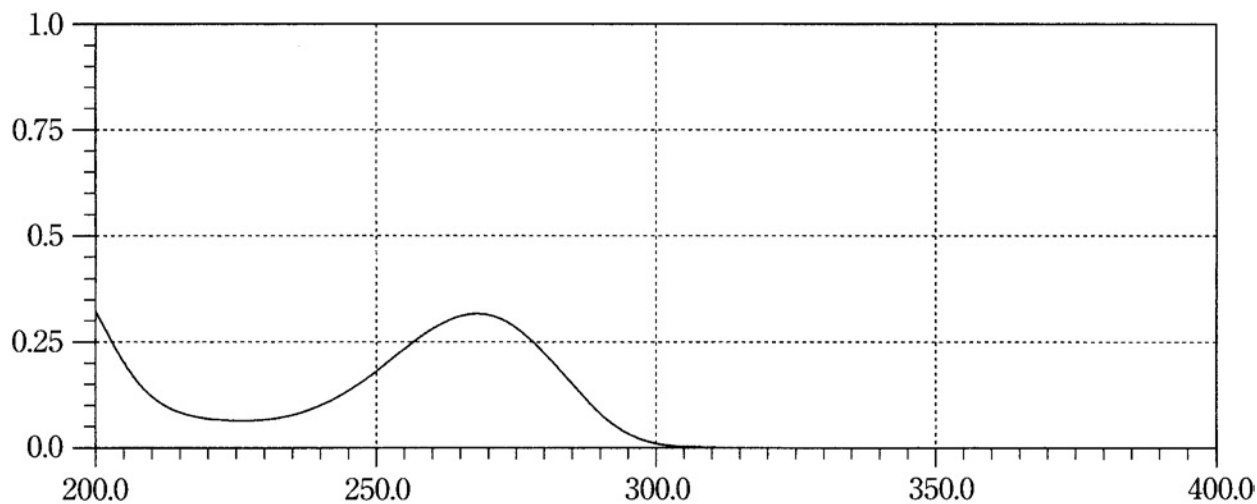
A solution prepared as follows: Dissolve 0.03 g in 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL (in a brown volumetric flask). To 5 mL of this solution add 10 mL of 0.1 mol/L hydrochloric acid TS and ethanol (95) to make 100 mL (in a brown volumetric flask).

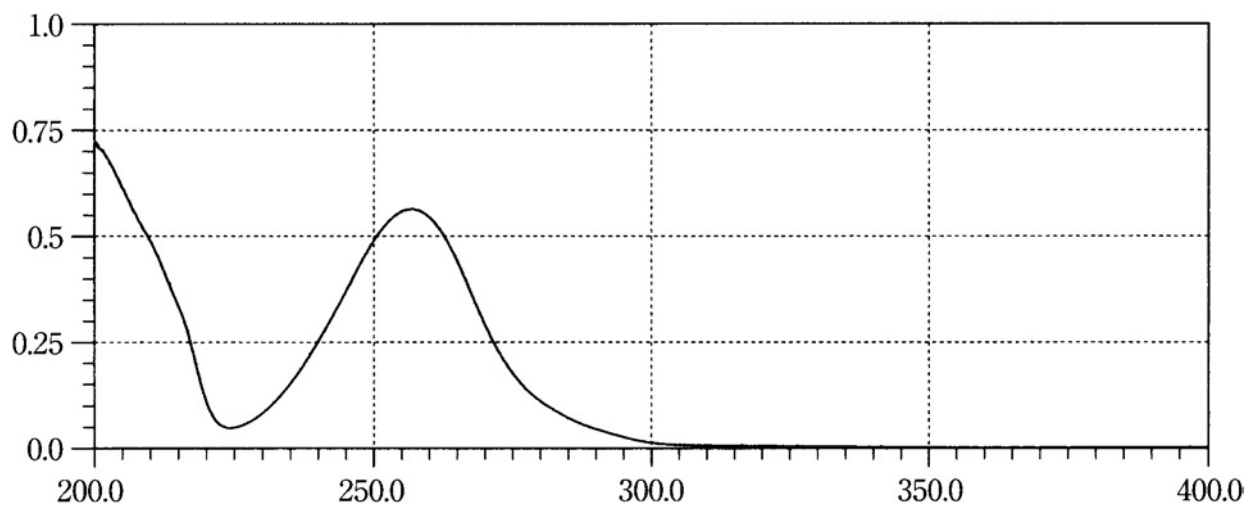
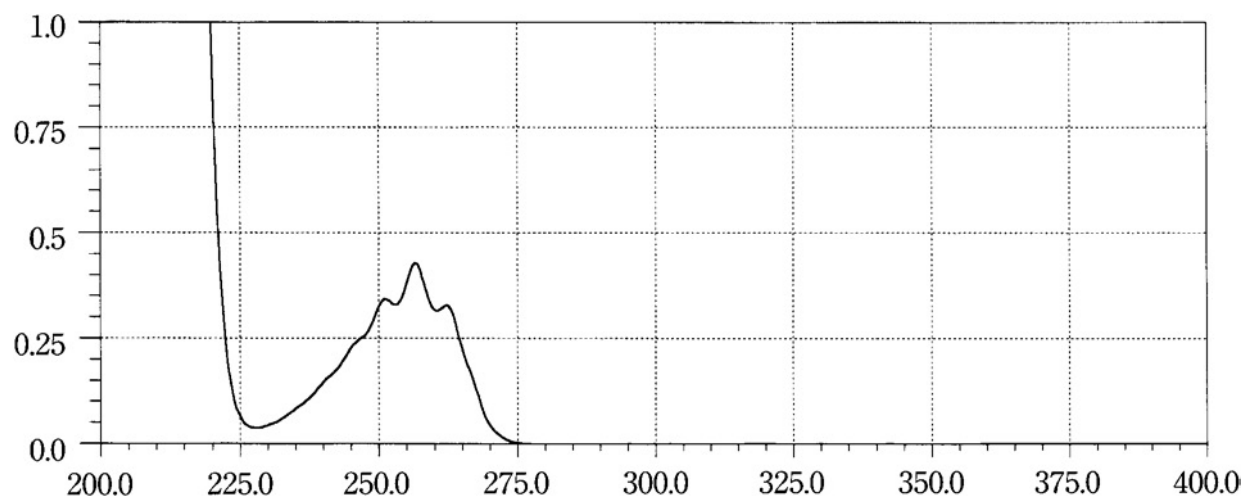
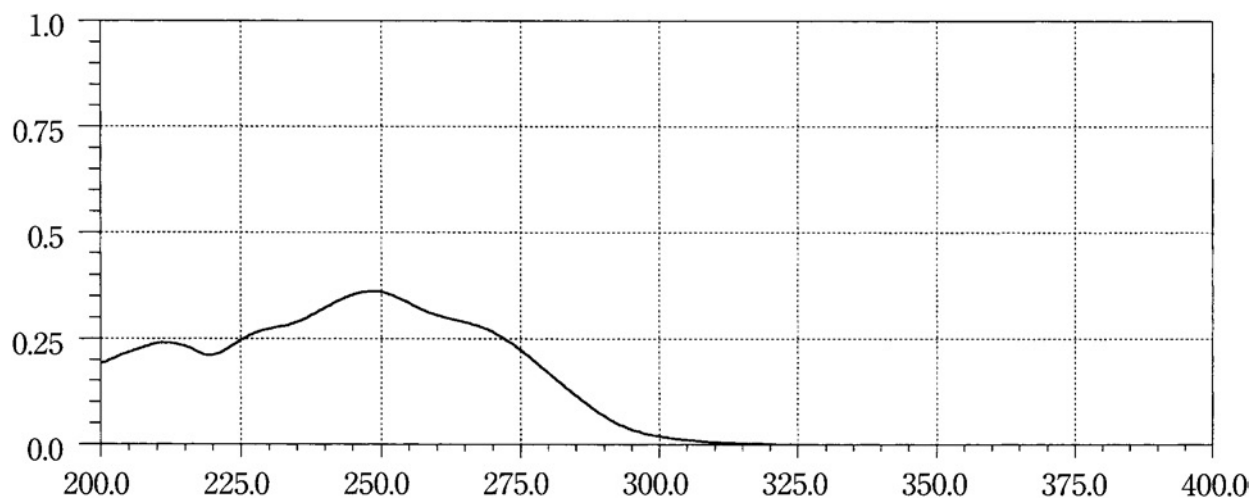
Dydrogesterone

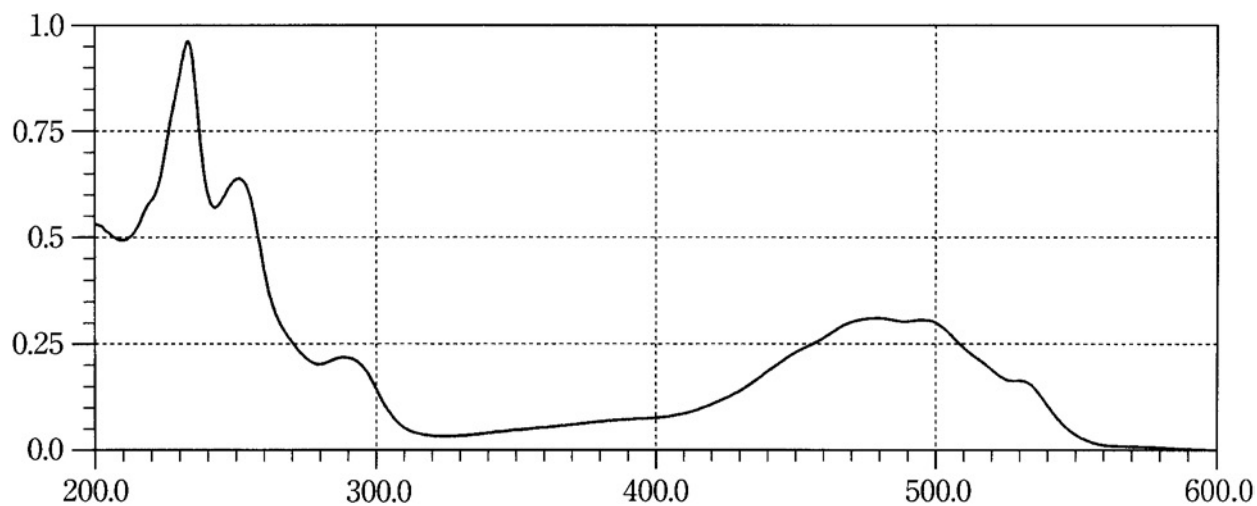
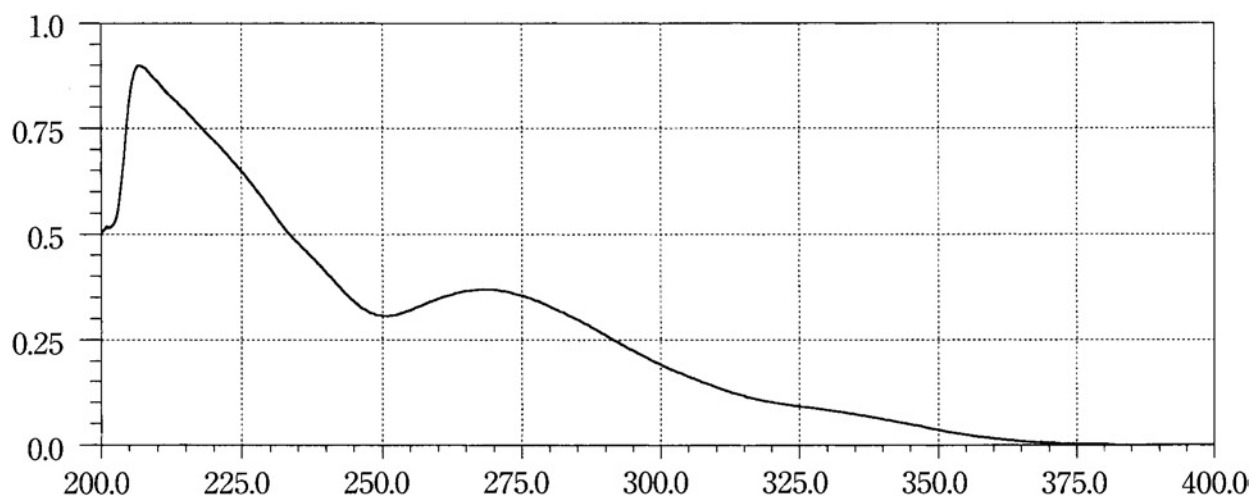
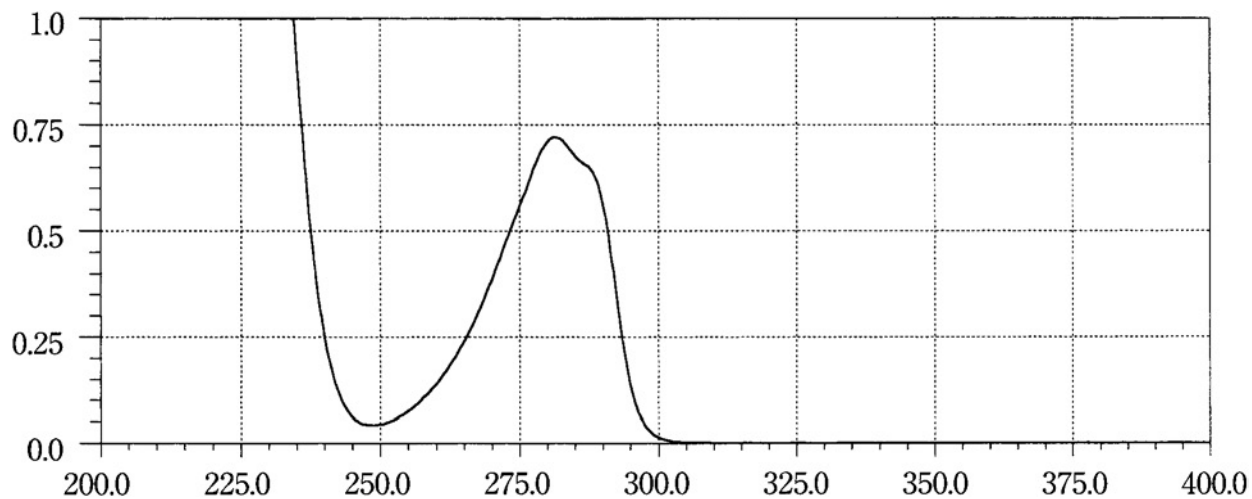
A solution in methanol (1 in 200,000)

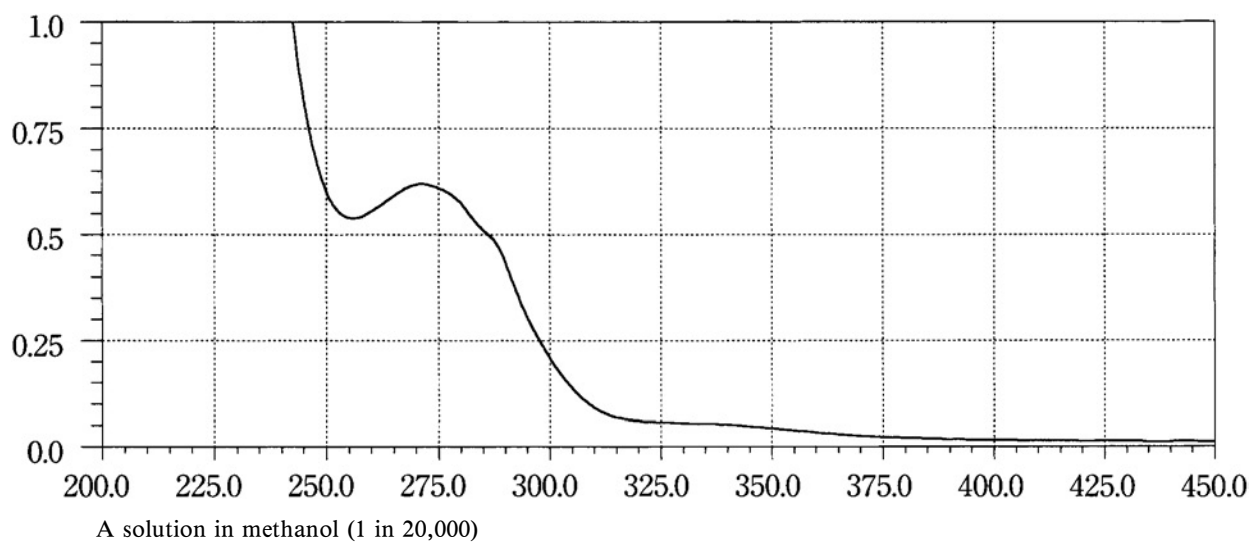
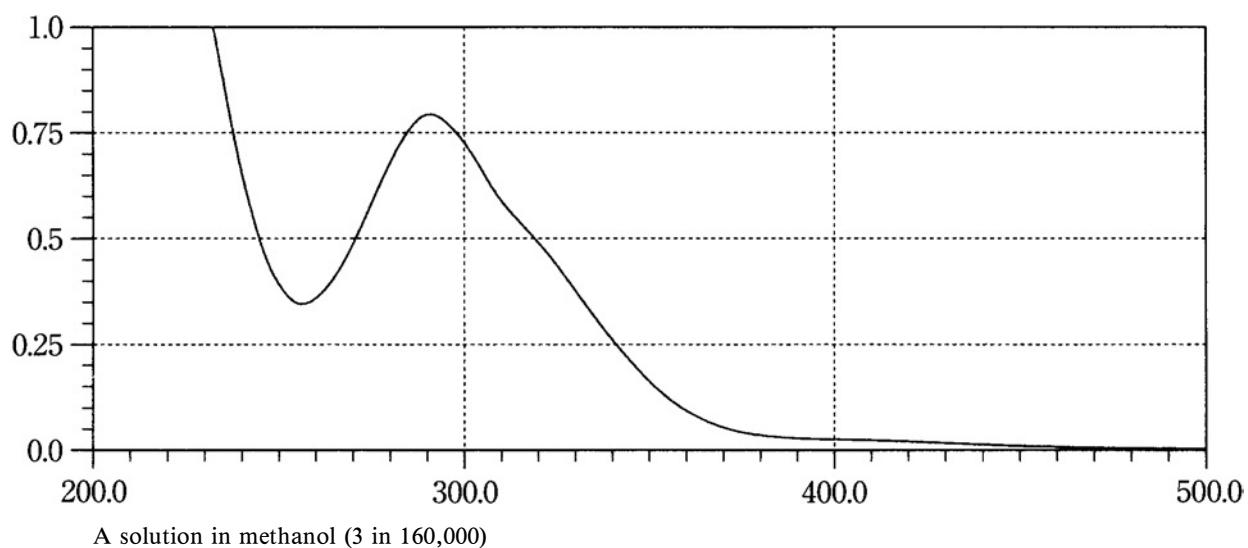
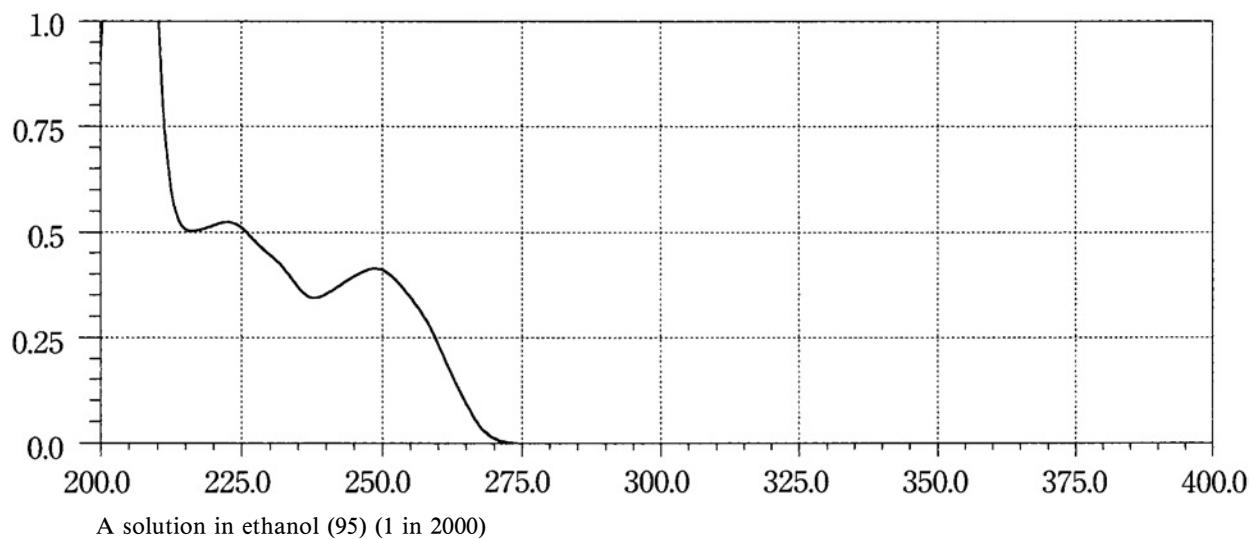
Edrophonium Chloride

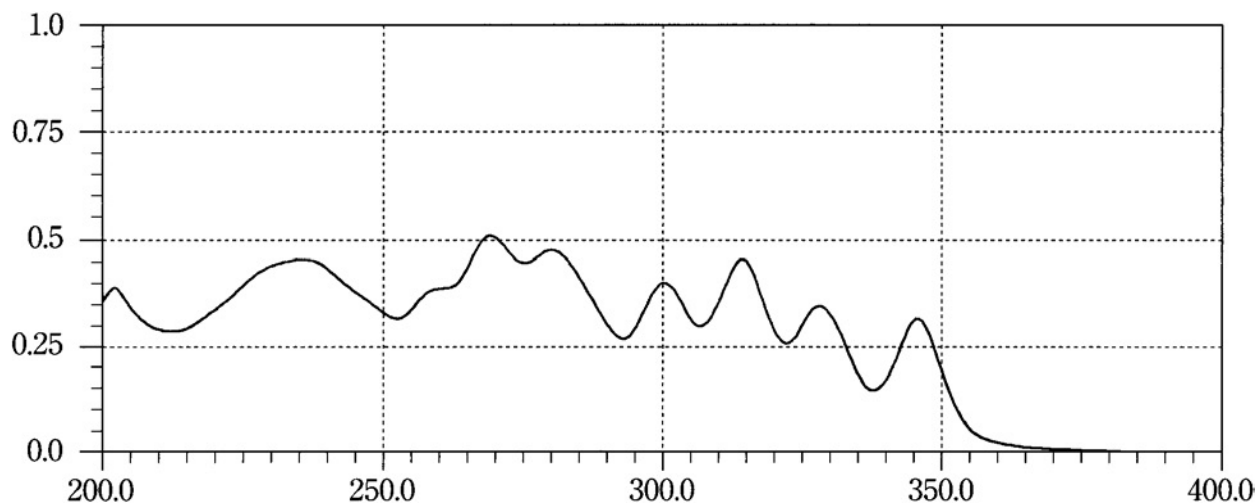
A solution in 0.1 mol/L hydrochloric acid TS (1 in 20,000)

Elcatonin**Enoxacin Hydrate****Enviomycin Sulfate**

Eperisone Hydrochloride**Ephedrine Hydrochloride****Epirizole**

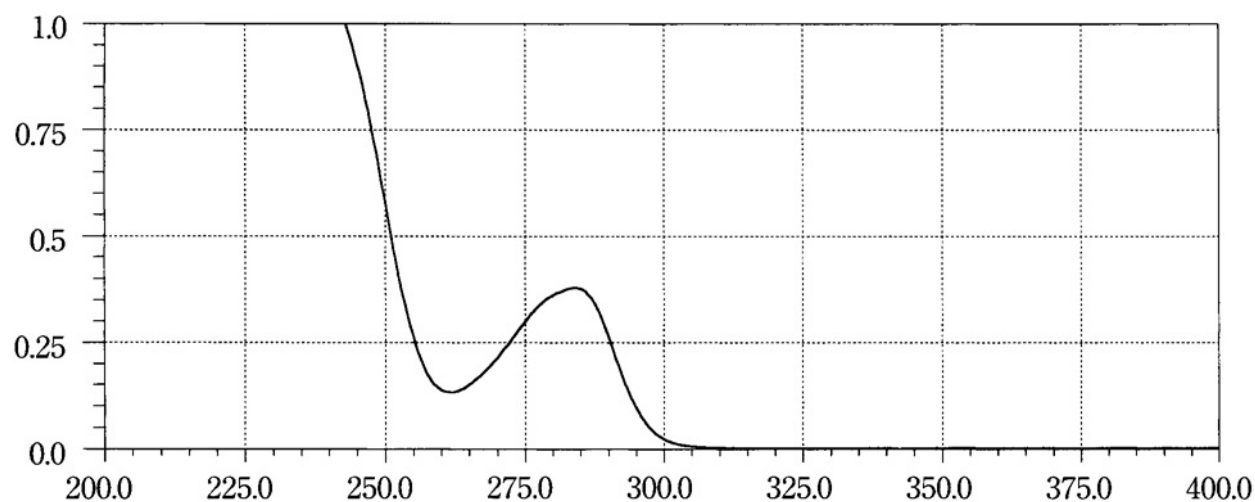
Epirubicin Hydrochloride**Estazolam****Estriol**

Etacrynic Acid**Ethionamide****Ethosuximide**

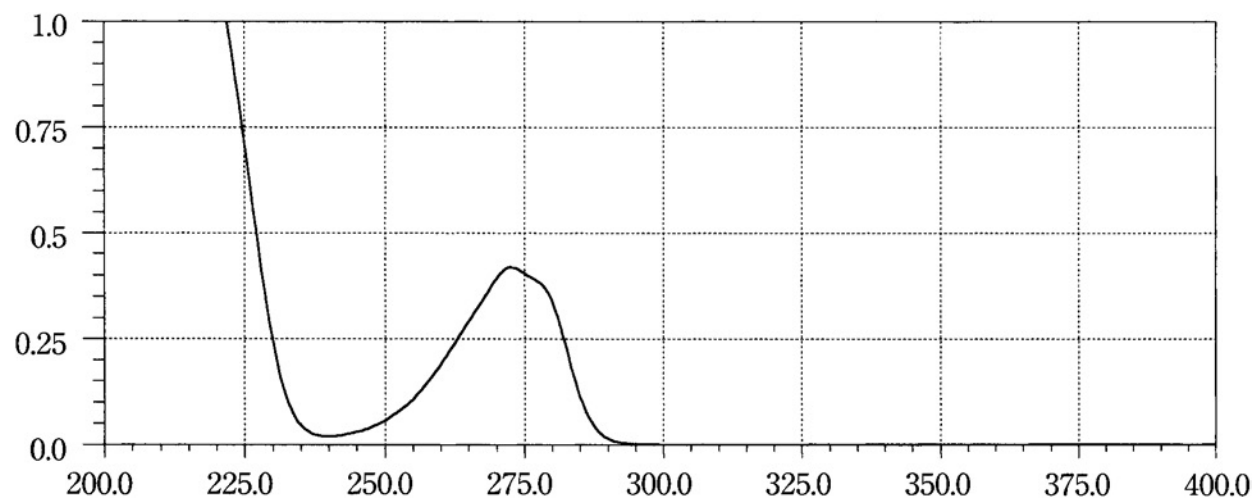
Ethyl Icosapentate

To 20 mg add 3 mL of a solution of potassium hydroxide in ethylene glycol (21 in 100), stopper tightly after passing of Nitrogen, heat at 180°C for 15 minutes, and add methanol to make 100 mL after cooling. To 4 mL of this solution add methanol to make 100 mL.

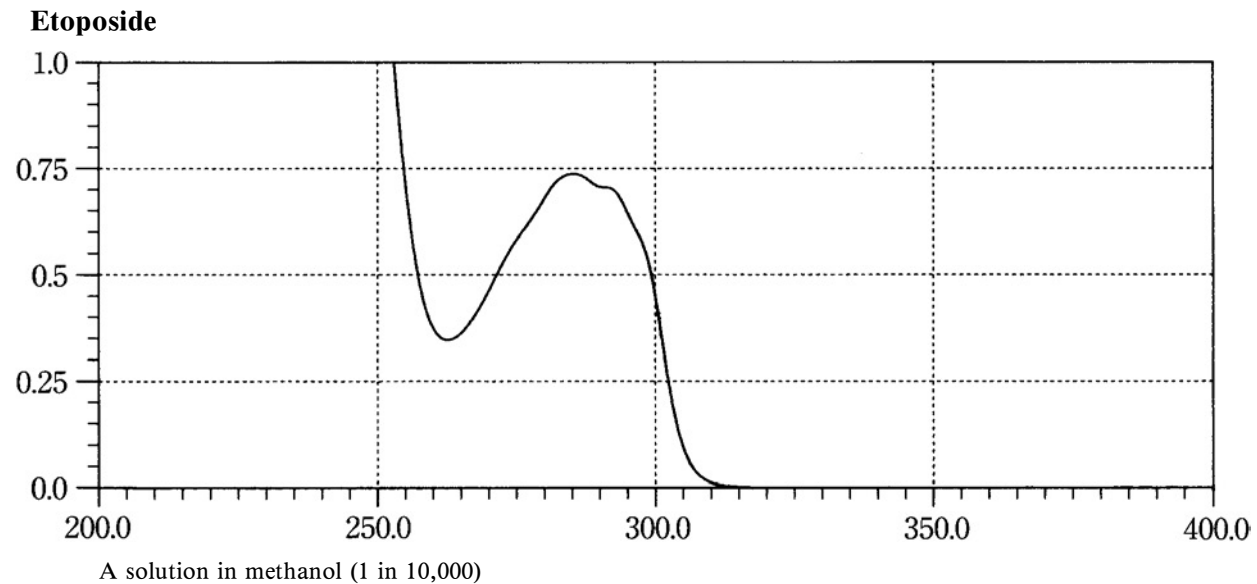
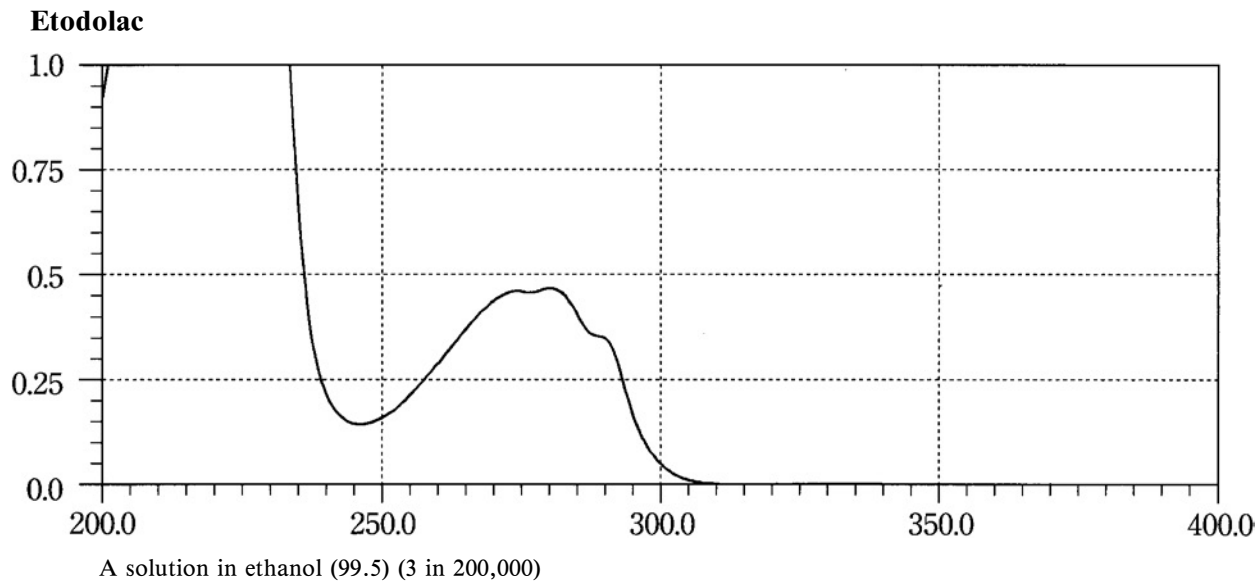
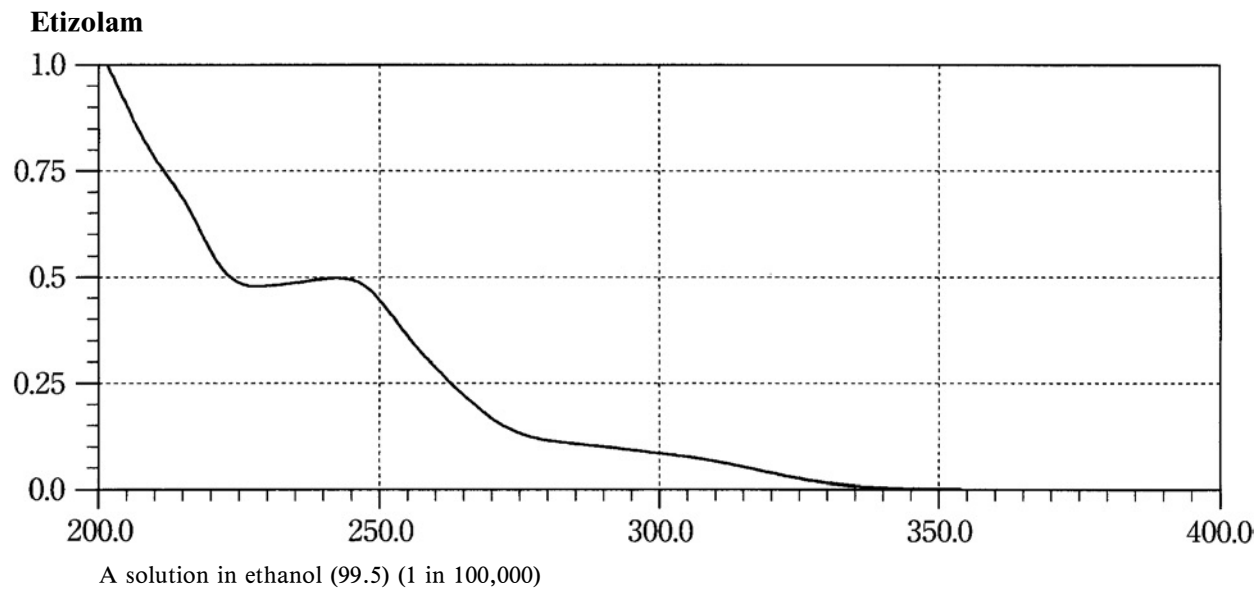
Blank: A solution obtained by proceeding in the same manner with 3 mL of the solution of potassium hydroxide in ethylene glycol (21 in 100).

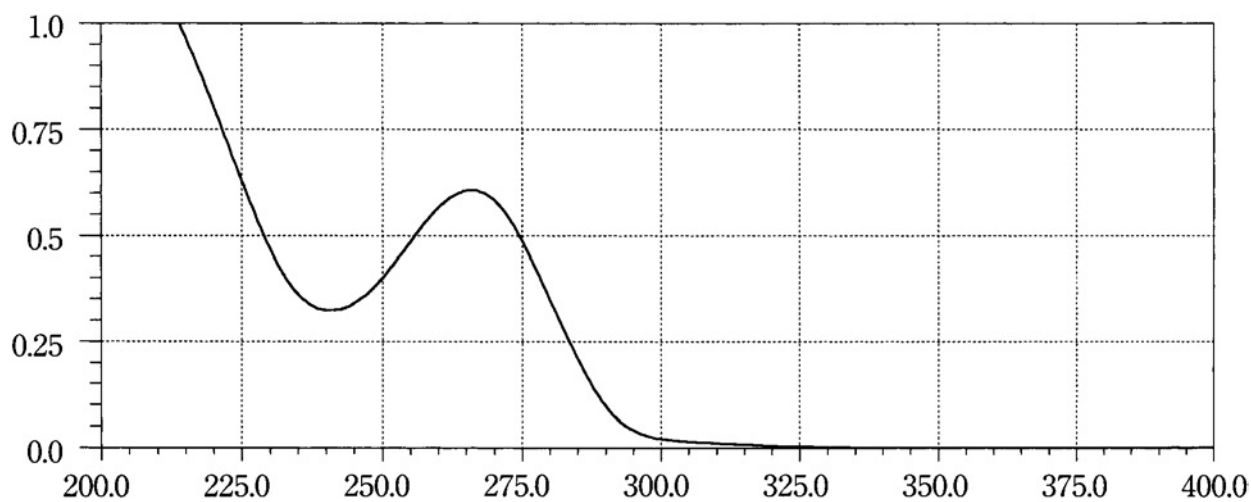
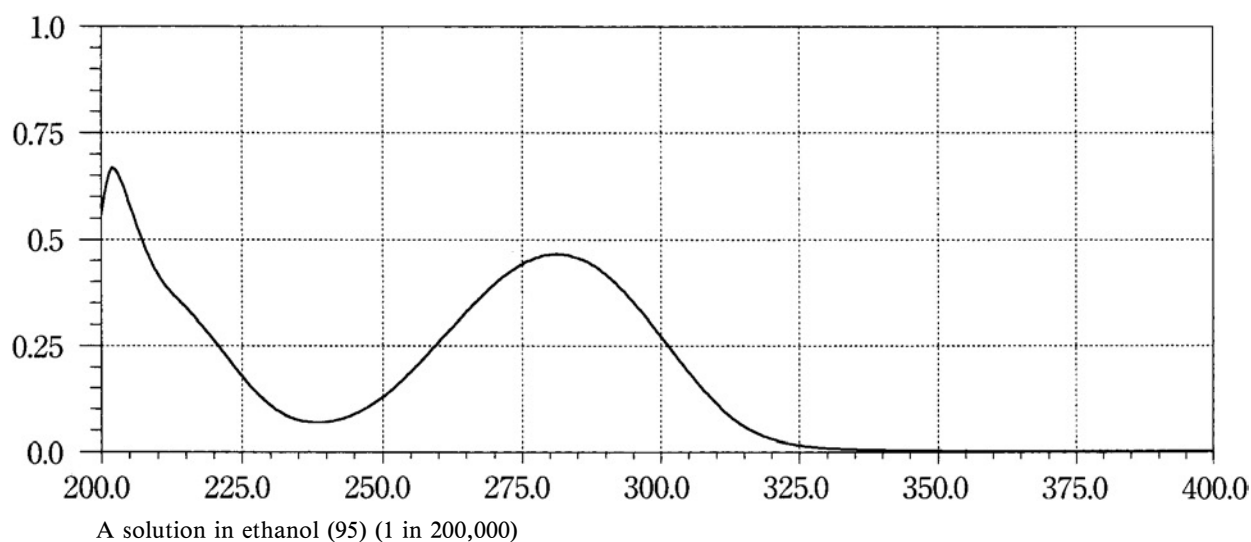
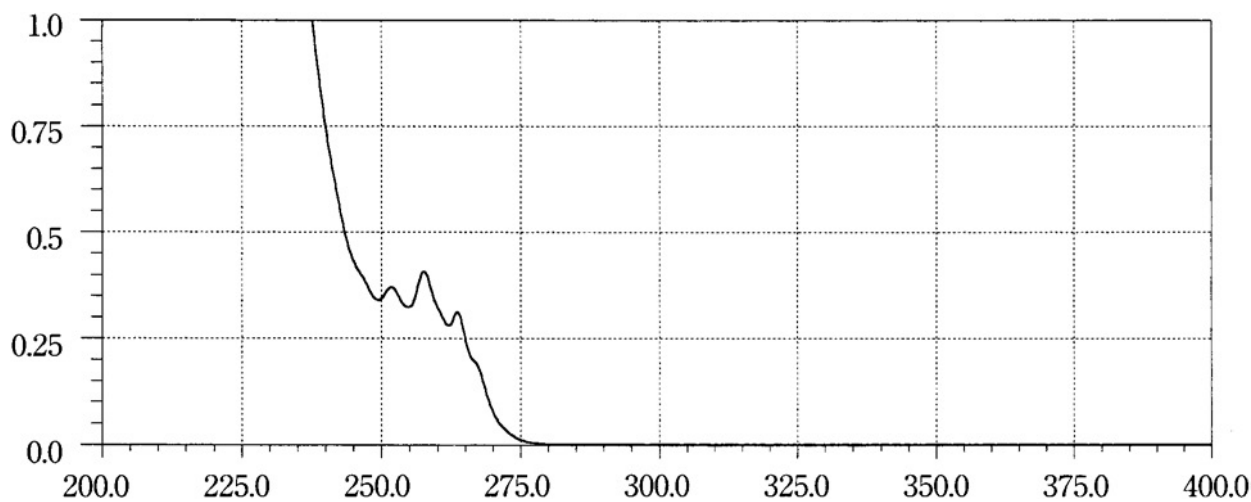
Ethylmorphine Hydrochloride Hydrate

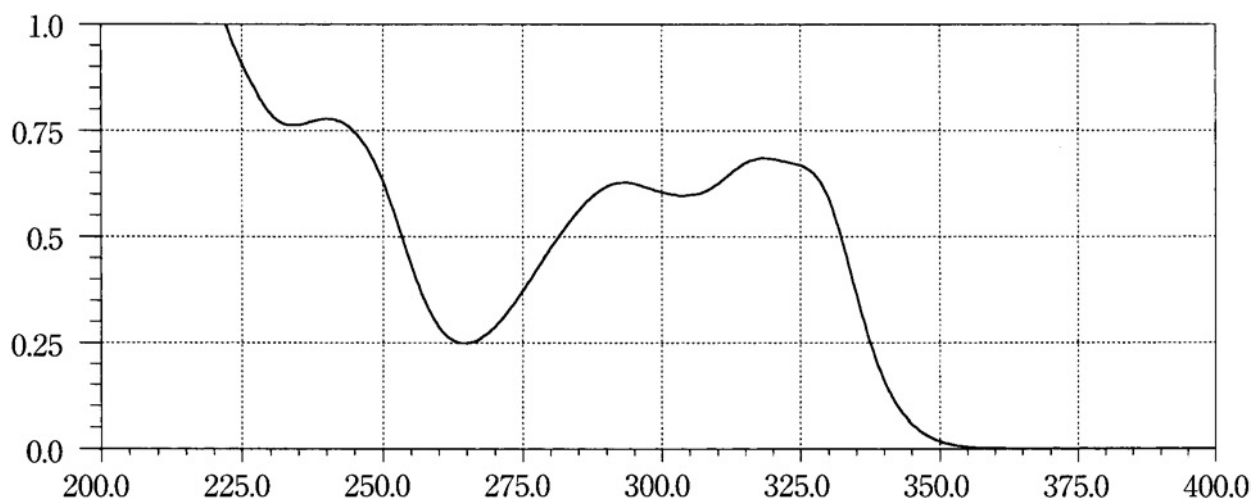
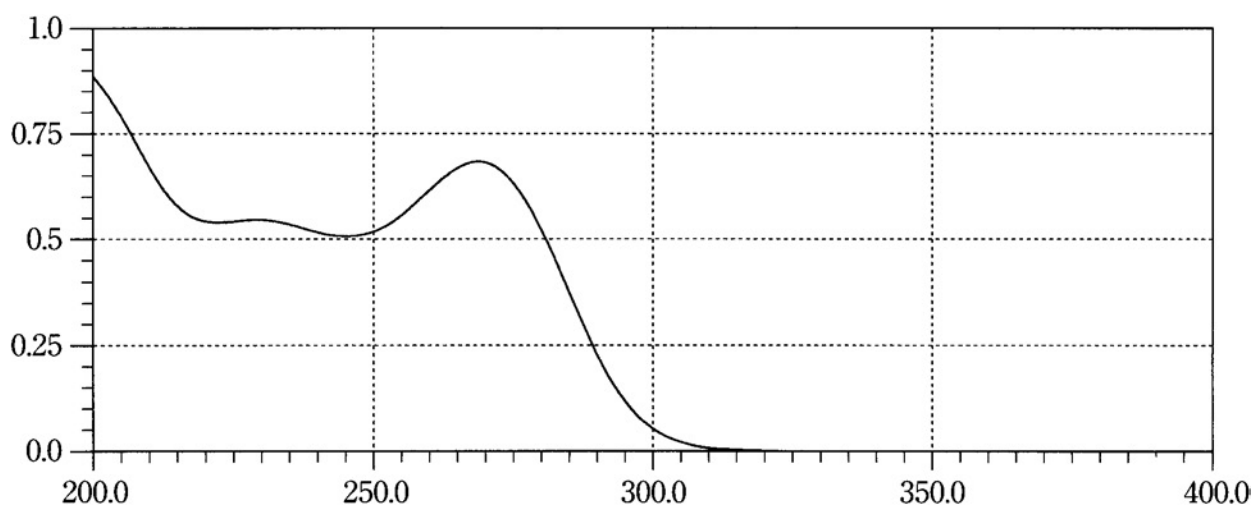
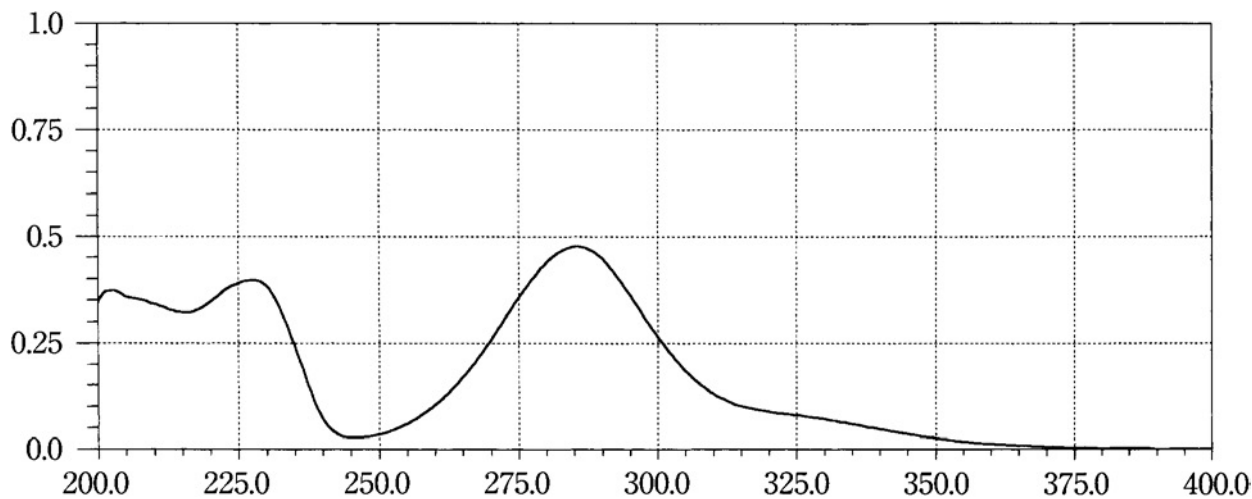
An aqueous solution (1 in 10,000)

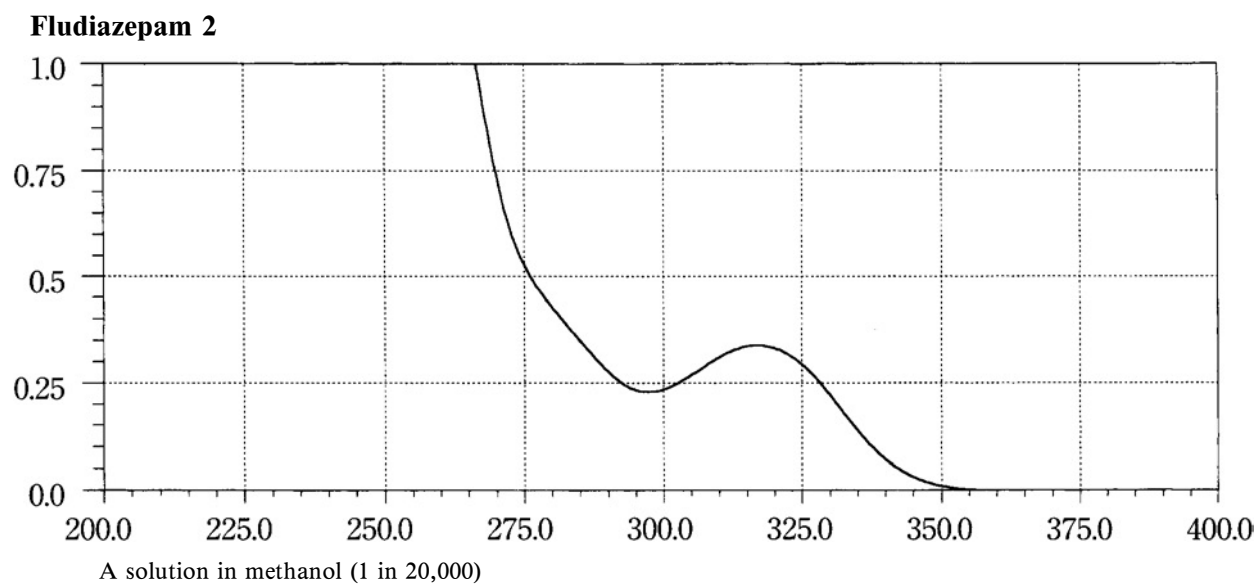
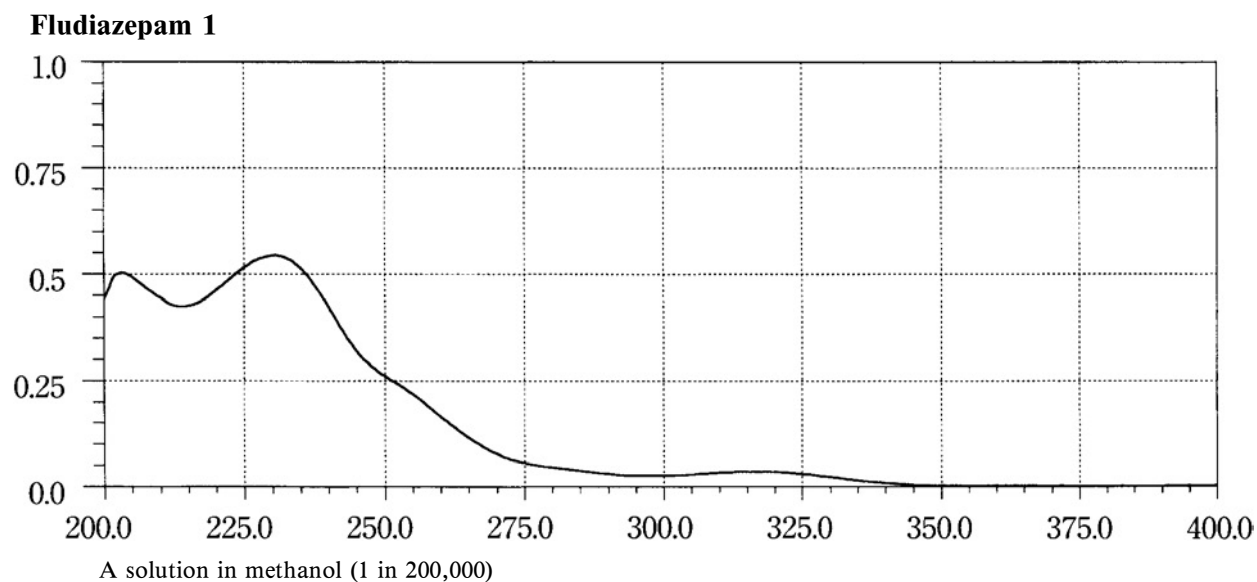
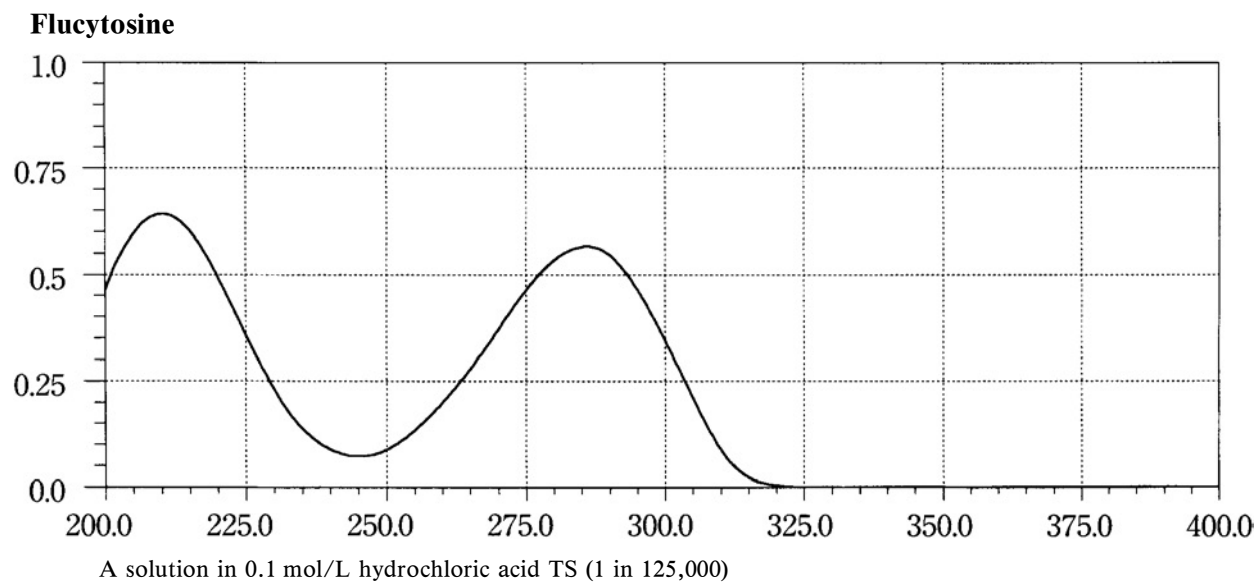
Etilefrine Hydrochloride

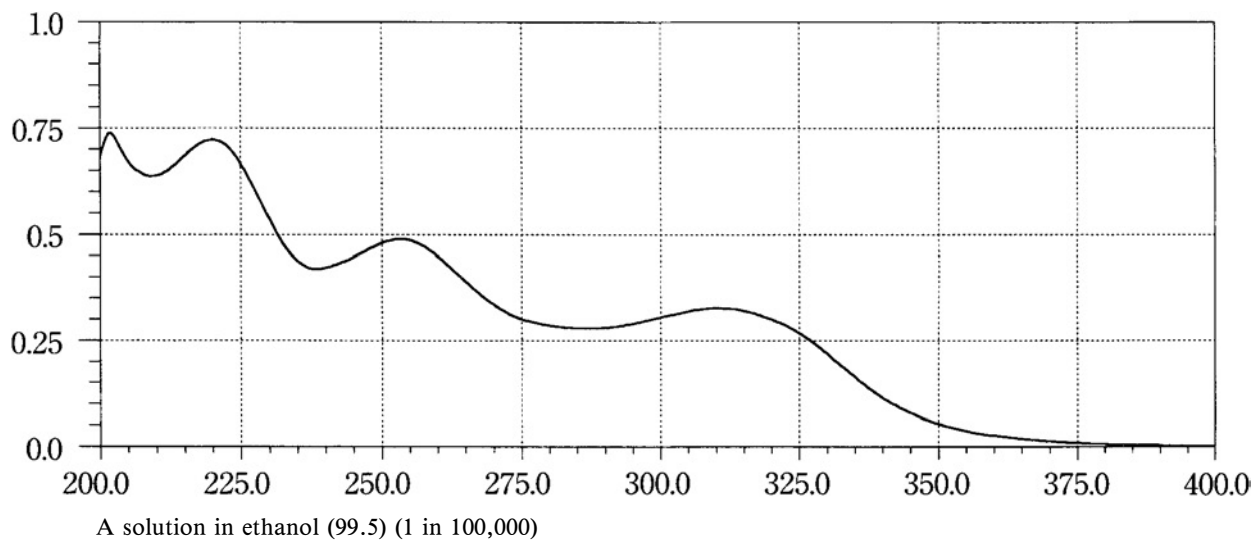
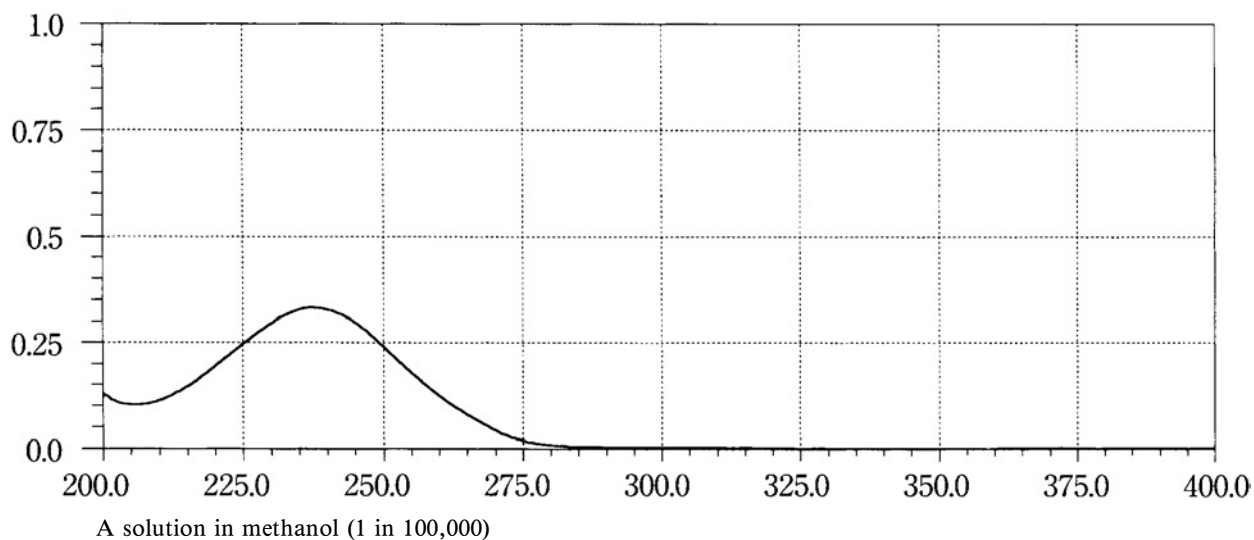
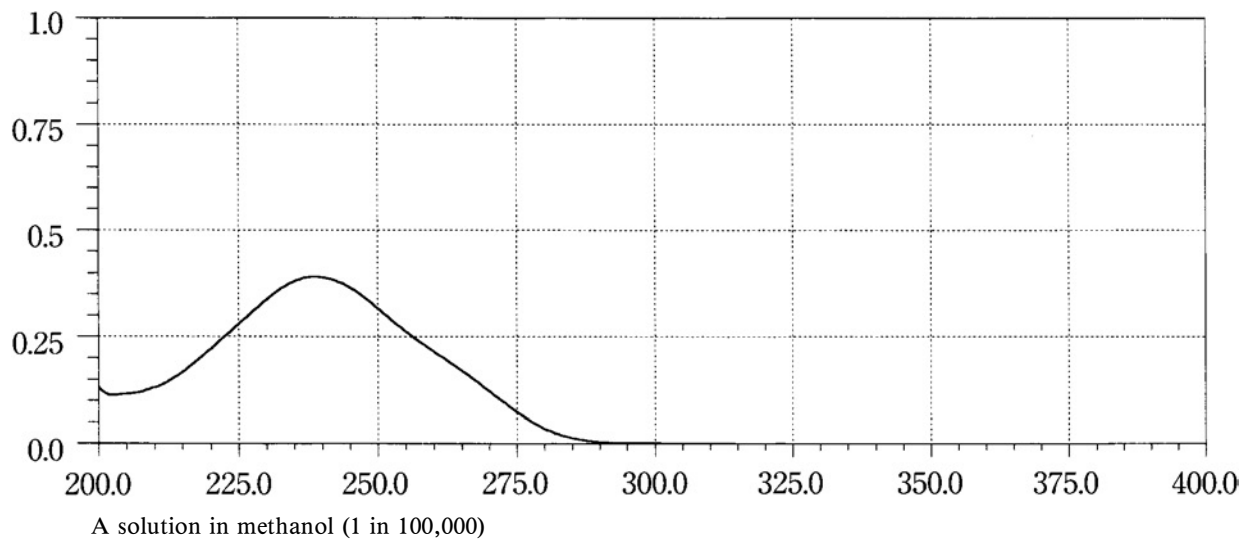
A solution prepared as follows: Dissolve 5 mg in 100 mL of diluted hydrochloric acid (1 in 1000)

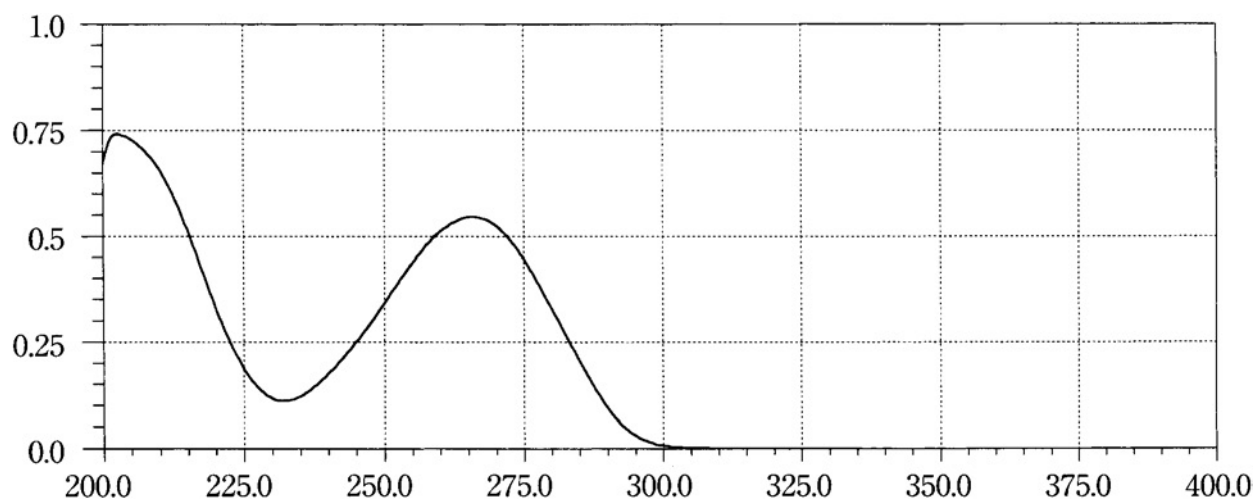
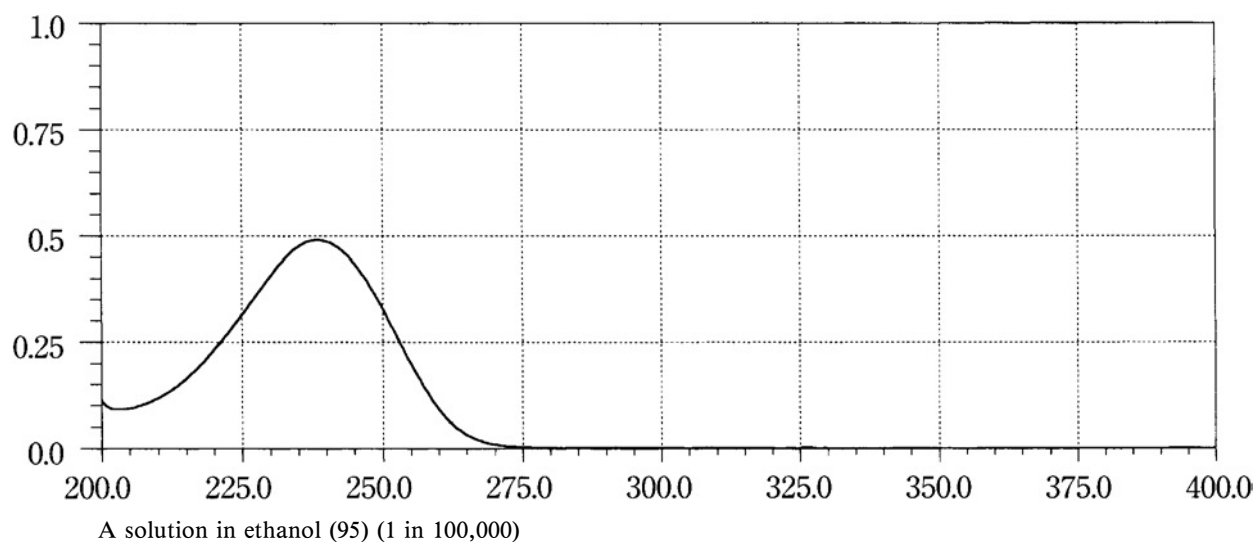
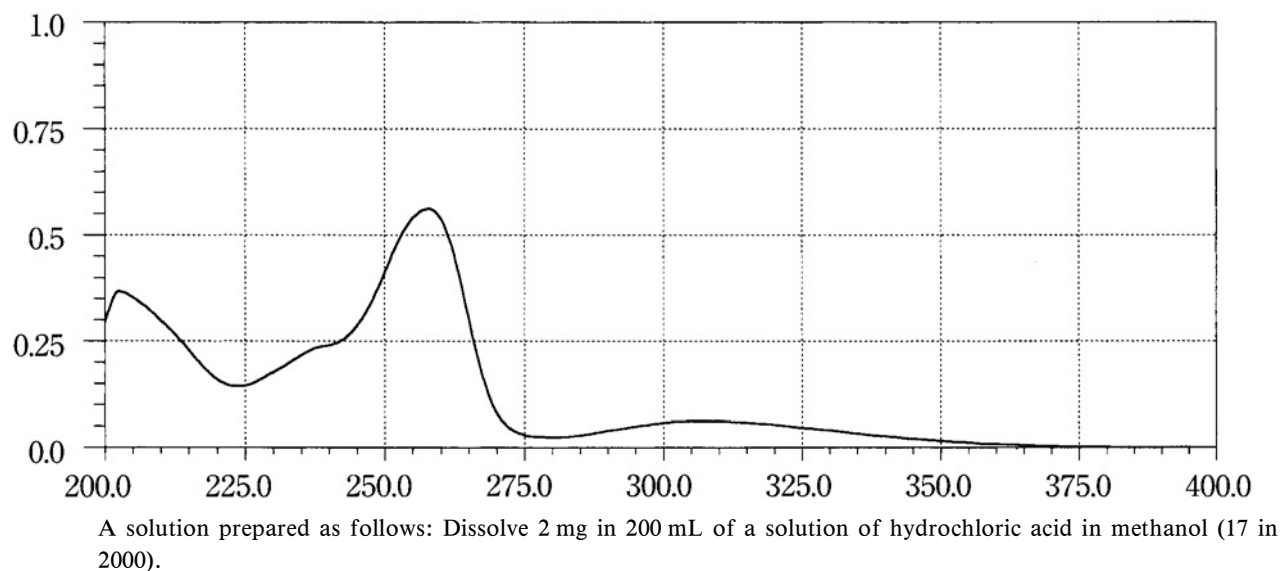


Famotidine**Fenbufen****Fentanyl Citrate**

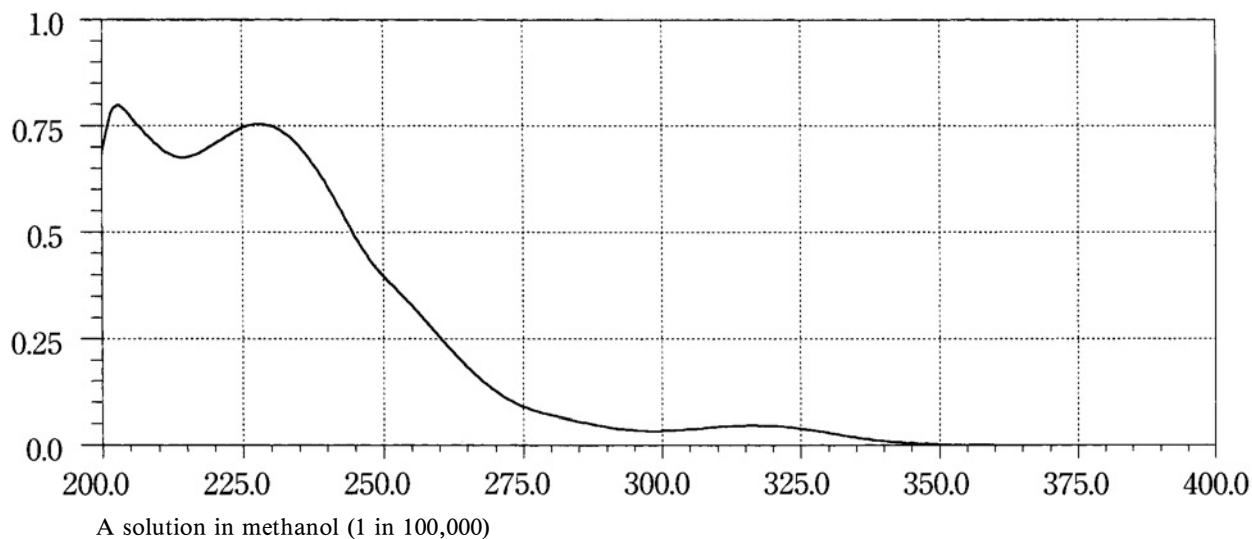
Flavoxate Hydrochloride**Flomoxef Sodium****Flopropione**



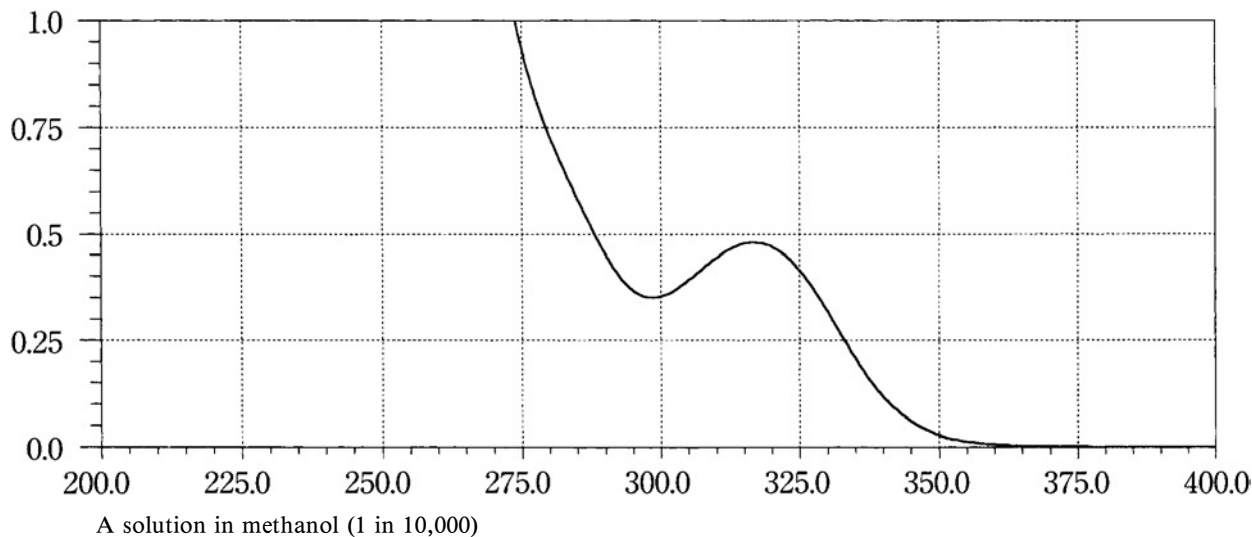
Flunitrazepam**Fluocinonide****Fluorometholone**

Fluorouracil**Fluoxymesterone****Fluphenazine Enanthate**

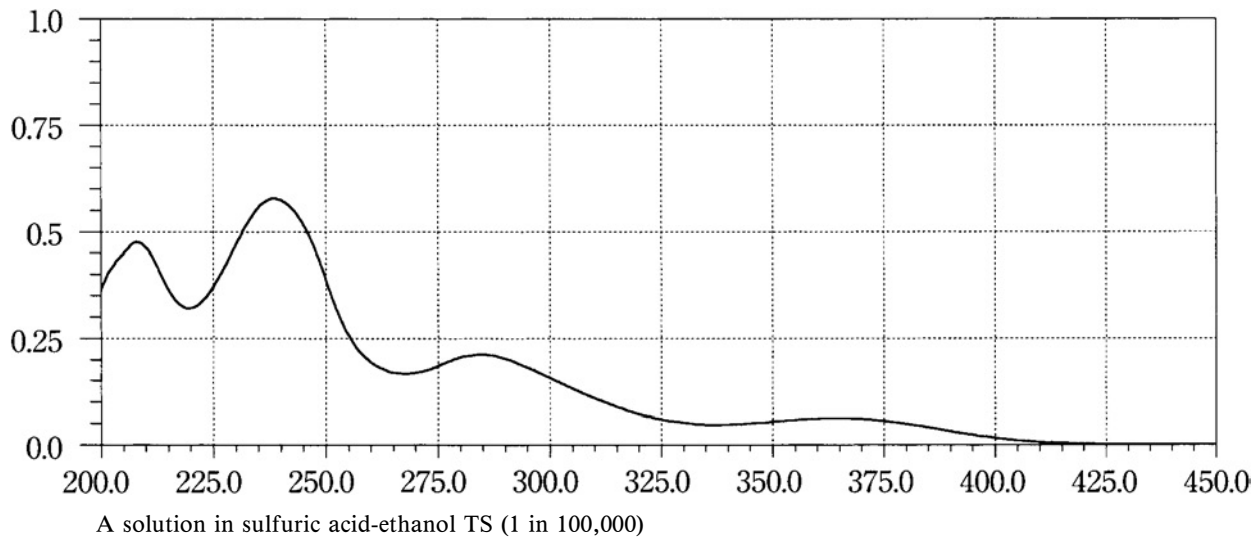
Flurazepam 1

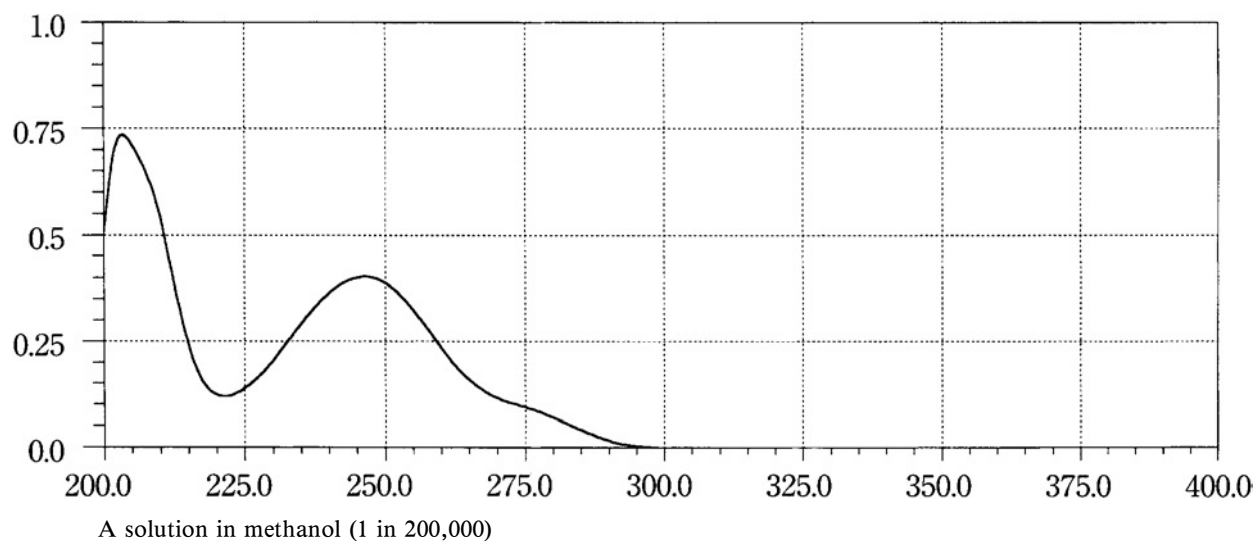
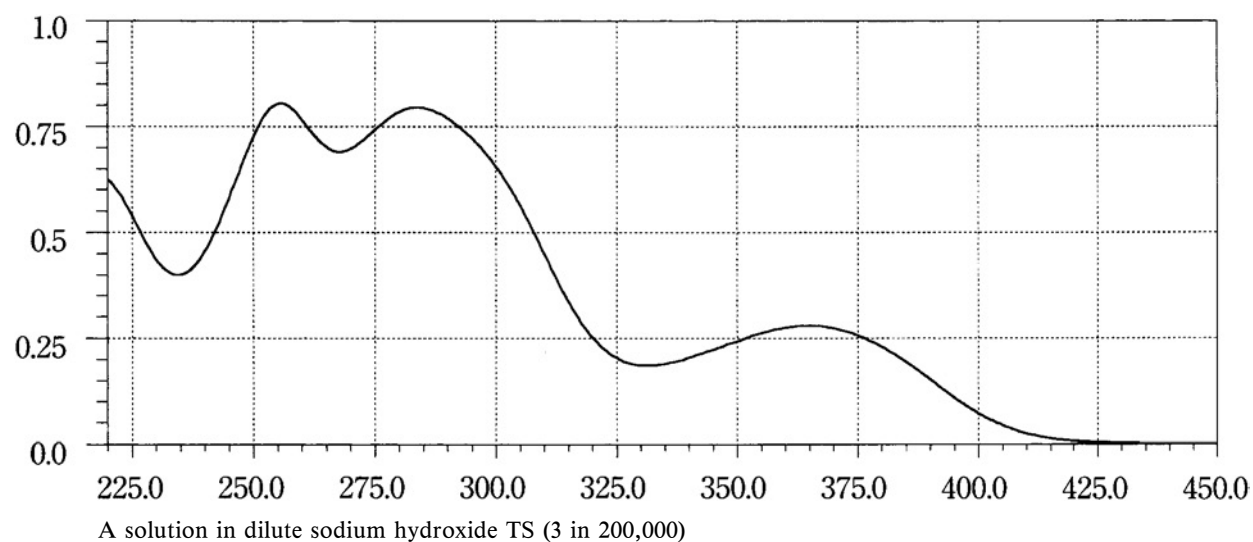
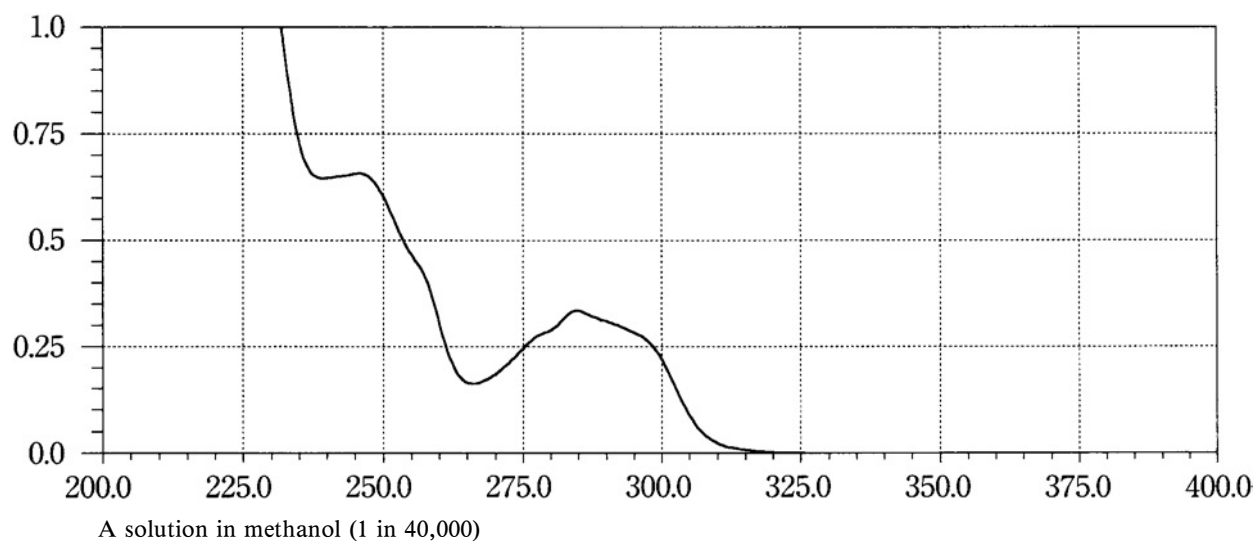


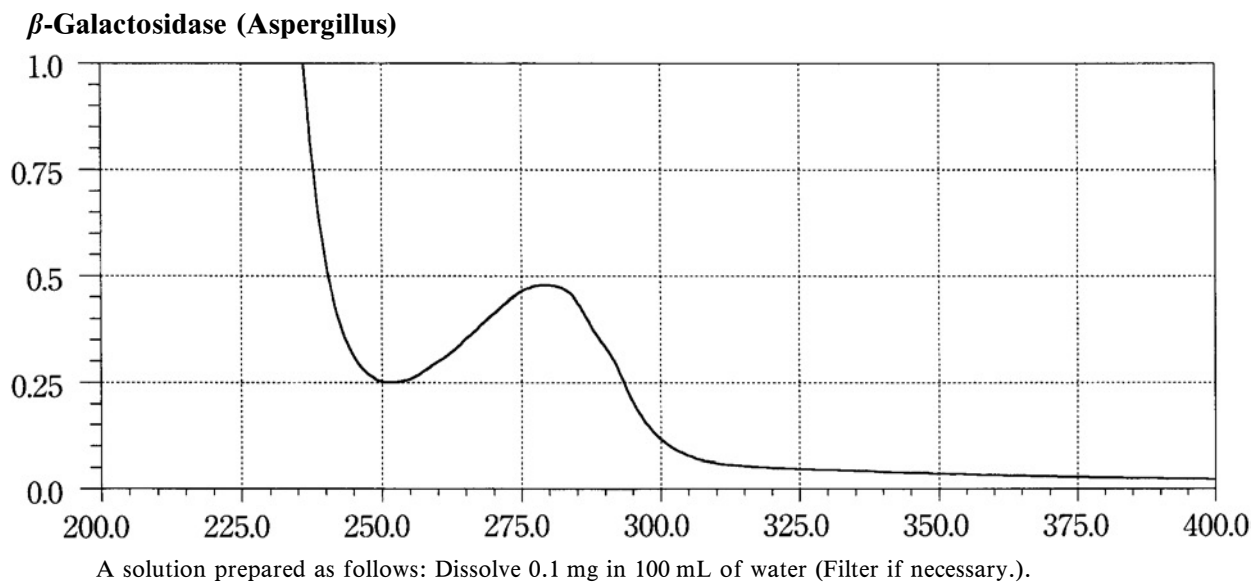
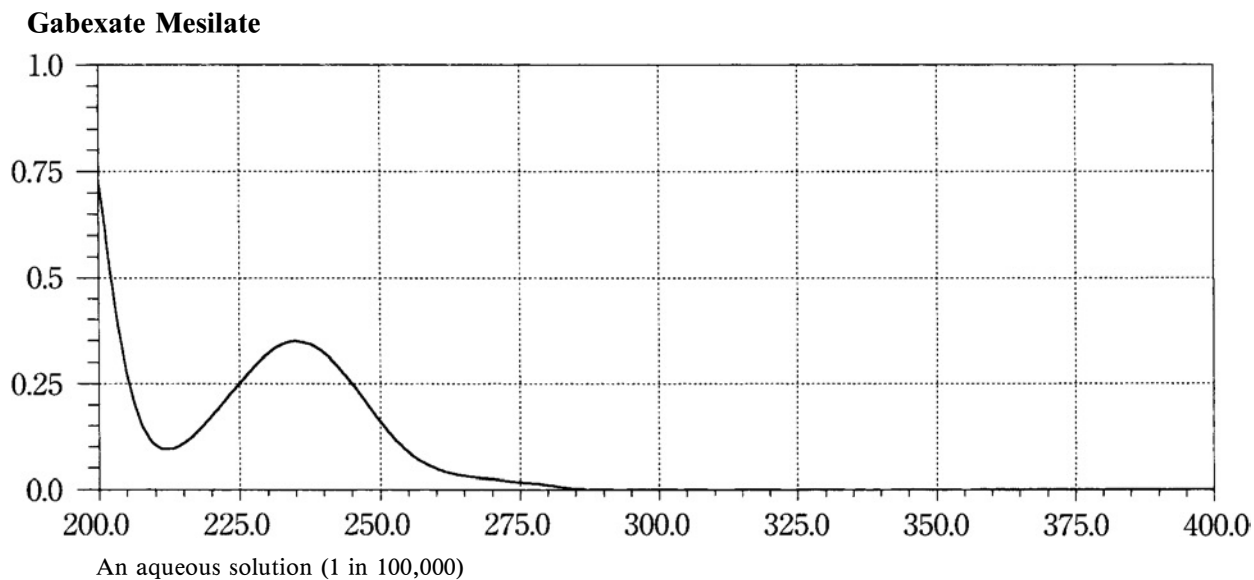
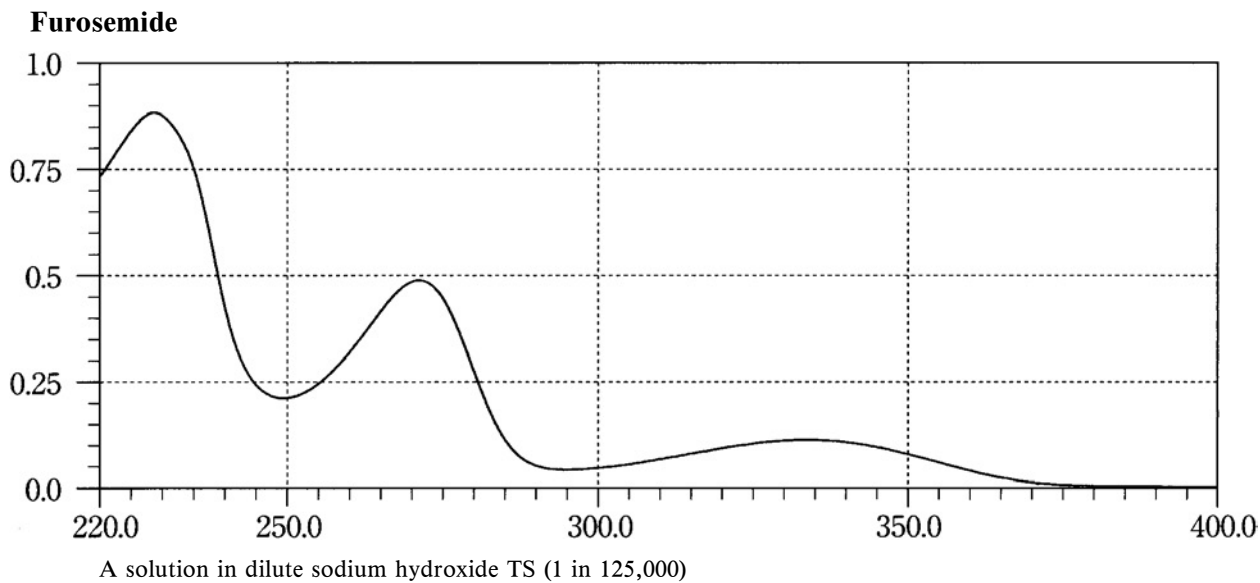
Flurazepam 2

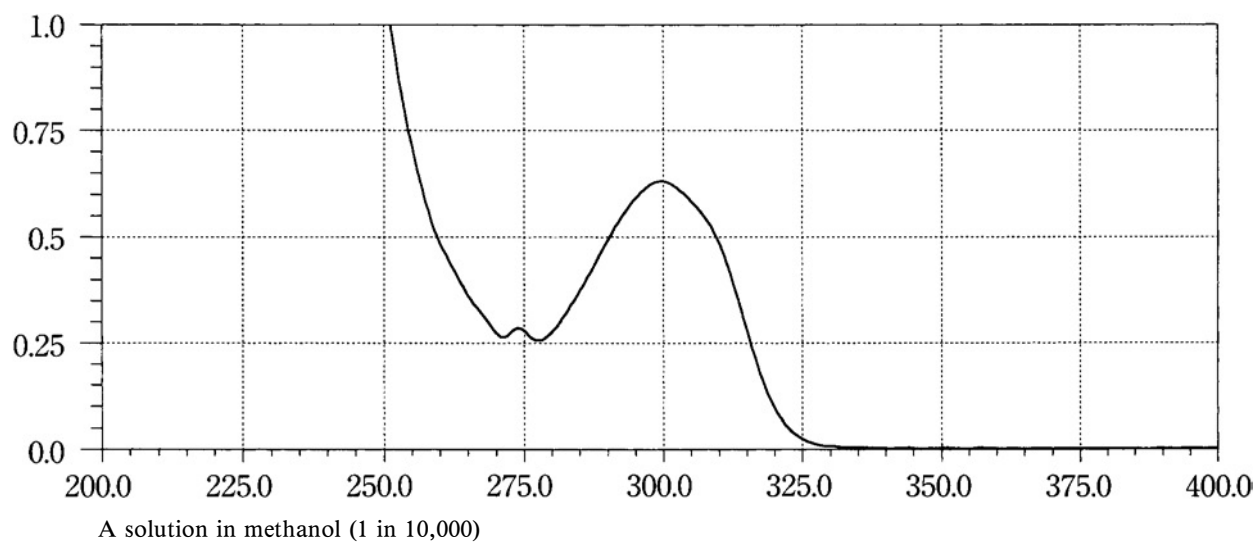
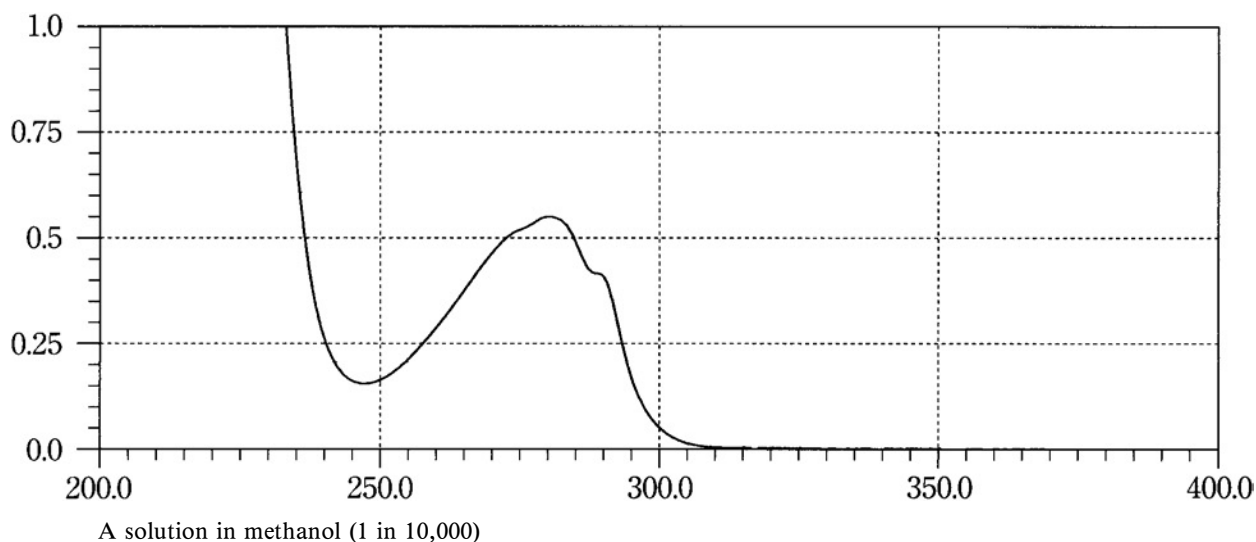
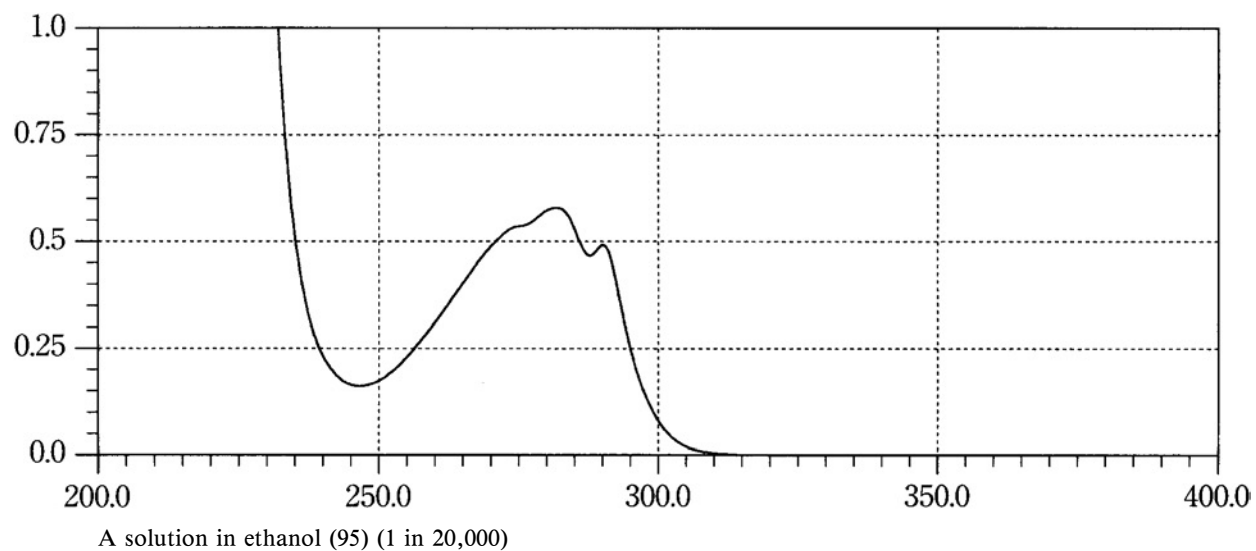


Flurazepam Hydrochloride

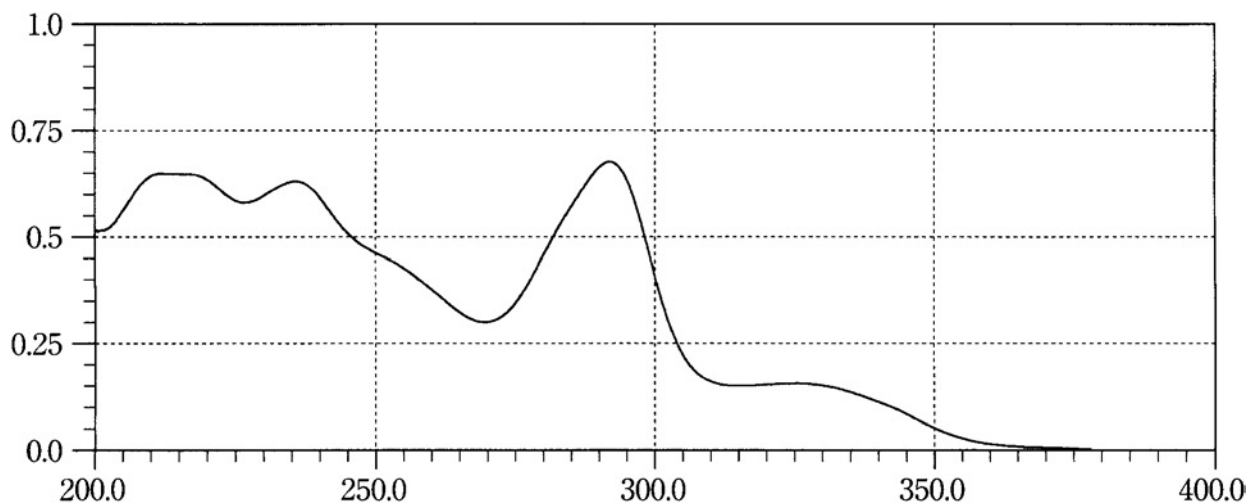


Flurbiprofen**Folic Acid****Formoterol Fumarate Hydrate**



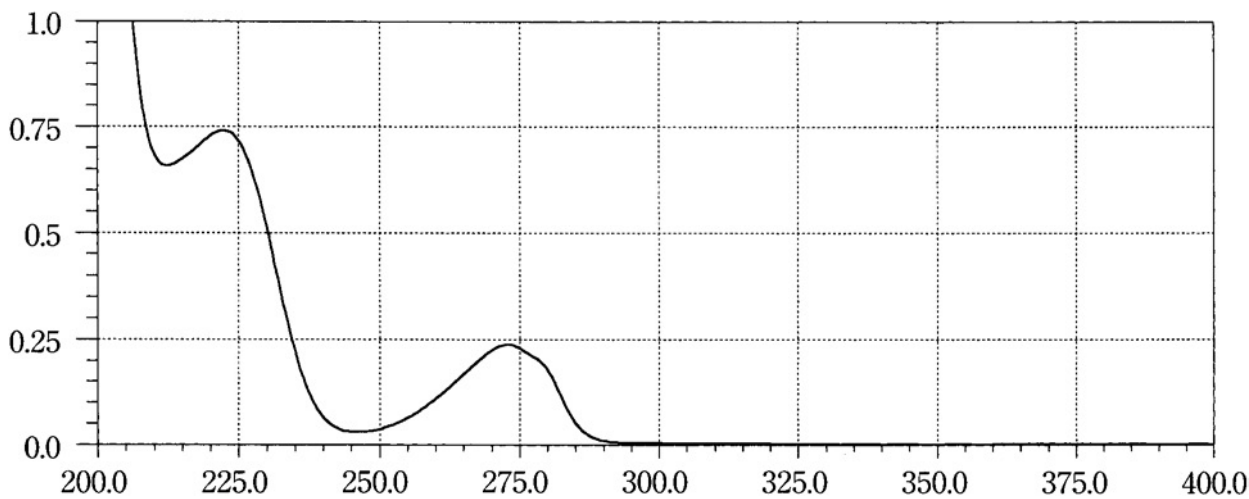
Glibenclamide**Gonadorelin Acetate****Gramicidin**

Griseofulvin



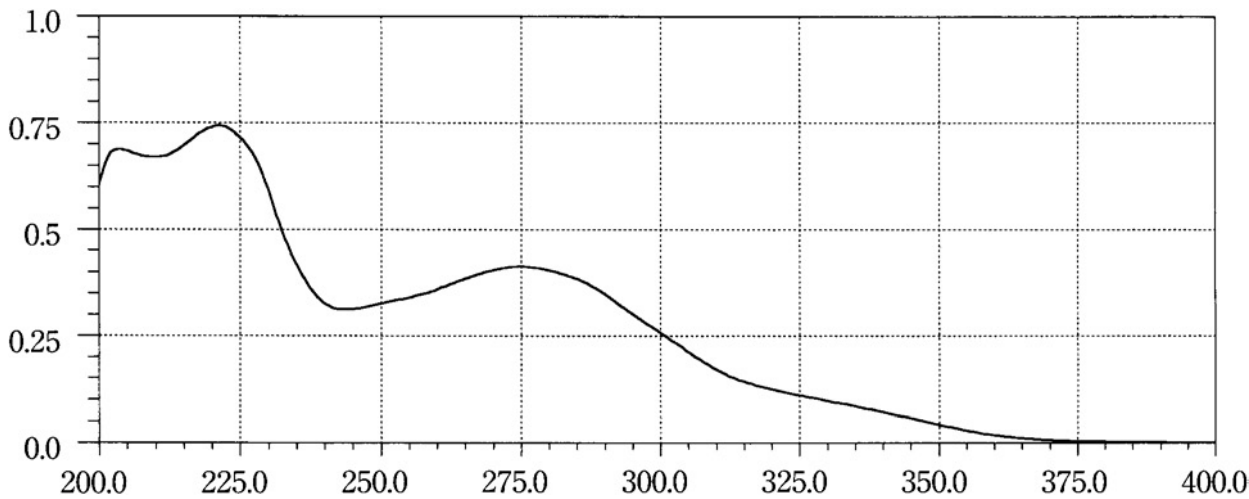
A solution in ethanol (95) (1 in 100,000)

Guaifenesin

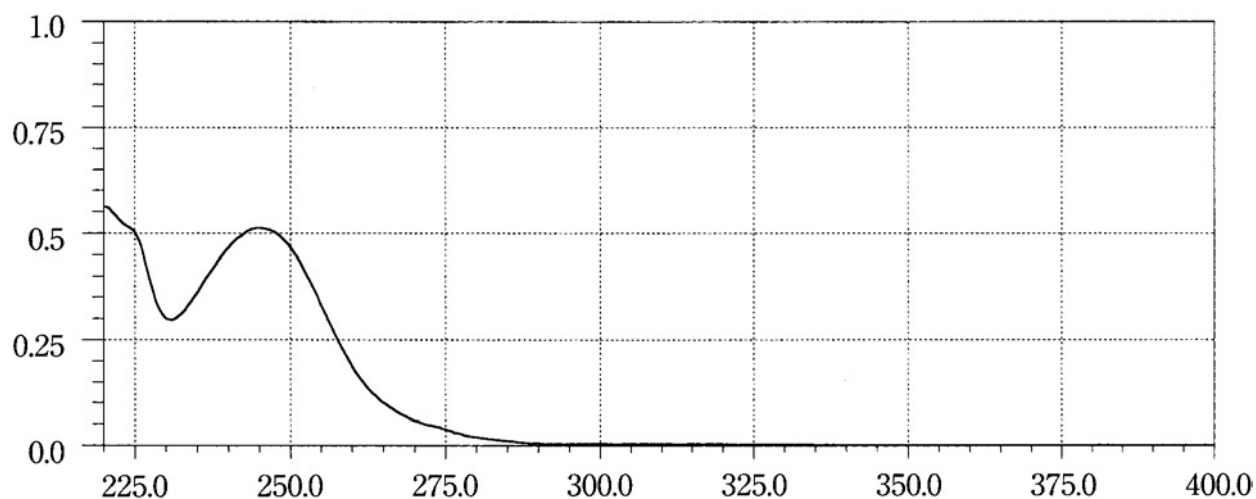


An aqueous solution (1 in 50,000)

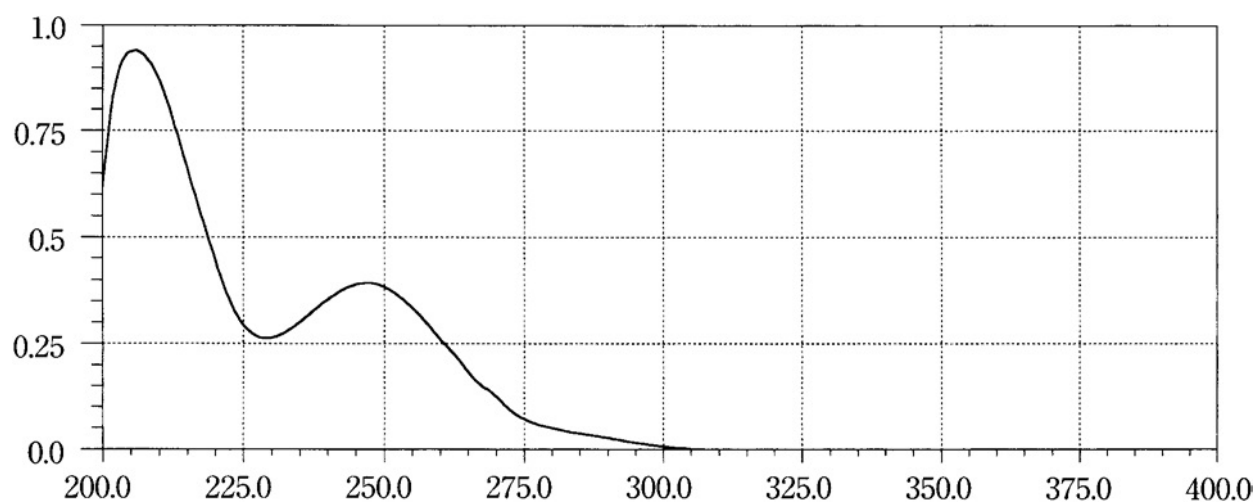
Guanabenz Acetate



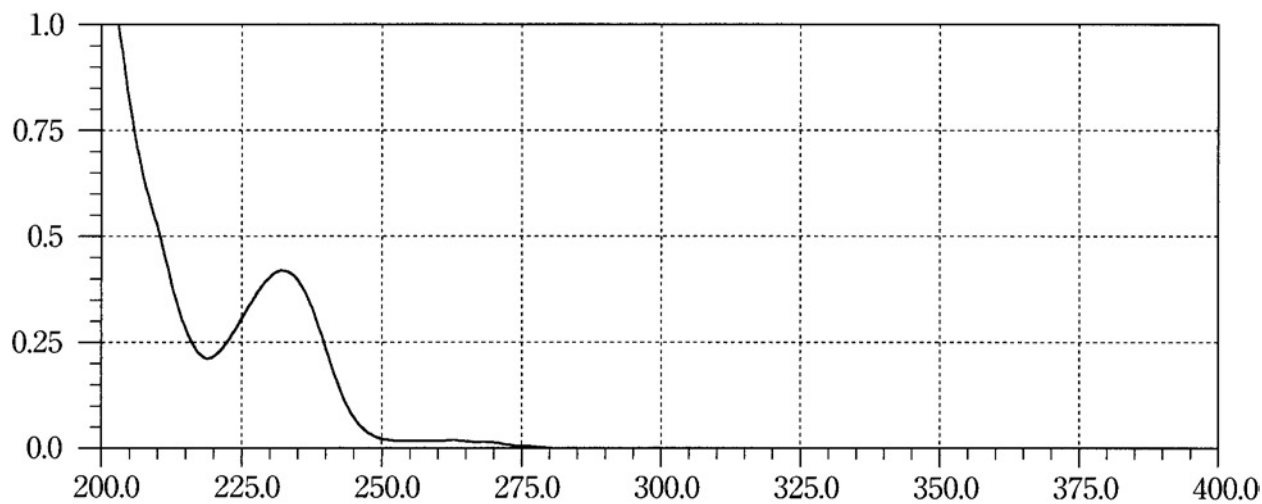
A solution in methanol (1 in 100,000)

Haloperidol

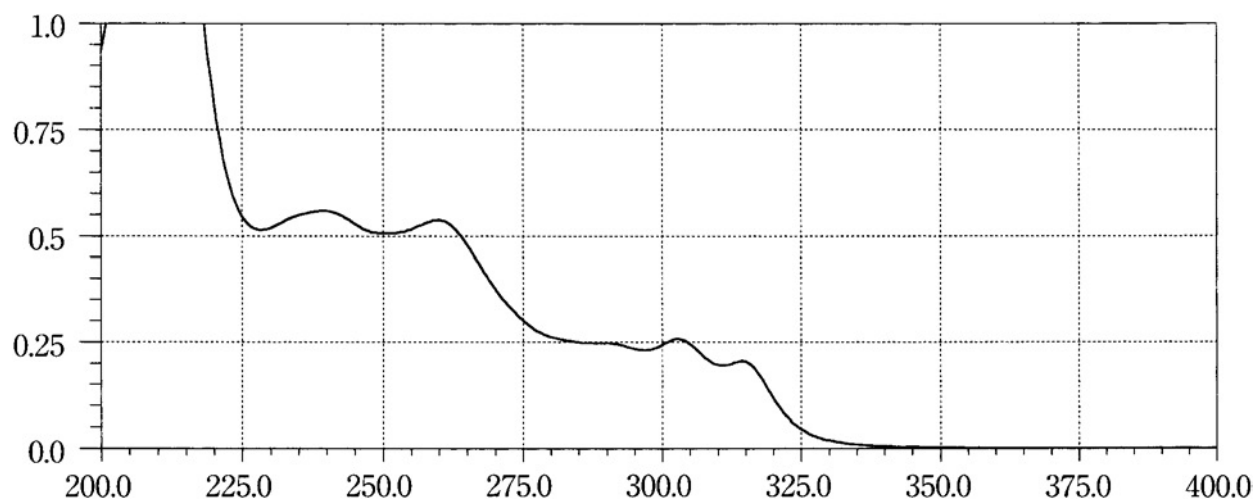
A solution prepared as follows: Dissolve 30 mg in 100 mL of 2-propanol. To 5 mL of this solution add 10 mL of 0.1 mol/L hydrochloric acid TS and 2-propanol to make 100 mL.

Haloxazolam

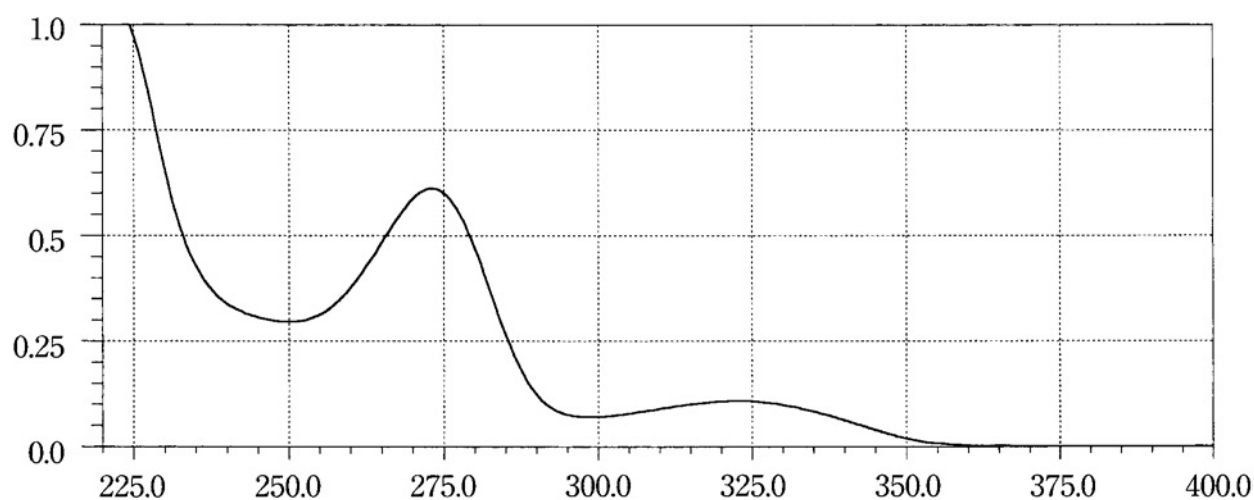
A solution in methanol (1 in 100,000)

Homochlorcyclizine Hydrochloride

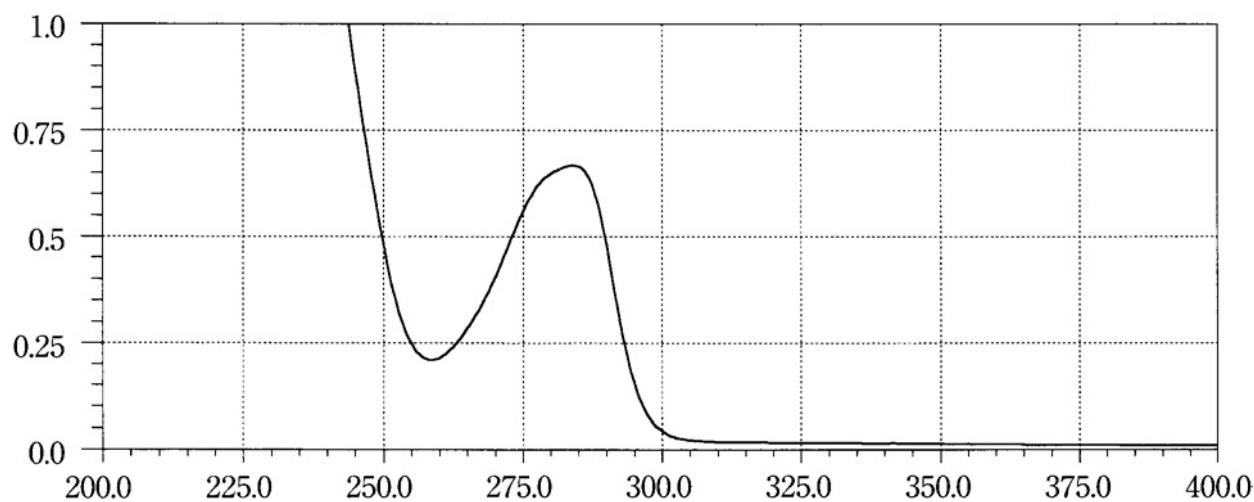
A solution in 0.1 mol/L hydrochloric acid TS (1 in 100,000)

Hydralazine Hydrochloride

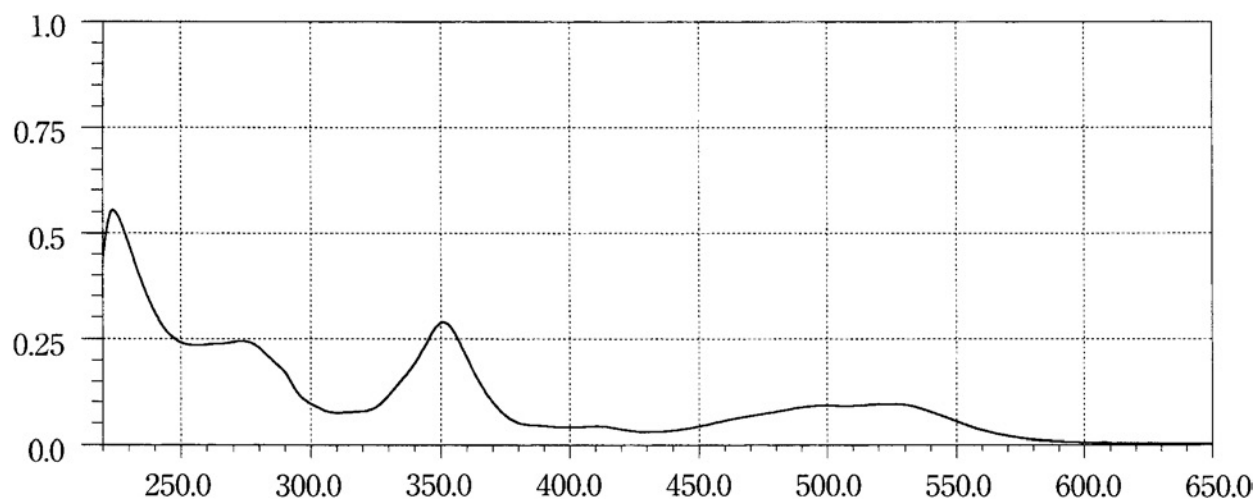
An aqueous solution (1 in 100,000)

Hydrochlorothiazide

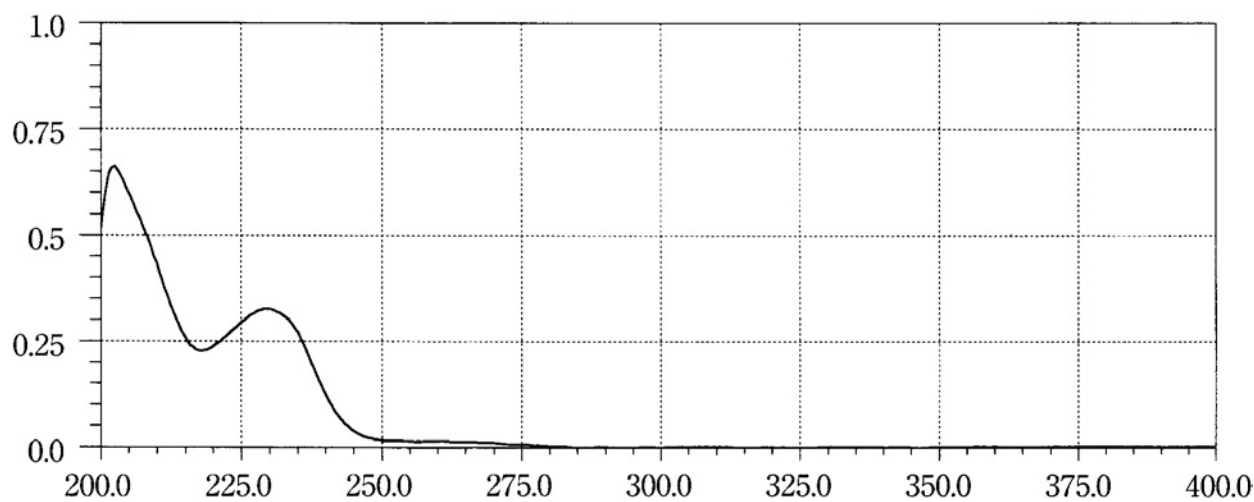
A solution prepared as follows: Dissolve 12 mg in 100 mL of sodium hydroxide TS. 10 mL of this solution add water to make 100 mL.

Hydrocotarnine Hydrochloride Hydrate

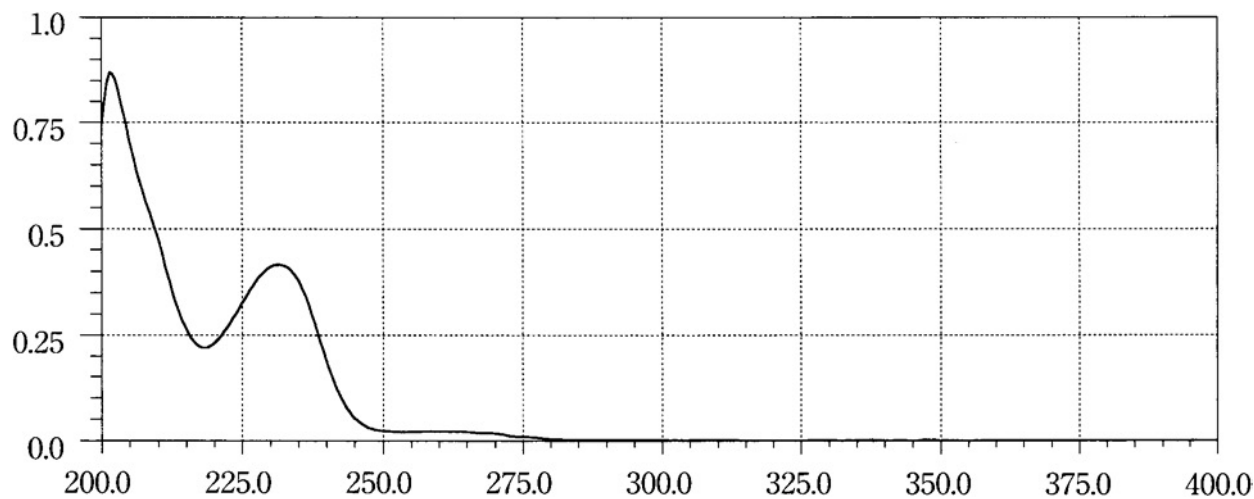
An aqueous solution (1 in 10,000)

Hydroxocobalamin Acetate

A solution in acetic acid-sodium acetate buffer solution, pH 4.5 (1 in 50,000)

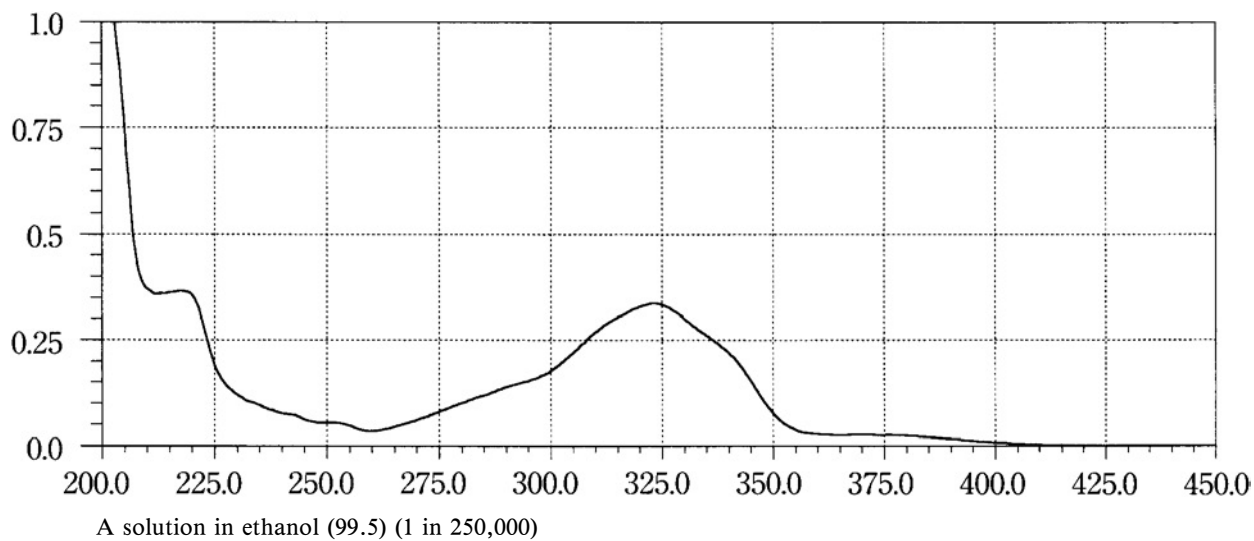
Hydroxyzine Hydrochloride

A solution in methanol (1 in 100,000)

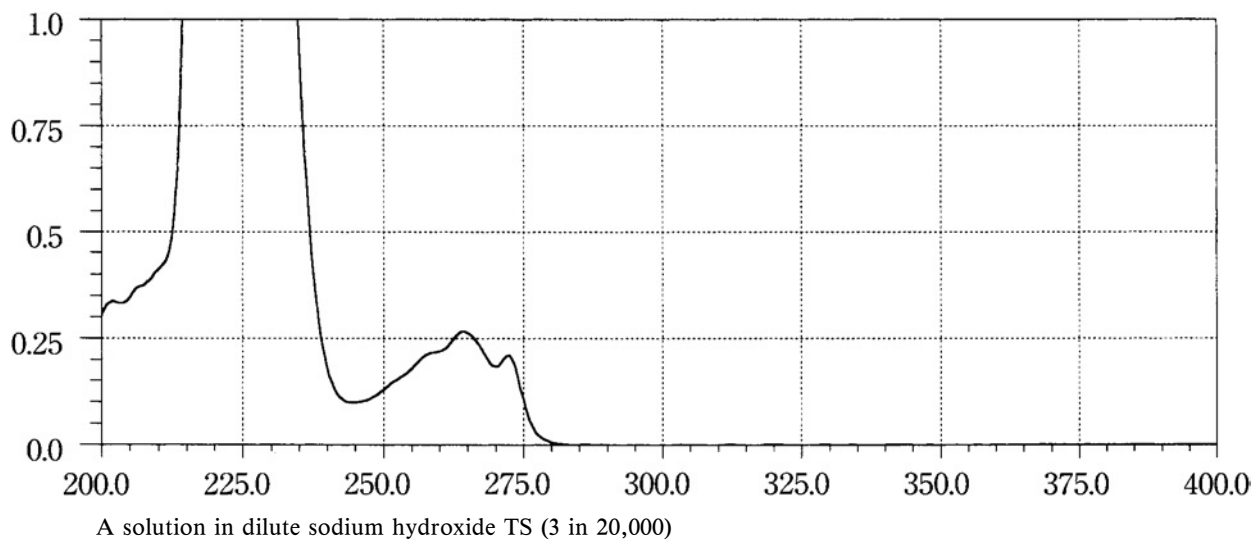
Hydroxyzine Pamoate

A solution prepared as follows: Evaporate 2 mL of the sample solution obtained in the Identification (1) on a water bath to dryness, and dissolve the residue in 0.1 mol/L hydrochloric acid TS to make 500 mL.

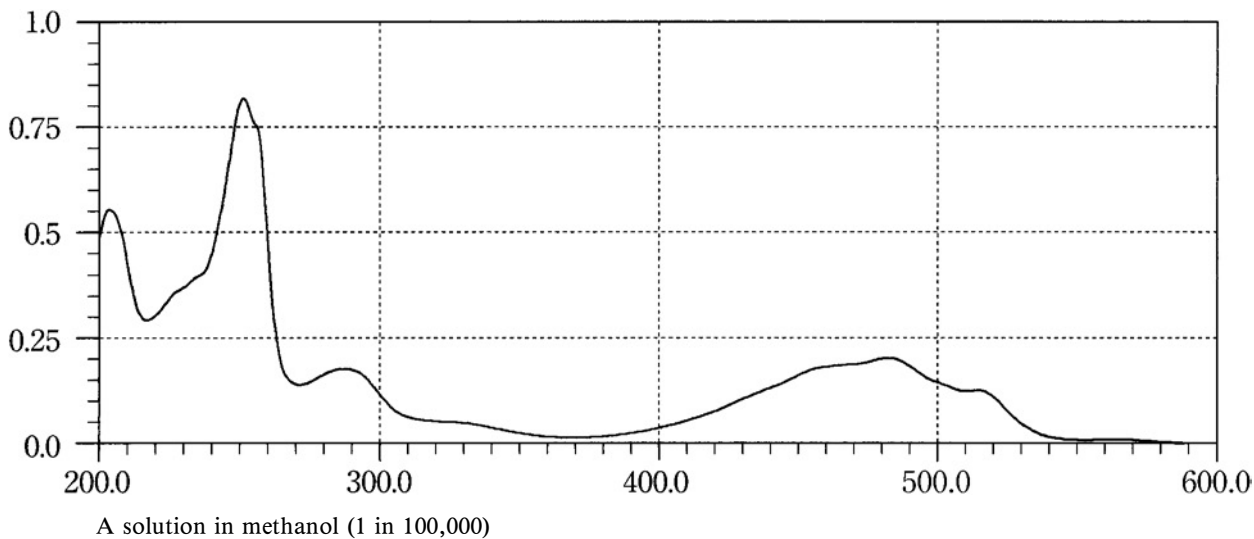
Hymecromone

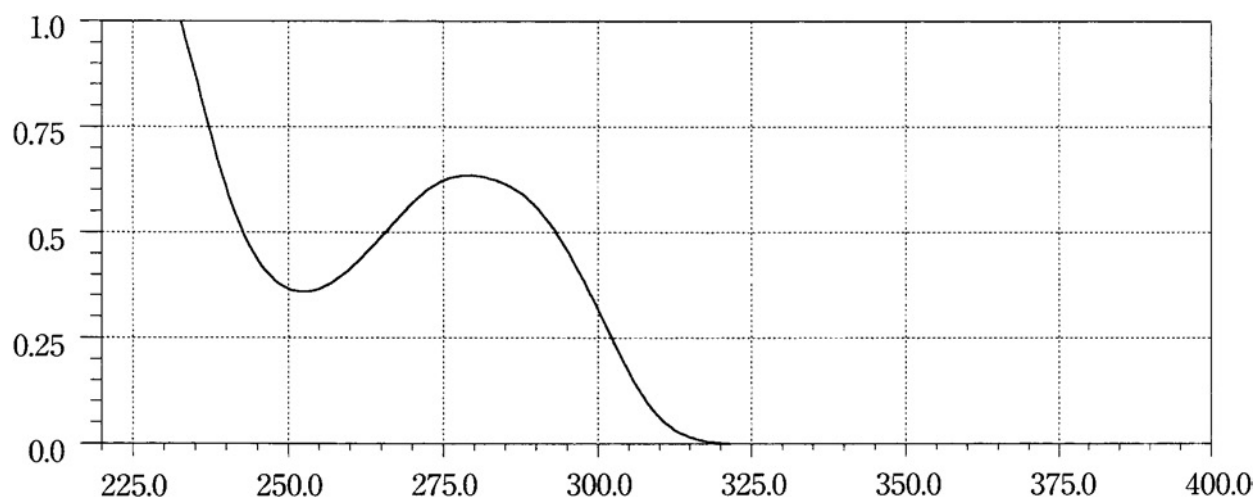


Ibuprofen

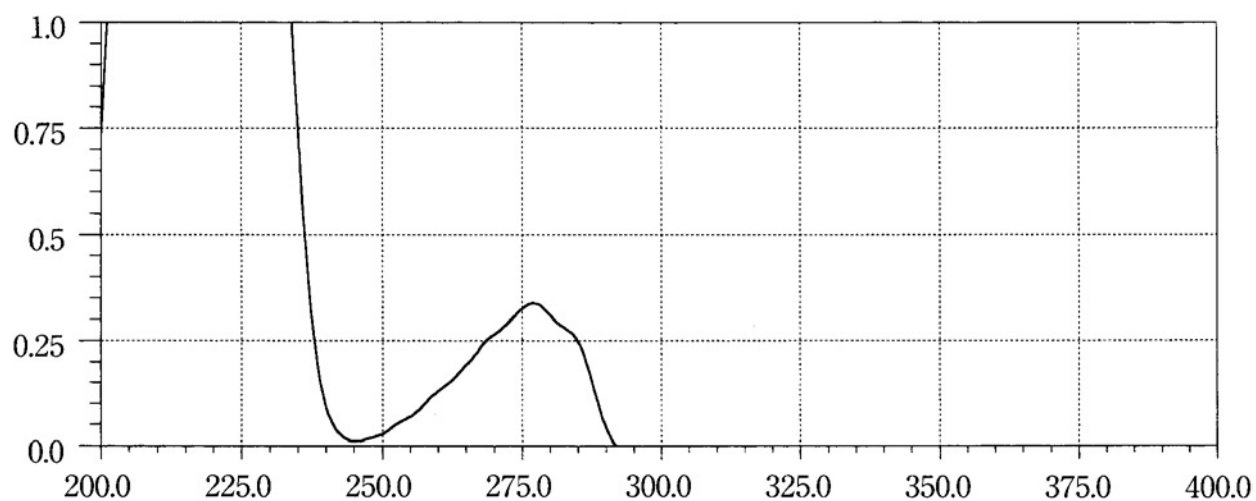


Idarubicin Hydrochloride

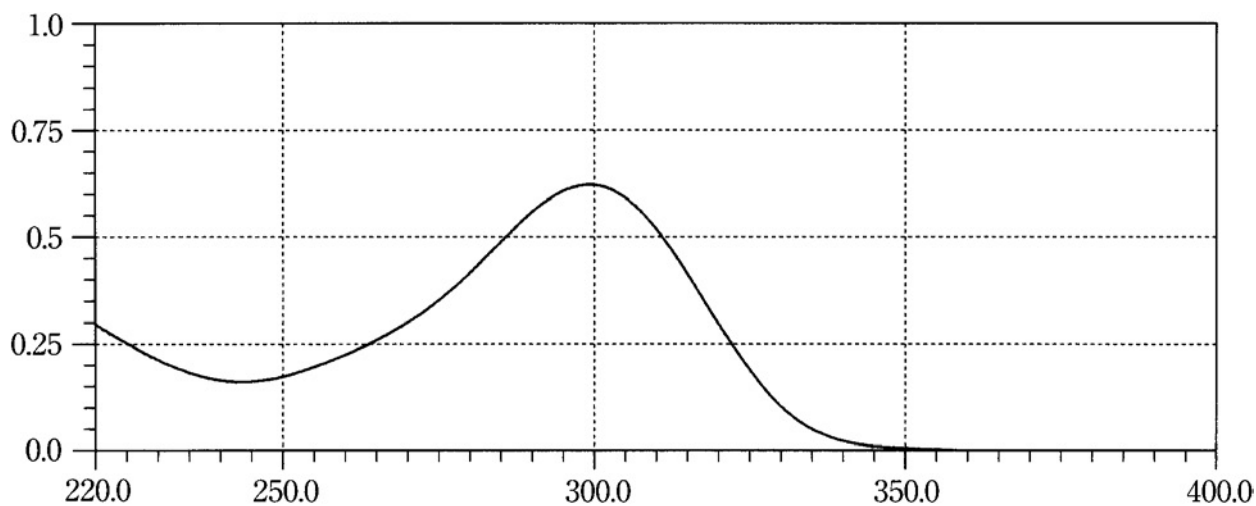


Idoxuridine

A solution in 0.01 mol/L sodium hydroxide TS (1 in 25,000)

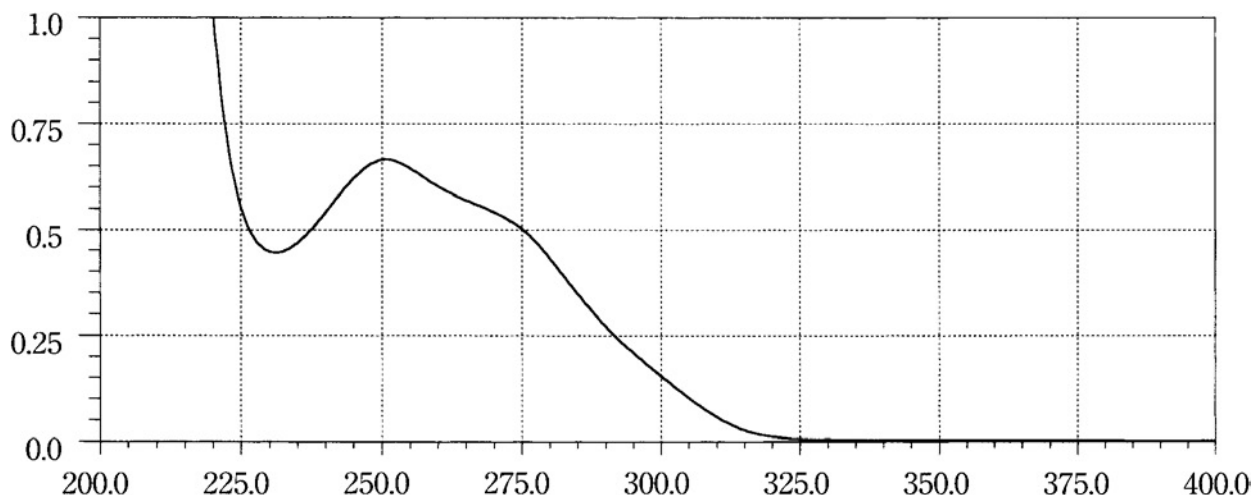
Ifenprodil Tartrate

A solution in methanol (1 in 100,000)

Imipenem Hydrate

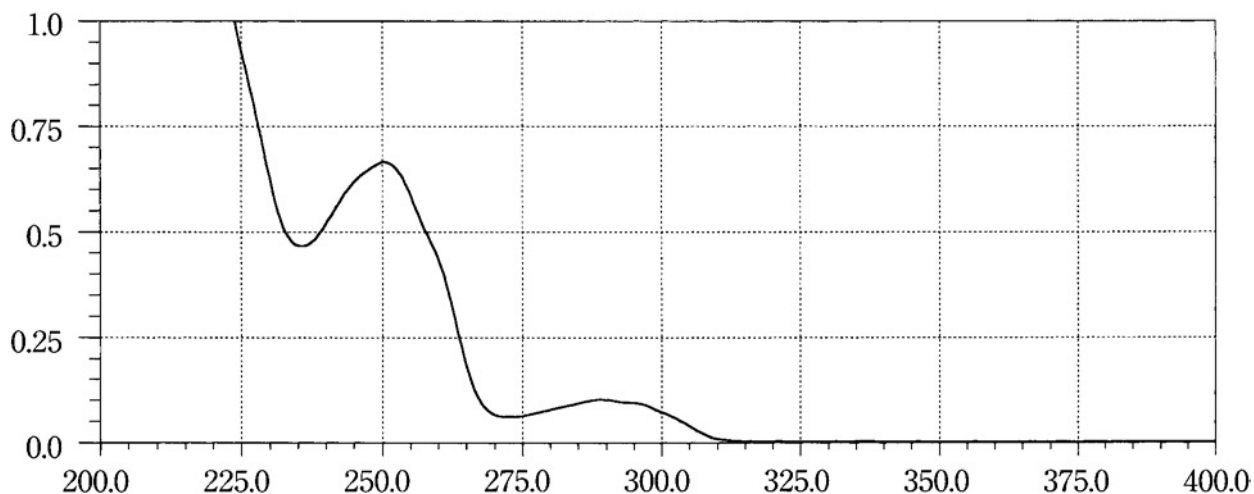
A solution in 0.1 mol/L 3-(*N*-morpholino)propanesulfonic acid buffer solution, pH 7.0 (1 in 50,000)

Imipramine Hydrochloride



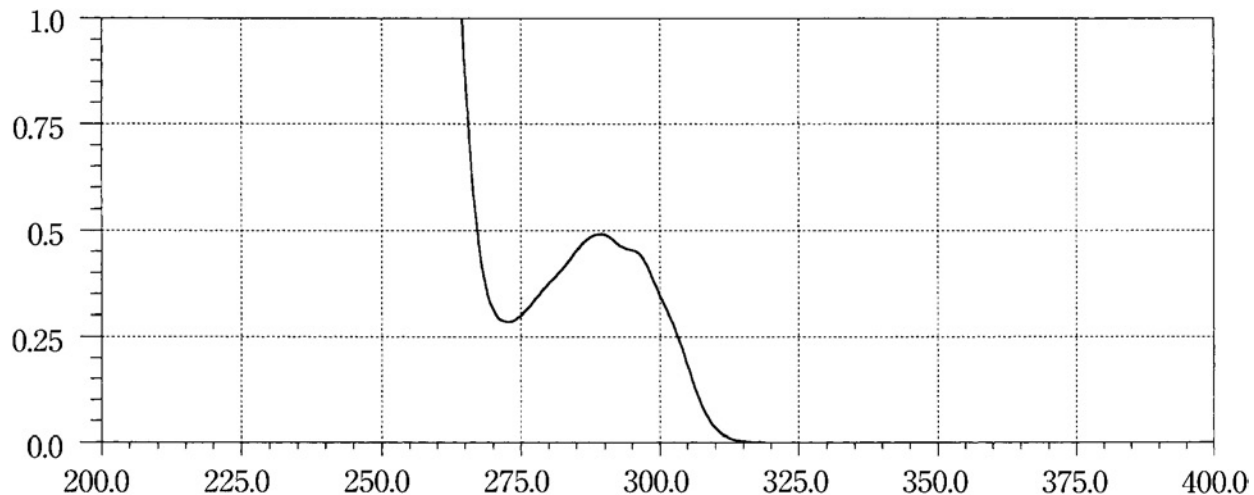
A solution prepared as follows: Dissolve 5 mg in 250 mL of 0.01 mol/L hydrochloric acid TS

Indenolol Hydrochloride 1

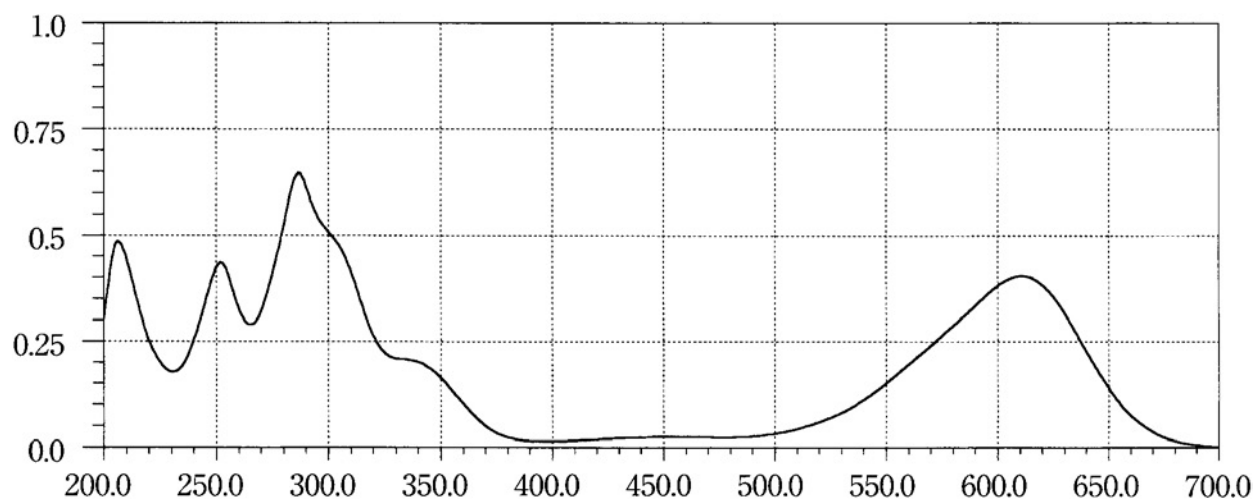


An aqueous solution (1 in 50,000)

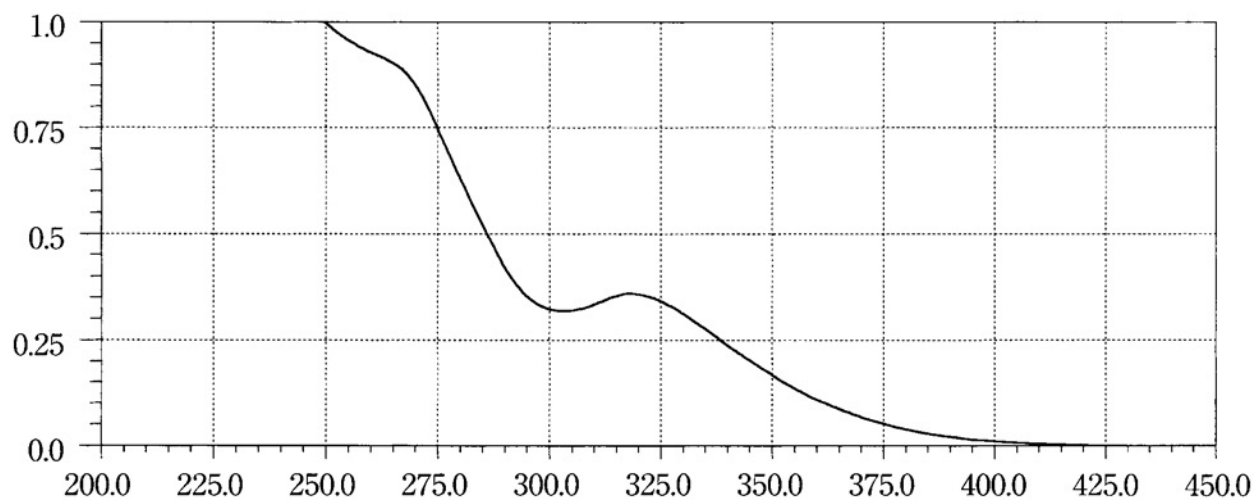
Indenolol Hydrochloride 2



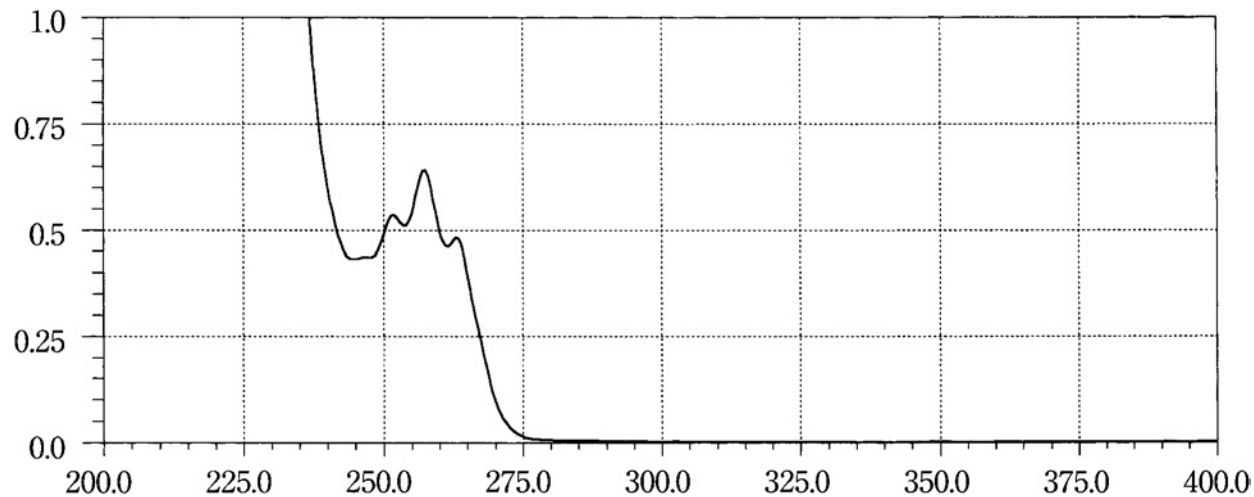
An aqueous solution (1 in 10,000)

Indigocarmine

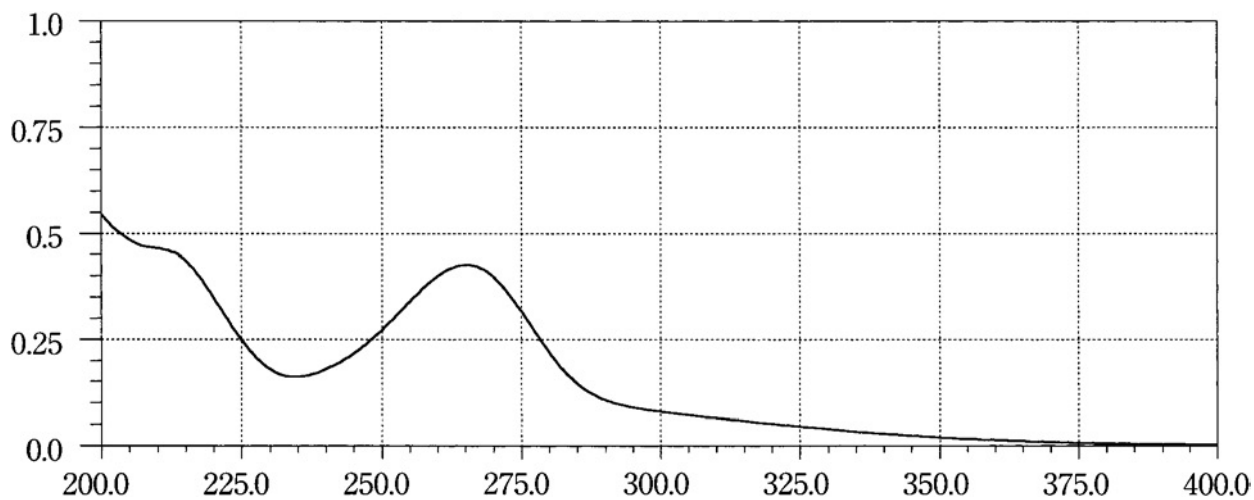
A solution prepared as follows: Dissolve 0.1 g in 100 mL of an aqueous solution of ammonium acetate (1 in 650). To 1 mL of this solution add the aqueous solution of ammonium acetate (1 in 650) to make 100 mL.

Indometacin

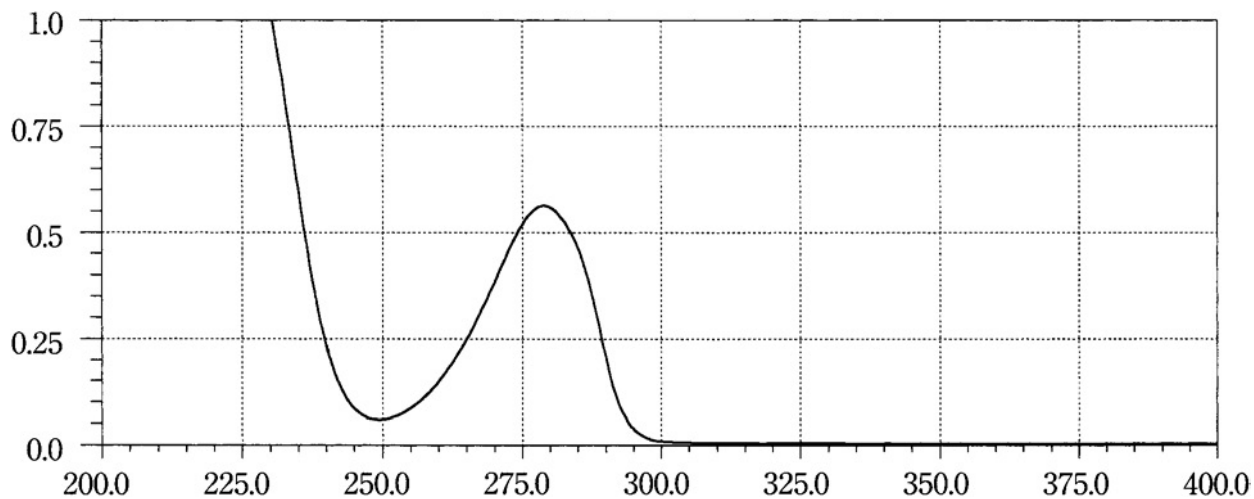
A solution prepared as follows: Dissolve 2 mg in 200 mL of methanol.

Ipratropium Bromide Hydrate

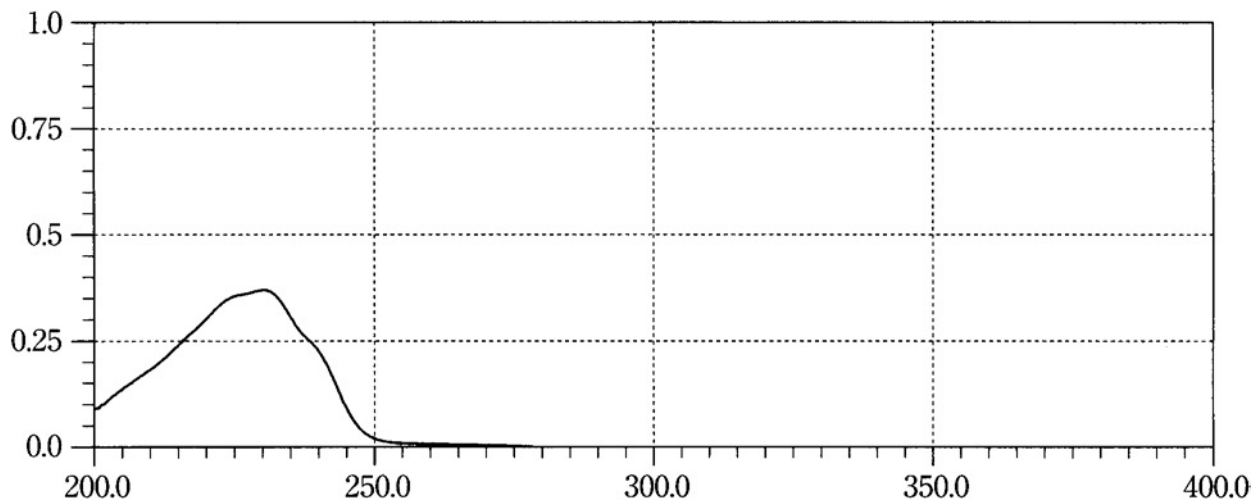
A solution in 0.01 mol/L hydrochloric acid TS (3 in 2000)

Isoniazid

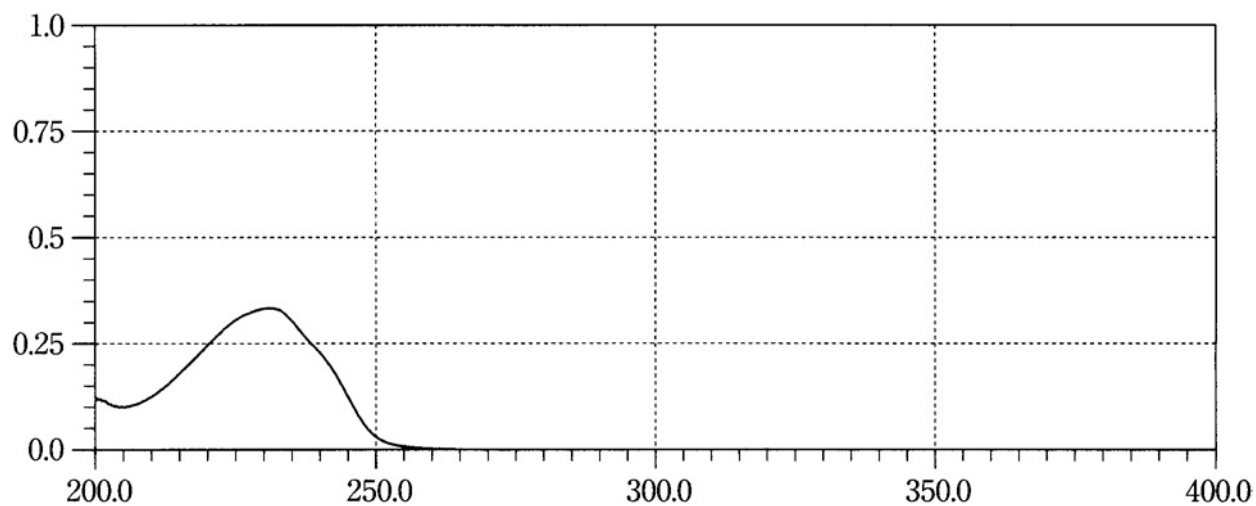
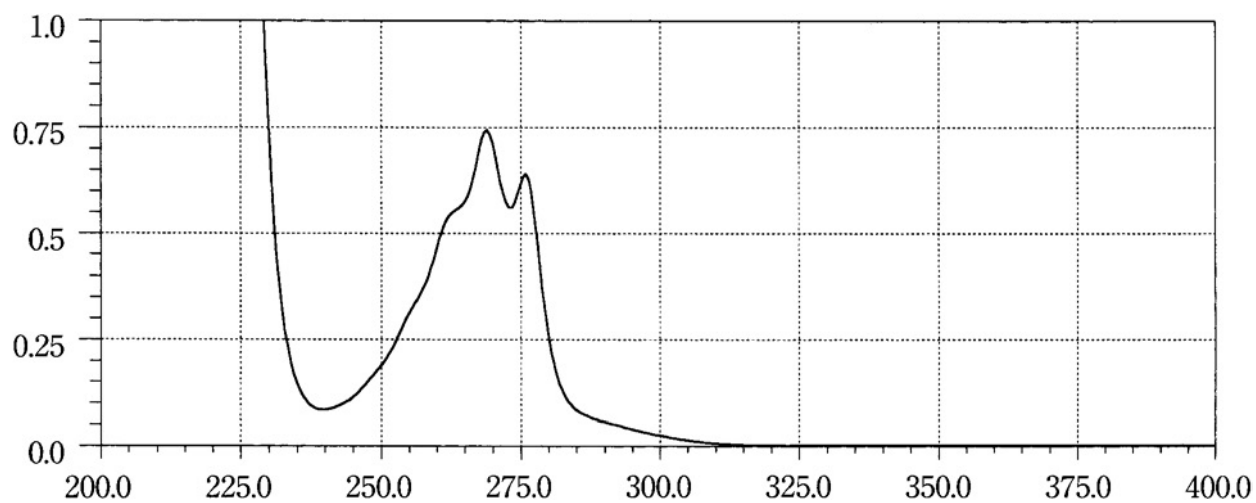
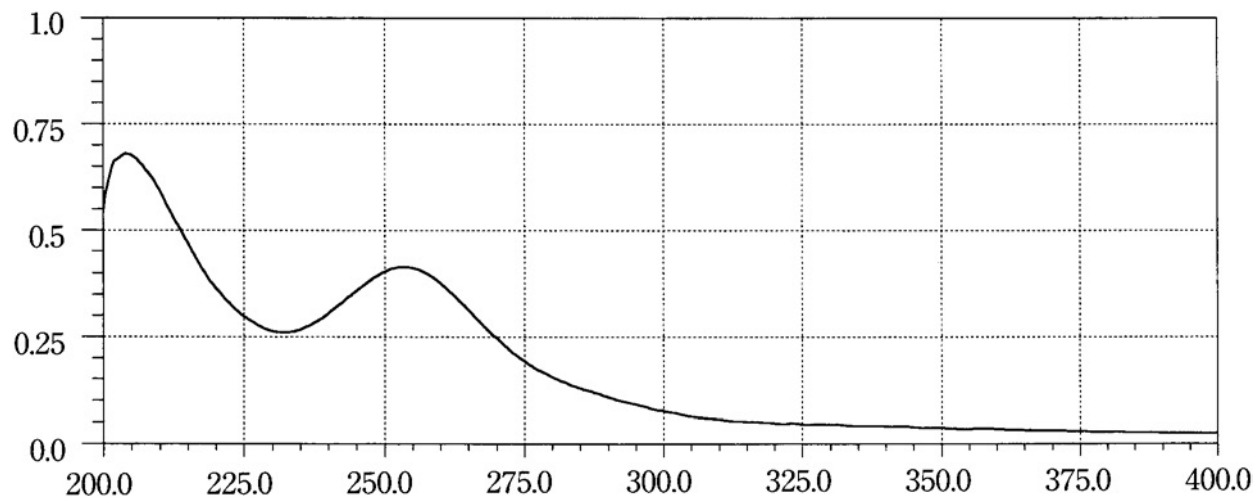
A solution prepared as follows: To 5 mL of an aqueous solution (1 in 10,000) add 1 mL of 0.1 mol/L hydrochloric acid TS and water to make 50 mL.

***l*-Isoprenaline Hydrochloride**

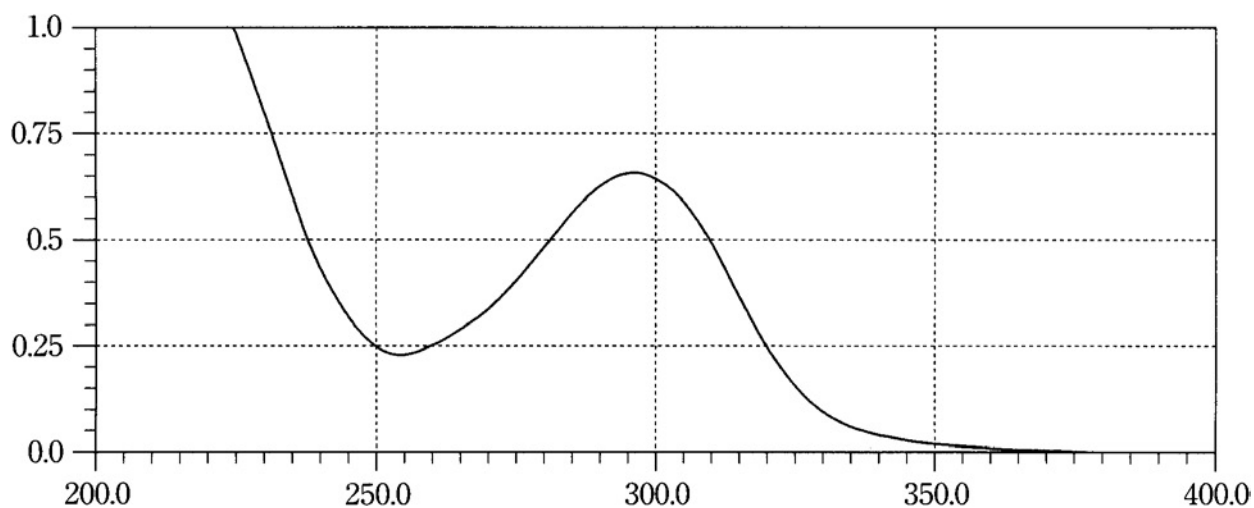
A solution in 0.1 mol/L hydrochloric acid TS (1 in 20,000)

Josamycin

A solution in methanol (1 in 100,000)

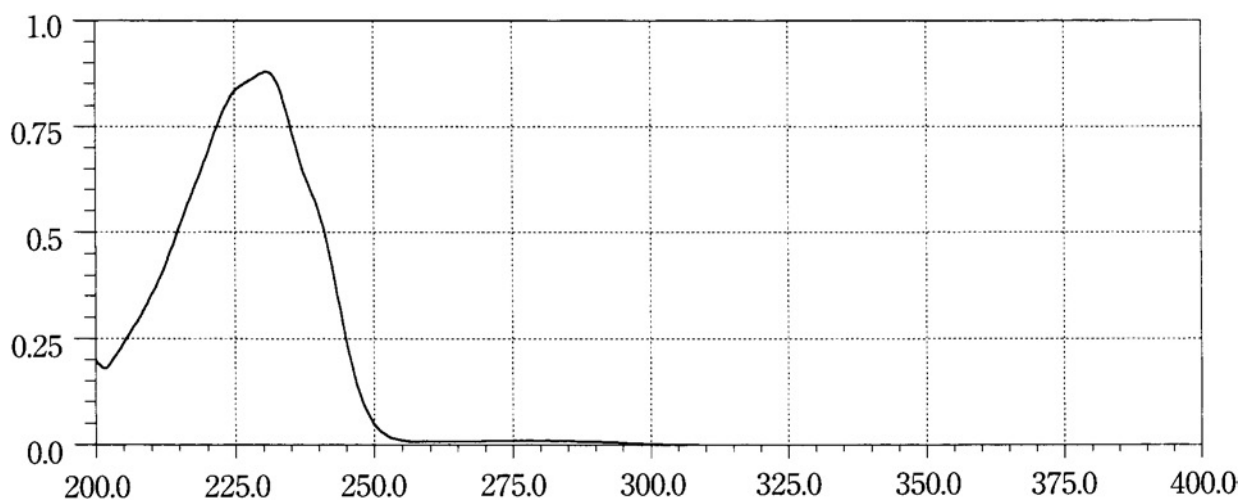
Josamycin Propionate**Ketamine Hydrochloride****Ketoprofen**

Ketotifen Fumarate



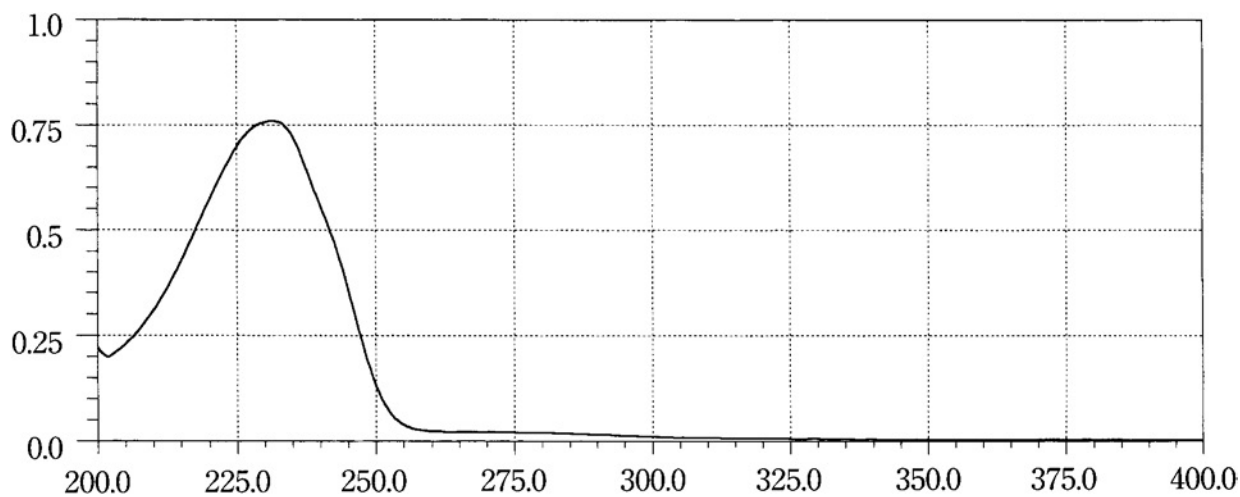
A solution in methanol (1 in 50,000)

Kitasamycin

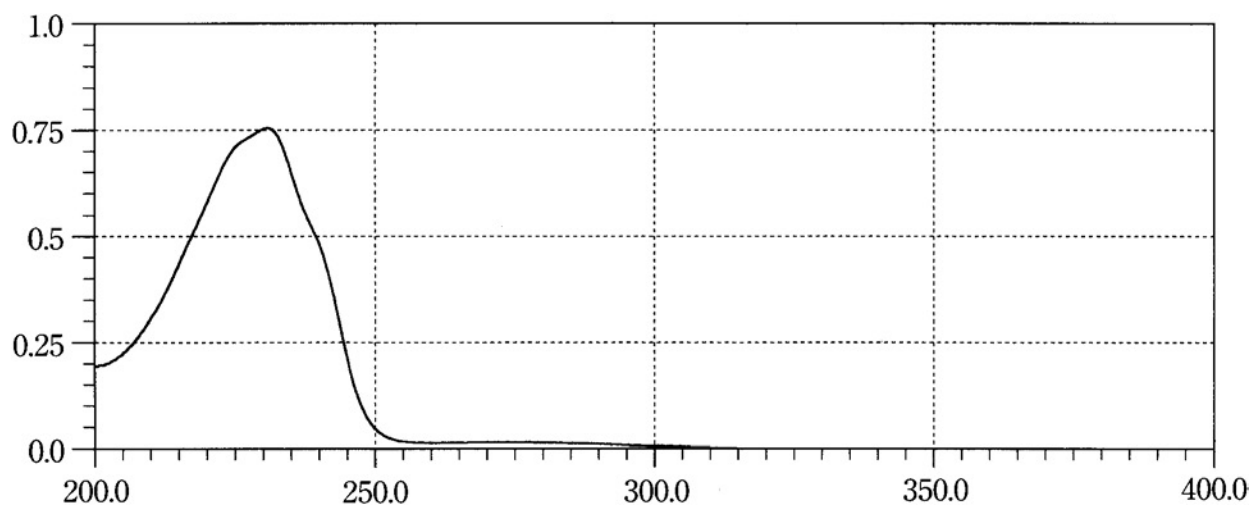
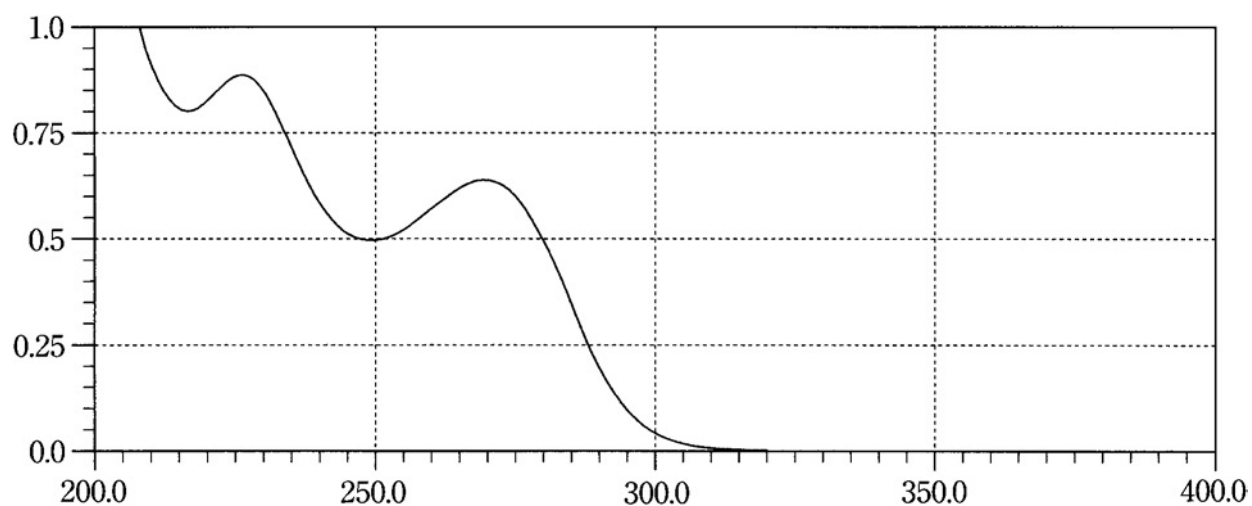
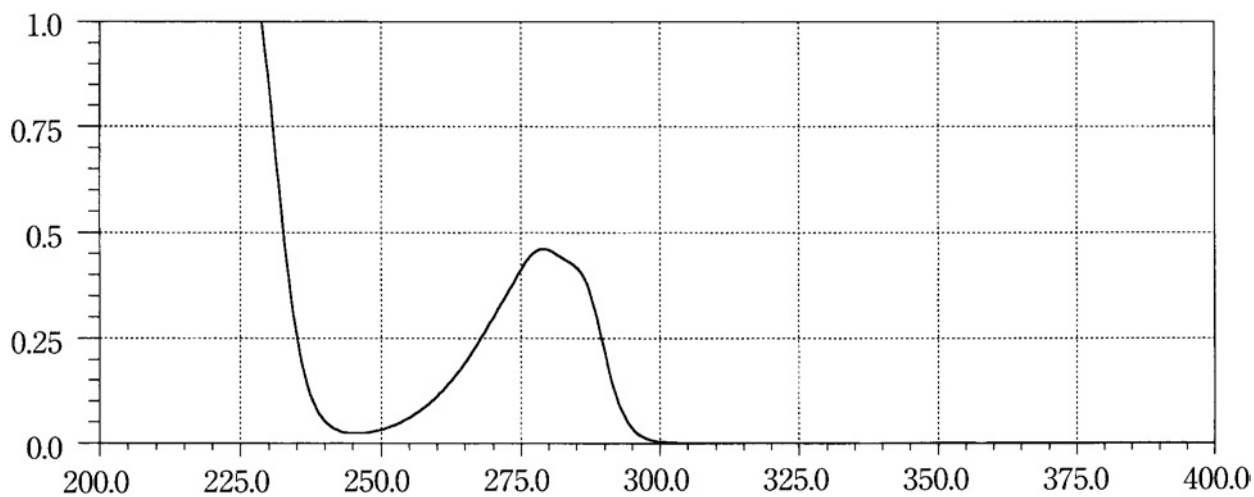


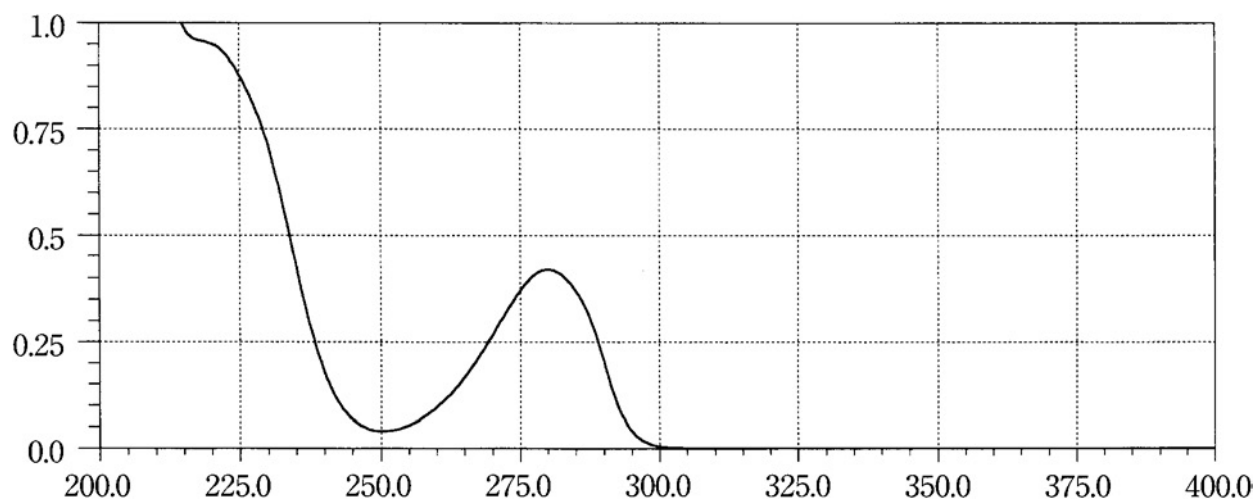
A solution in methanol (1 in 40,000)

Kitasamycin Acetate

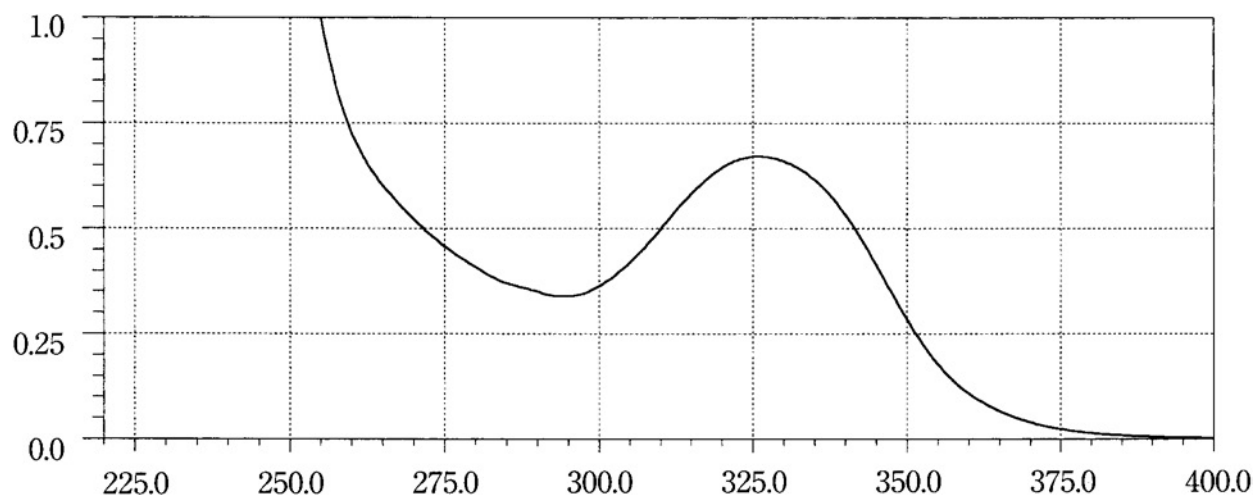


A solution in methanol (1 in 40,000)

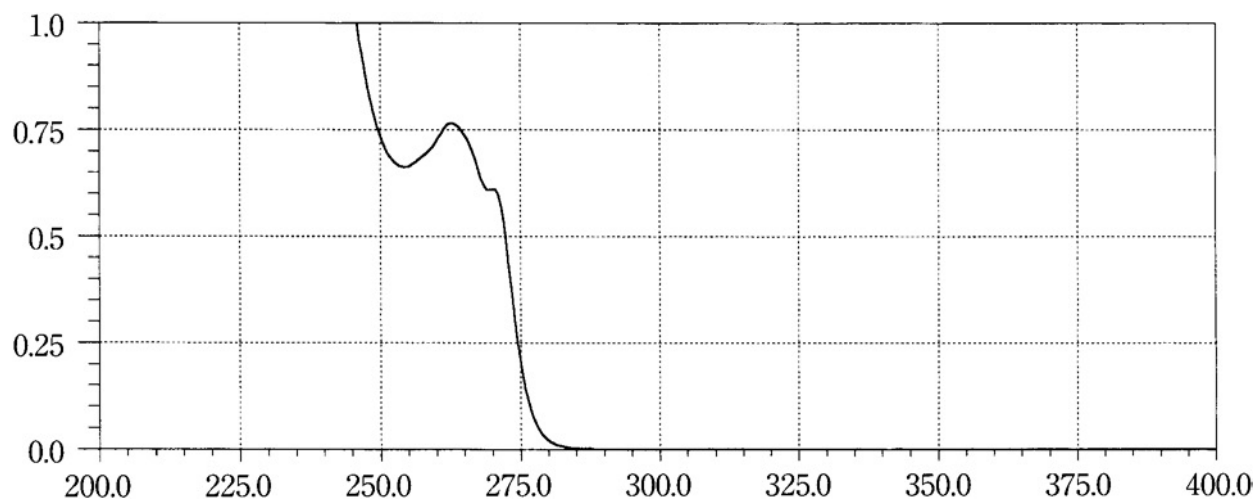
Kitasamycin Tartrate**Latamoxef Sodium****Levallorphan Tartrate**

Levodopa

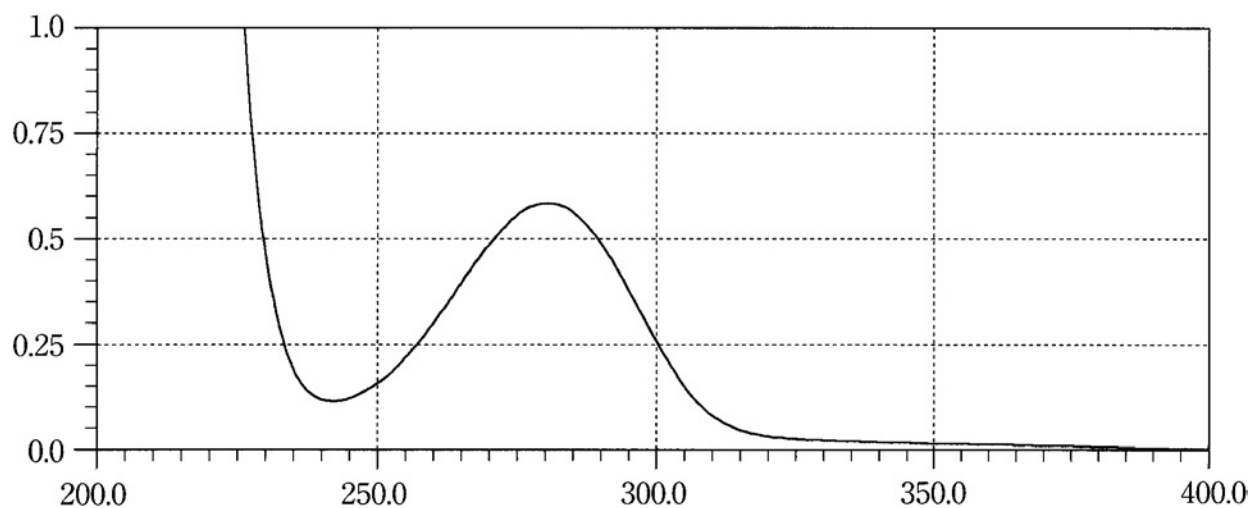
A solution in 0.001 mol/L hydrochloric acid TS (3 in 100,000)

Levothyroxine Sodium Hydrate

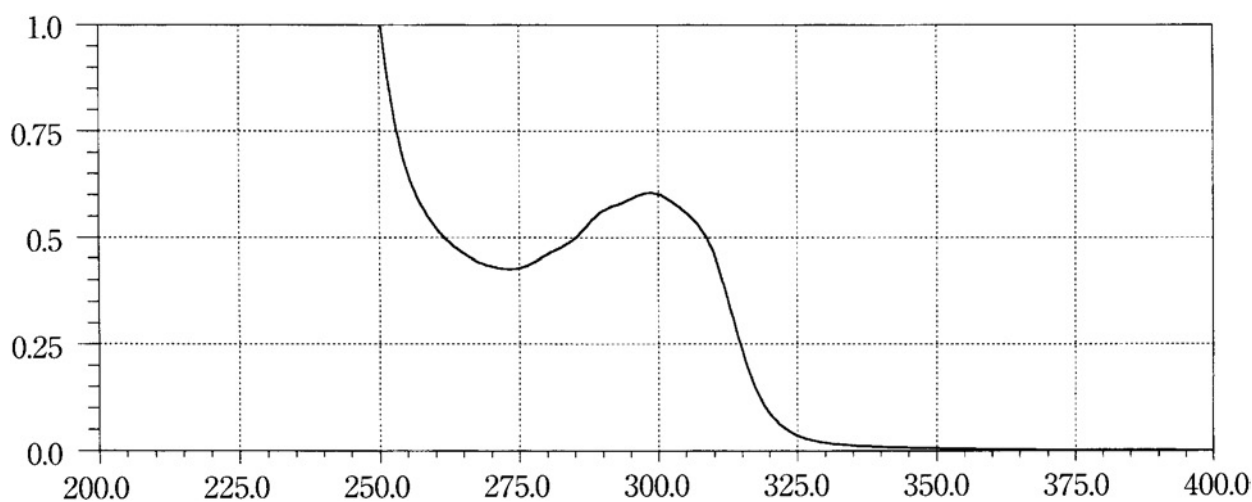
A solution in dilute sodium hydroxide TS (1 in 10,000)

Lidocaine

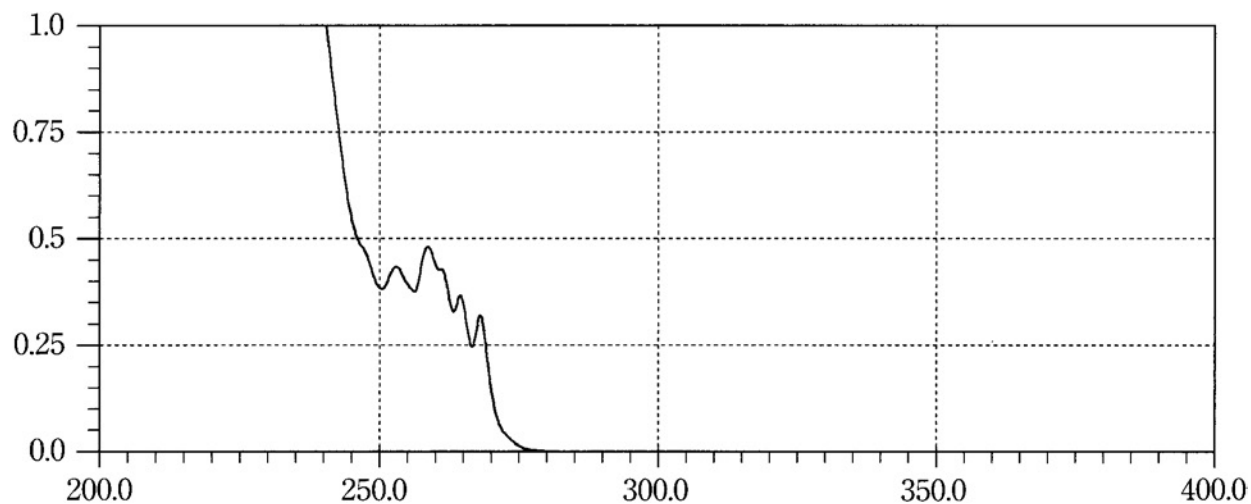
A solution prepared as follows: Dissolve 0.04 g in 10 mL of 1 mol/L hydrochloric acid TS and water to make 100 mL.

Limaprost Alfadex

A solution prepared as follows: To 10 mL of a solution in dilute ethanol (3 in 10,000) add 1 mL of potassium hydroxide-ethanol TS, and allow to stand for 15 minutes.

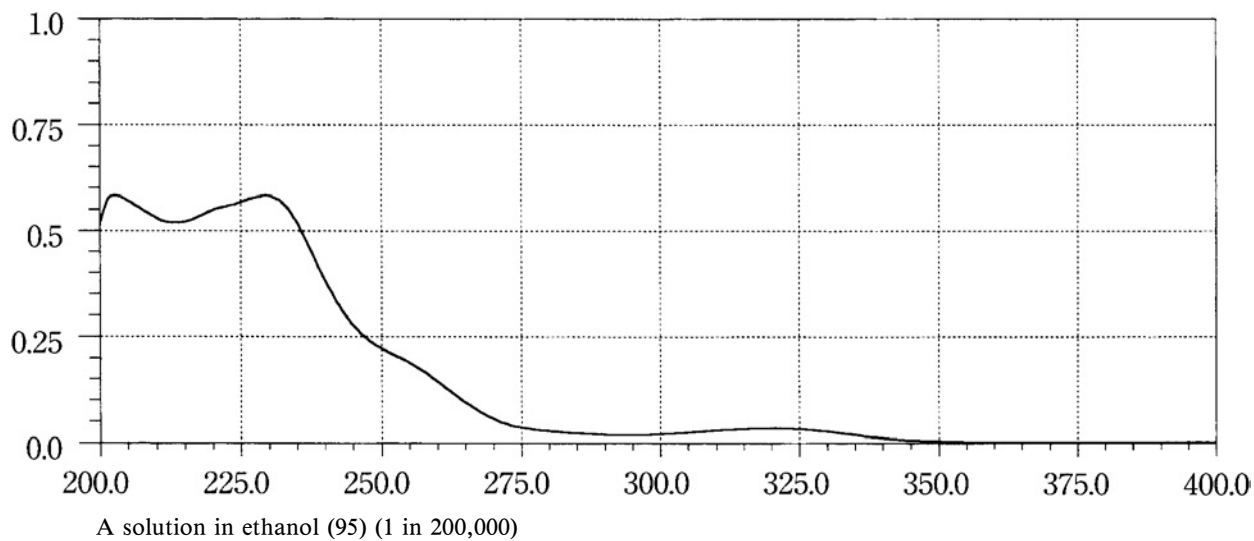
Liothyronine Sodium

A solution in ethanol (95) (1 in 10,000)

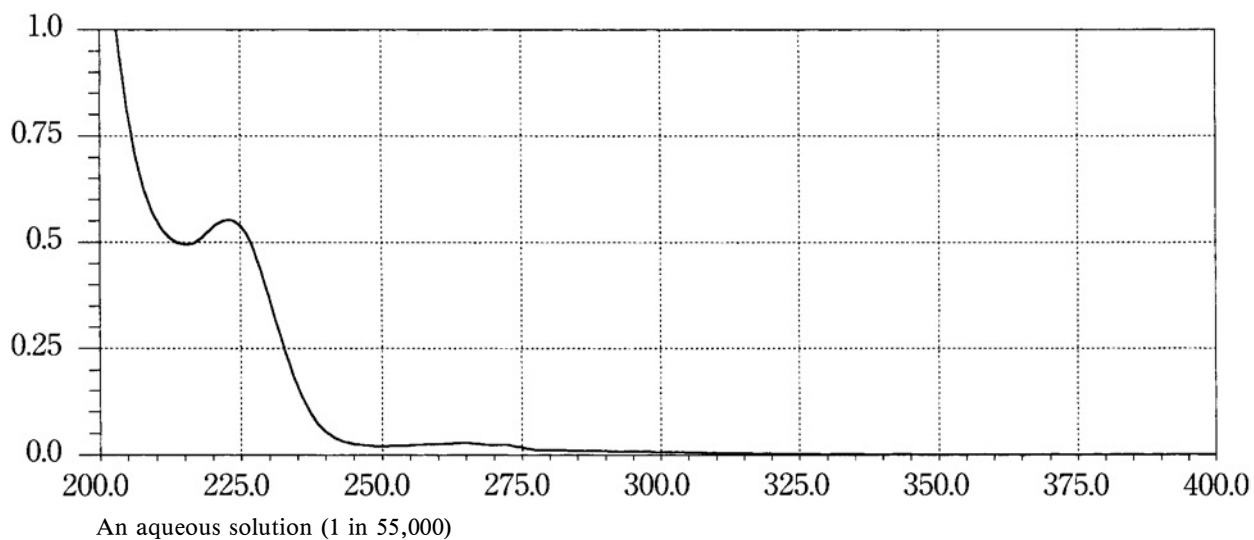
Lisinopril Hydrate

A solution in methanol (1 in 1000)

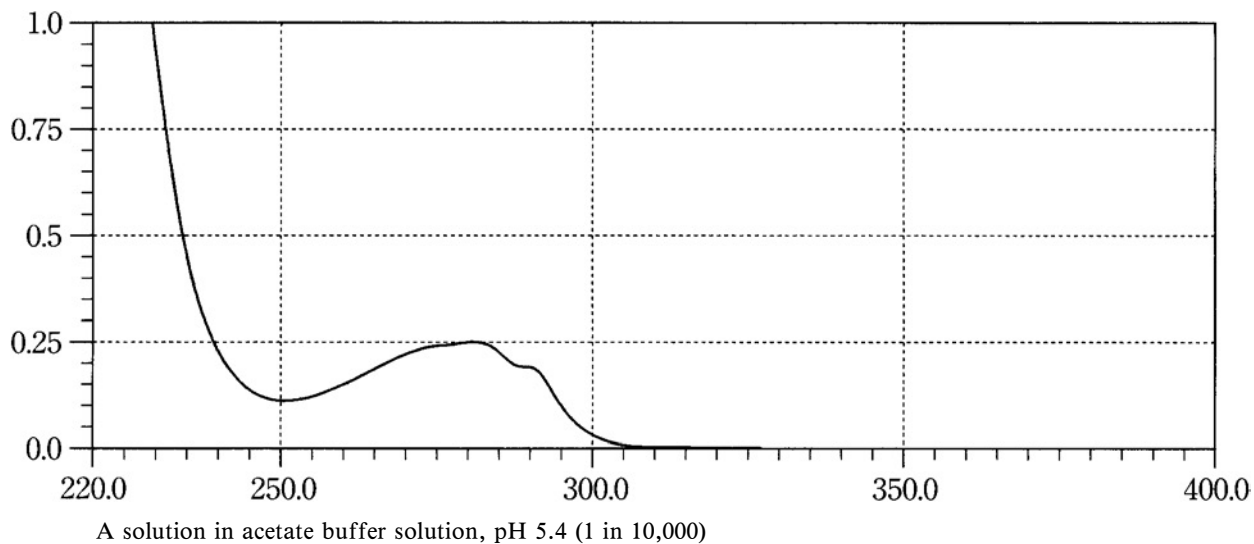
Lorazepam

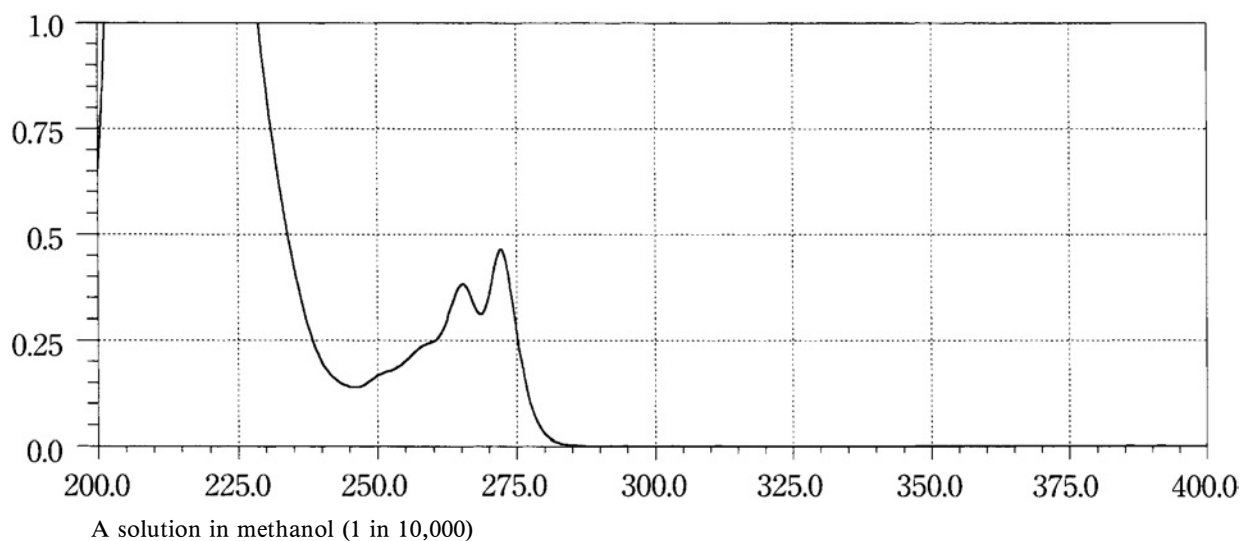
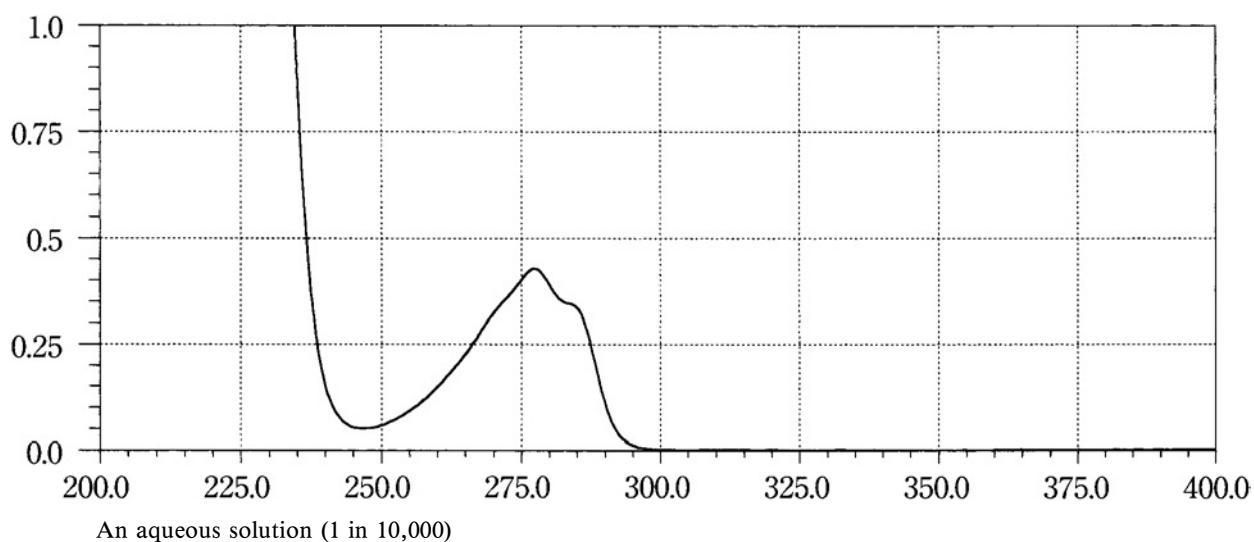
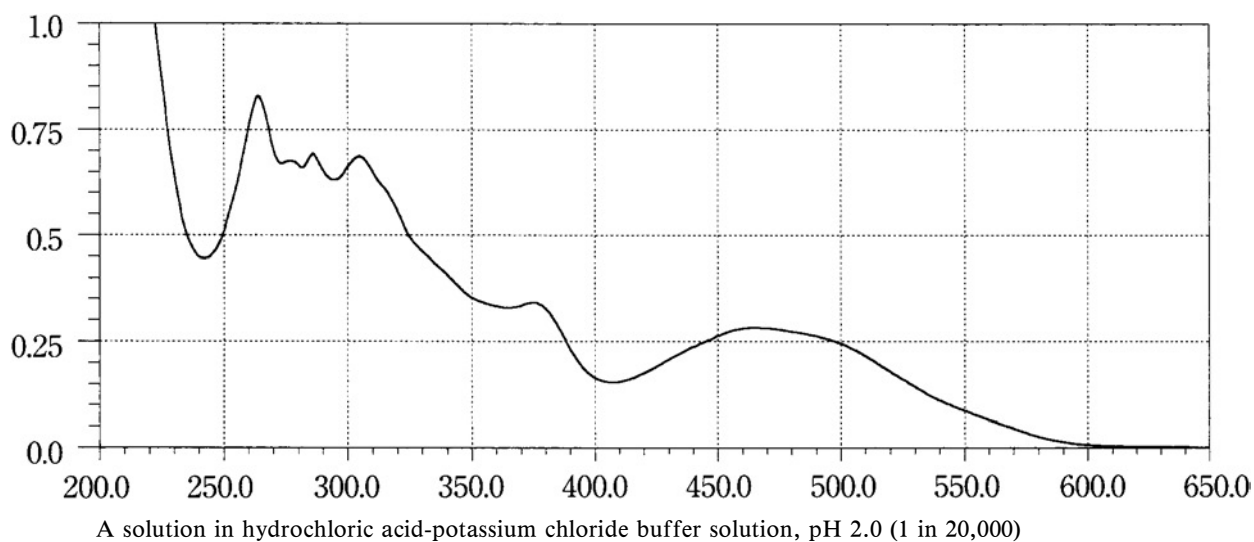


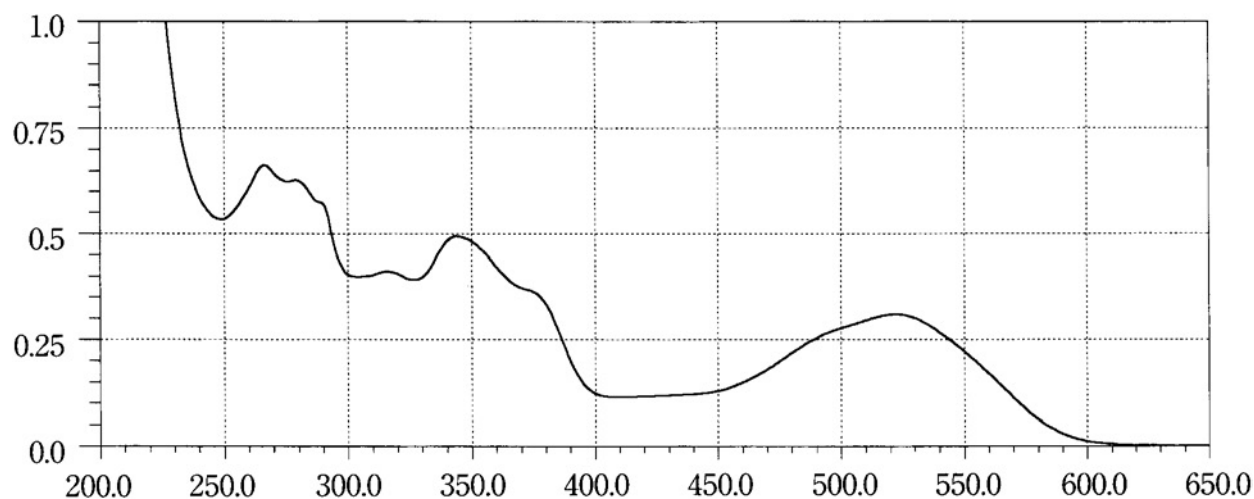
Loxoprofen Sodium Hydrate



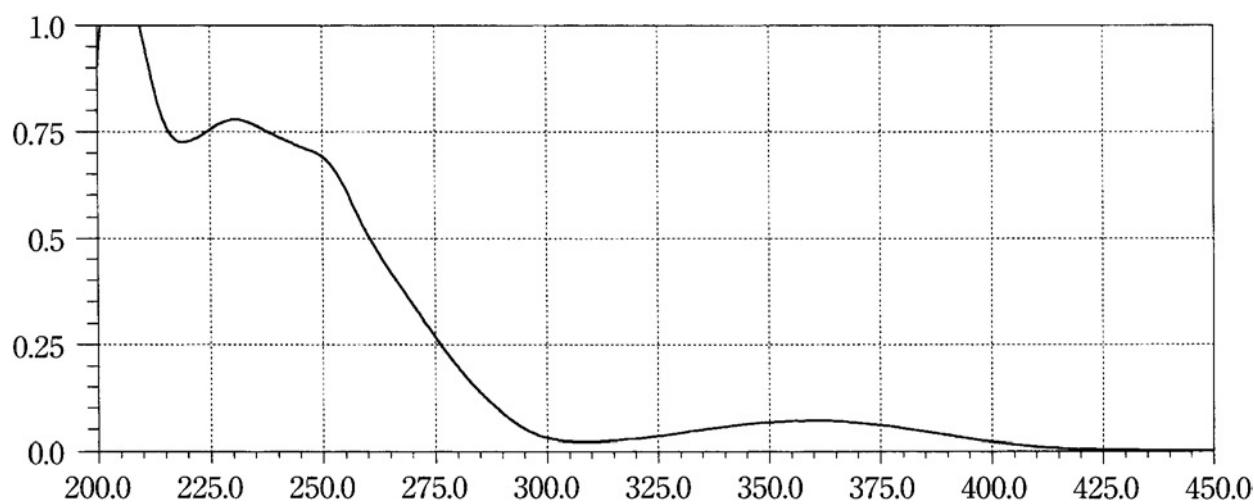
Lysozyme Hydrochloride



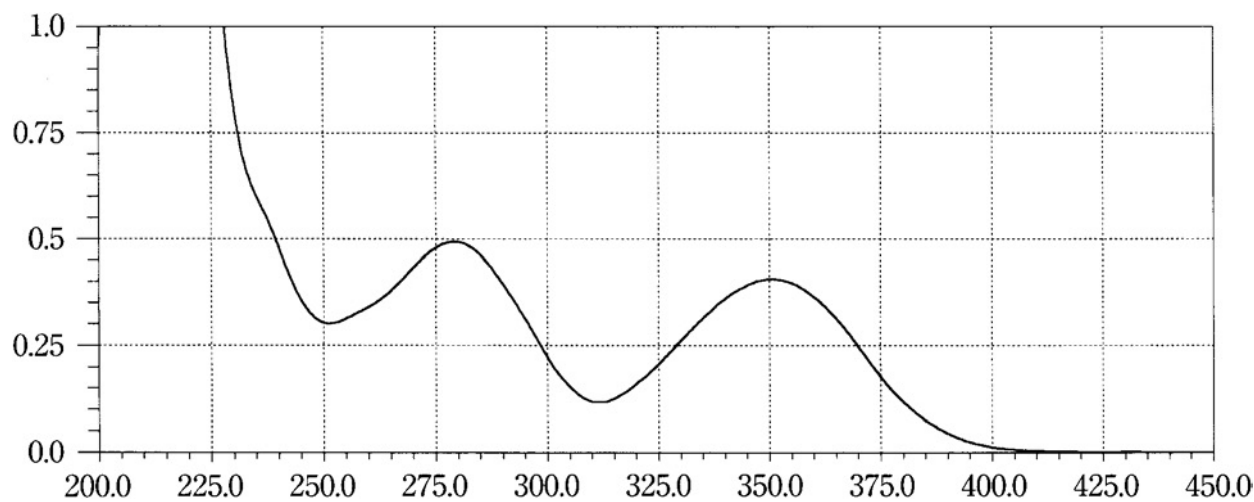
Maprotiline Hydrochloride**Meclofenoxate Hydrochloride****Mecobalamin 1**

Mecobalamin 2

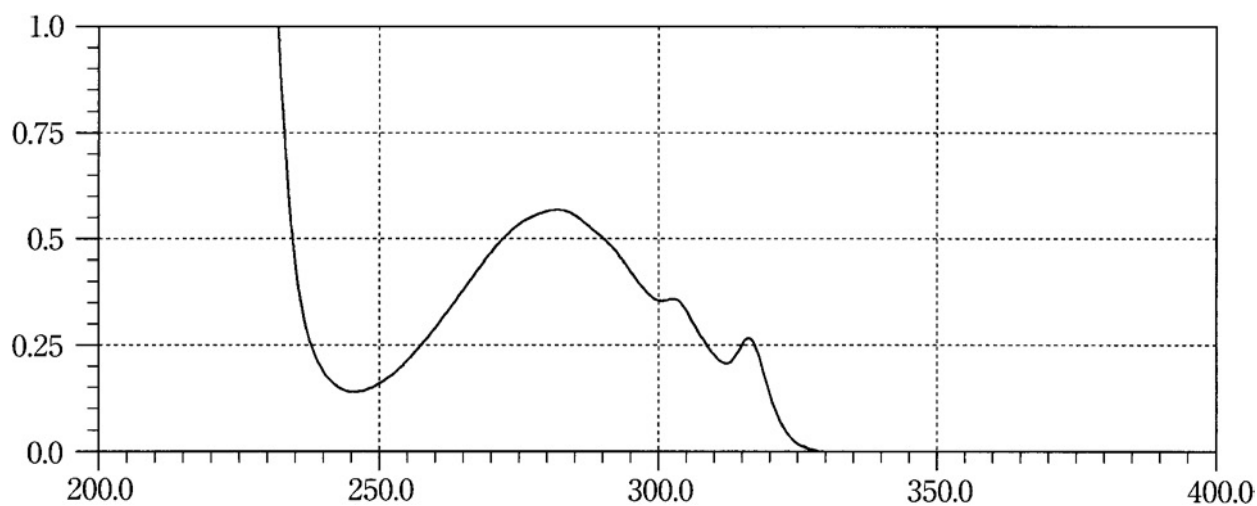
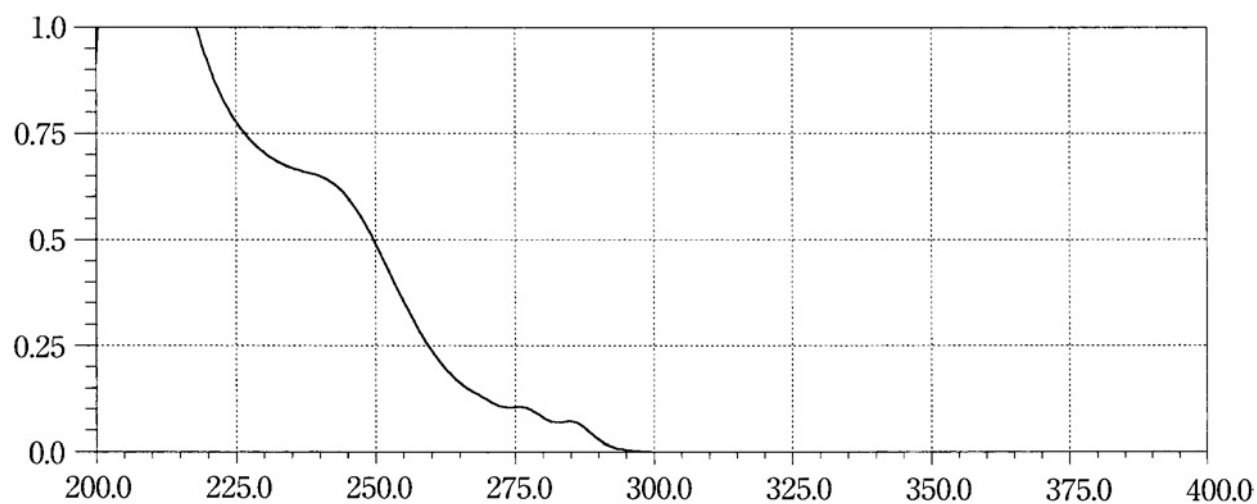
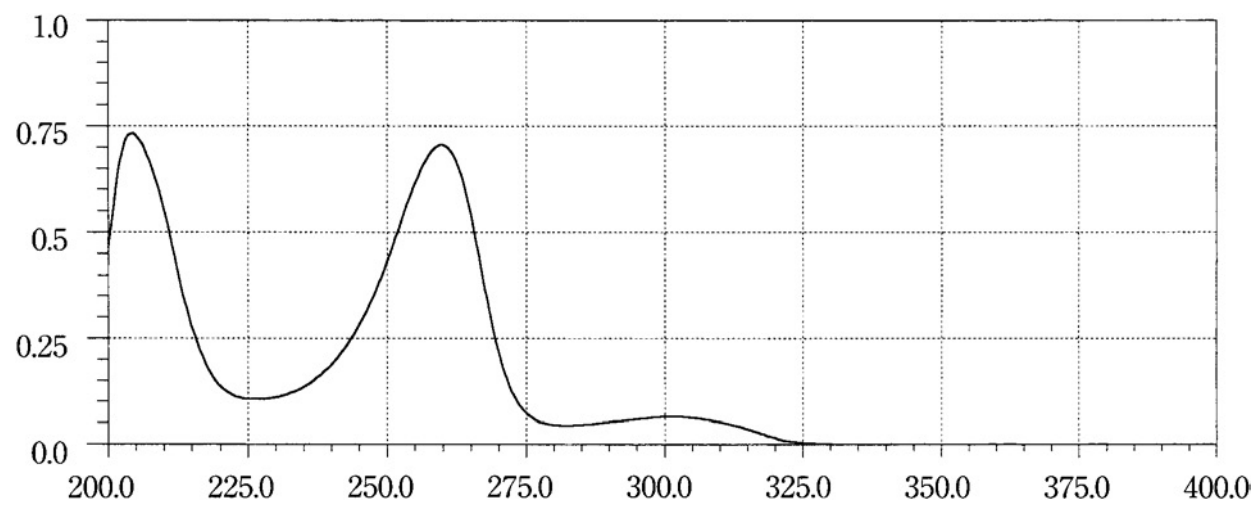
A solution in phosphate buffer solution, pH 7.0 (1 in 20,000)

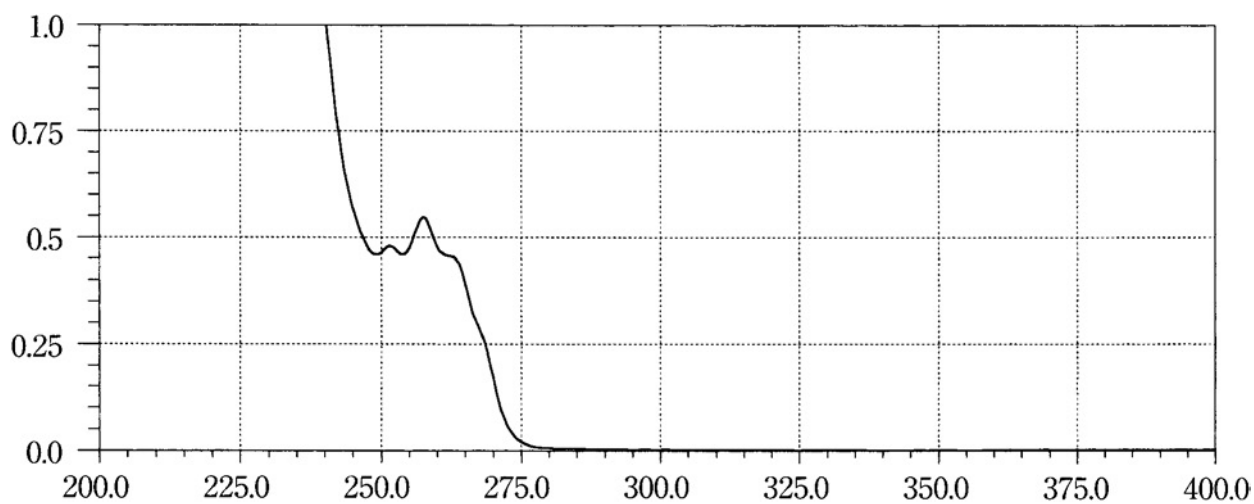
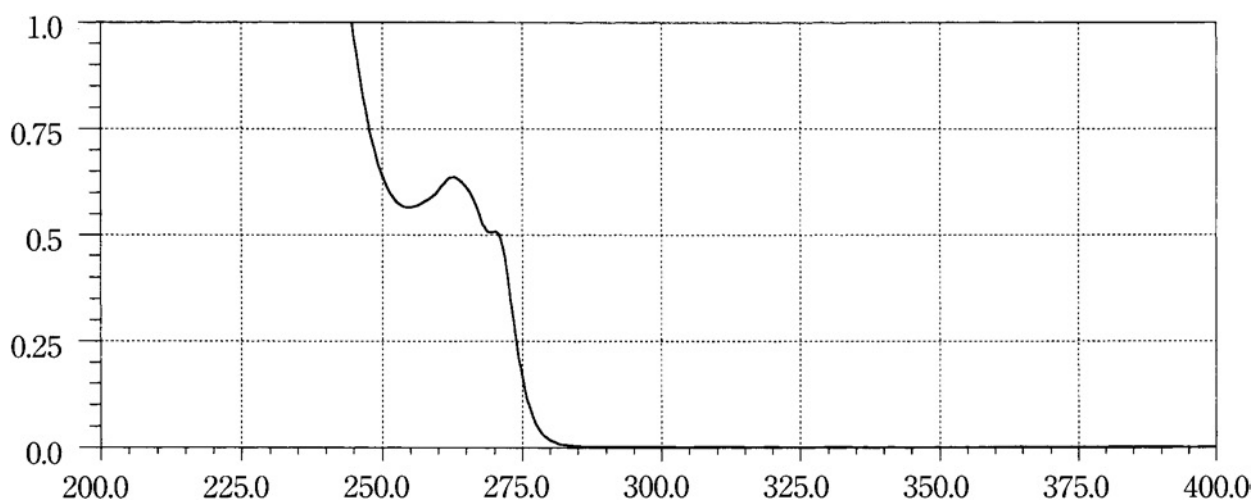
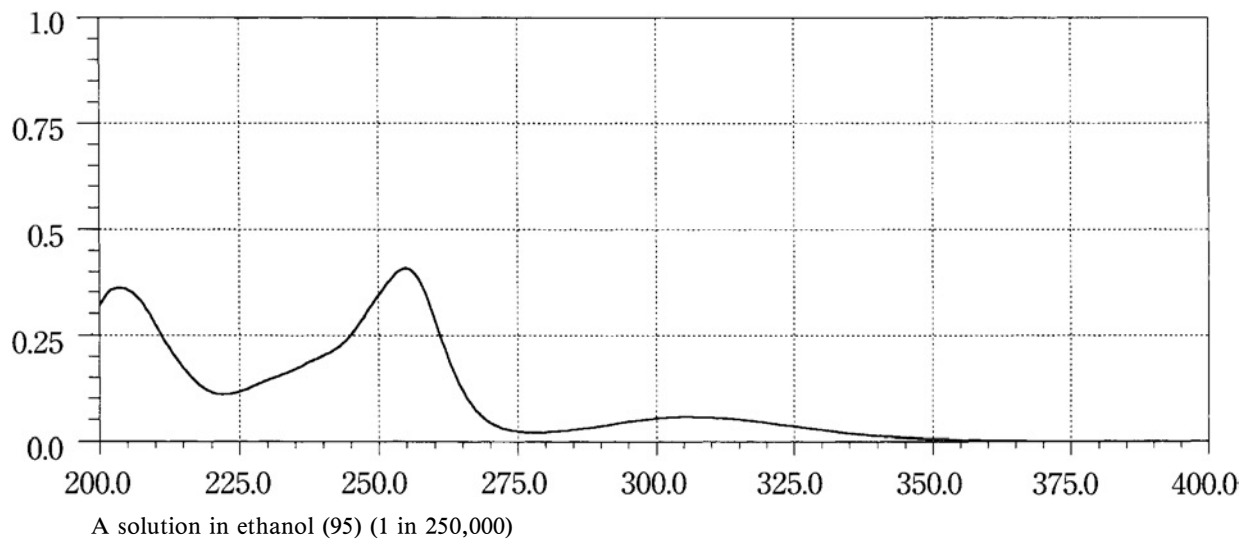
Medazepam

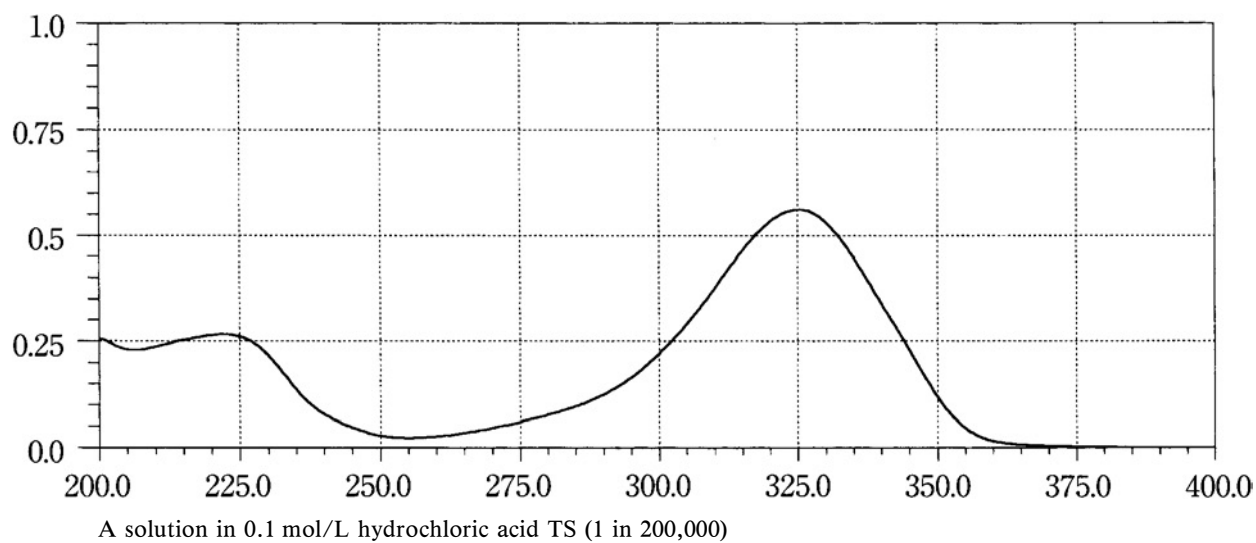
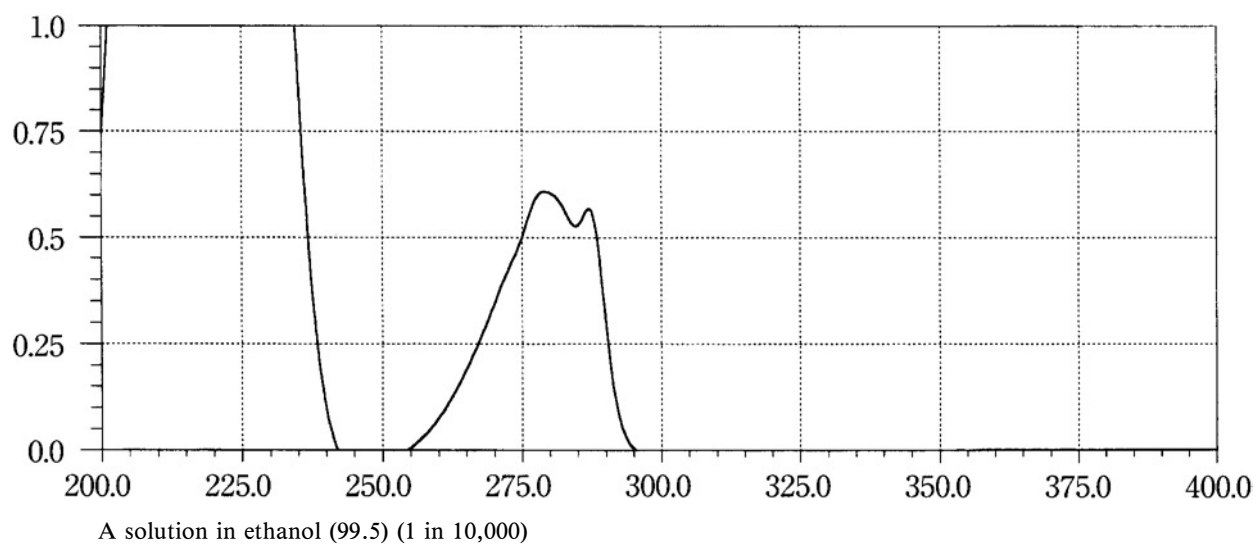
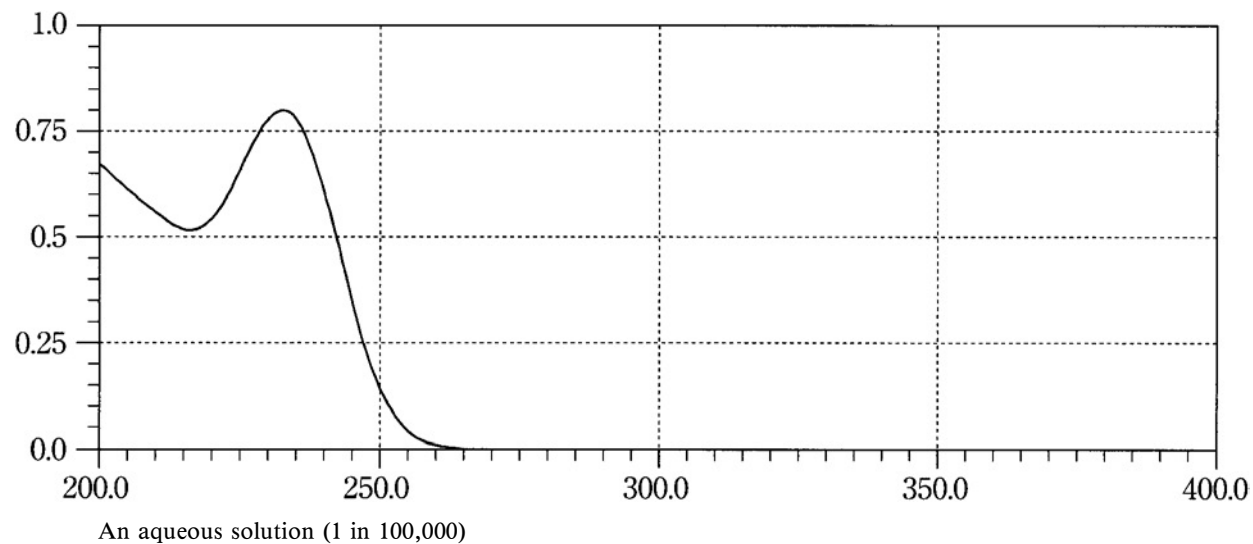
A solution in methanol (1 in 100,000)

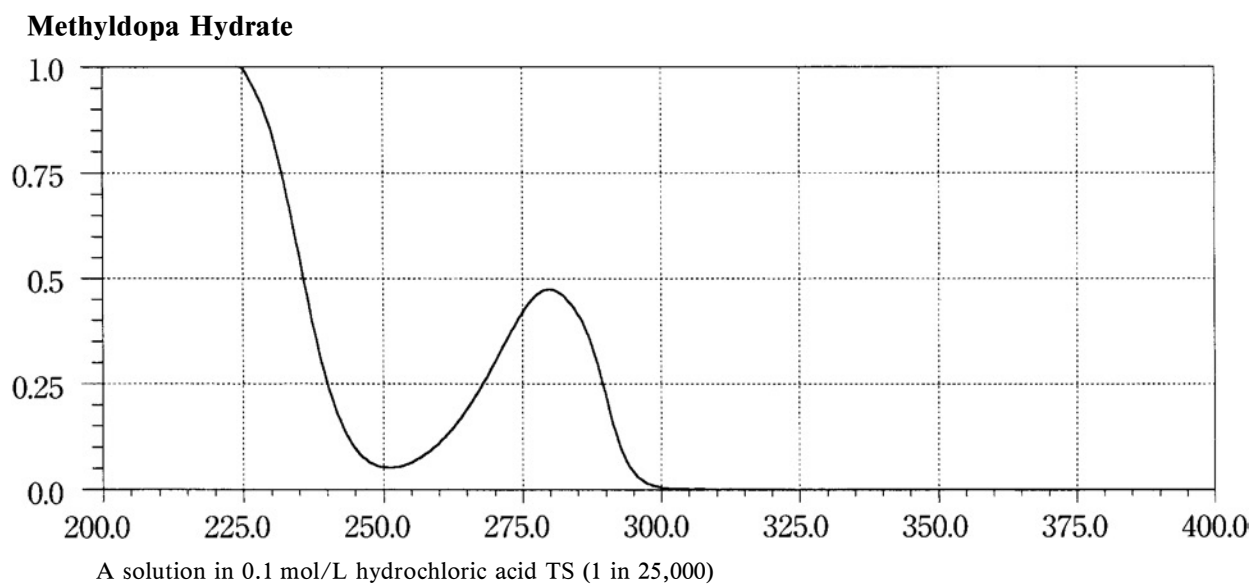
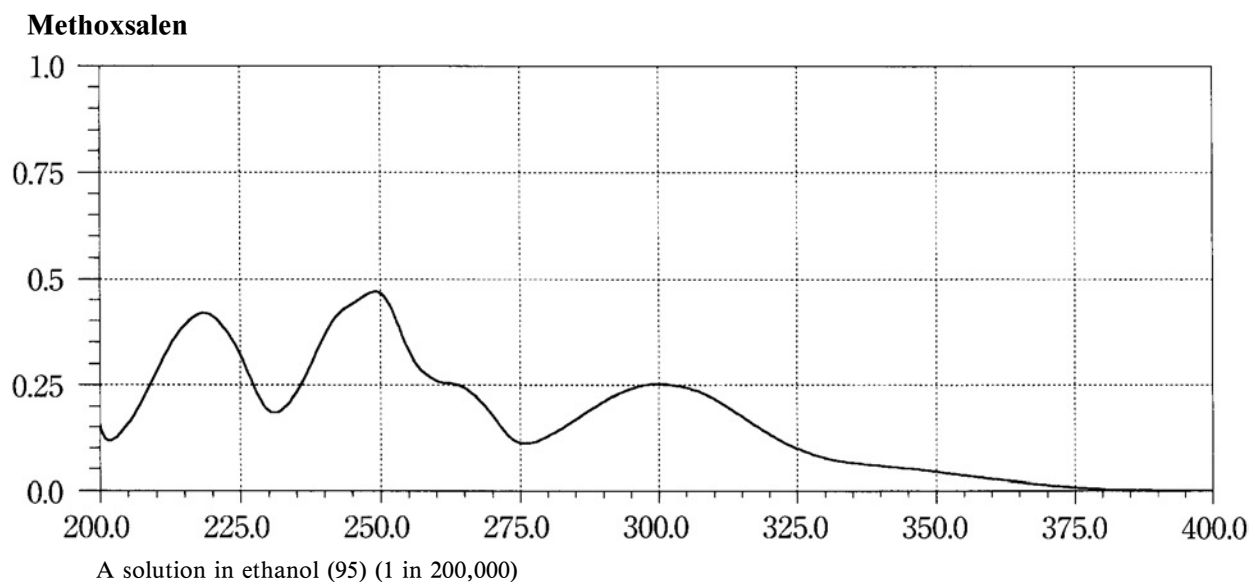
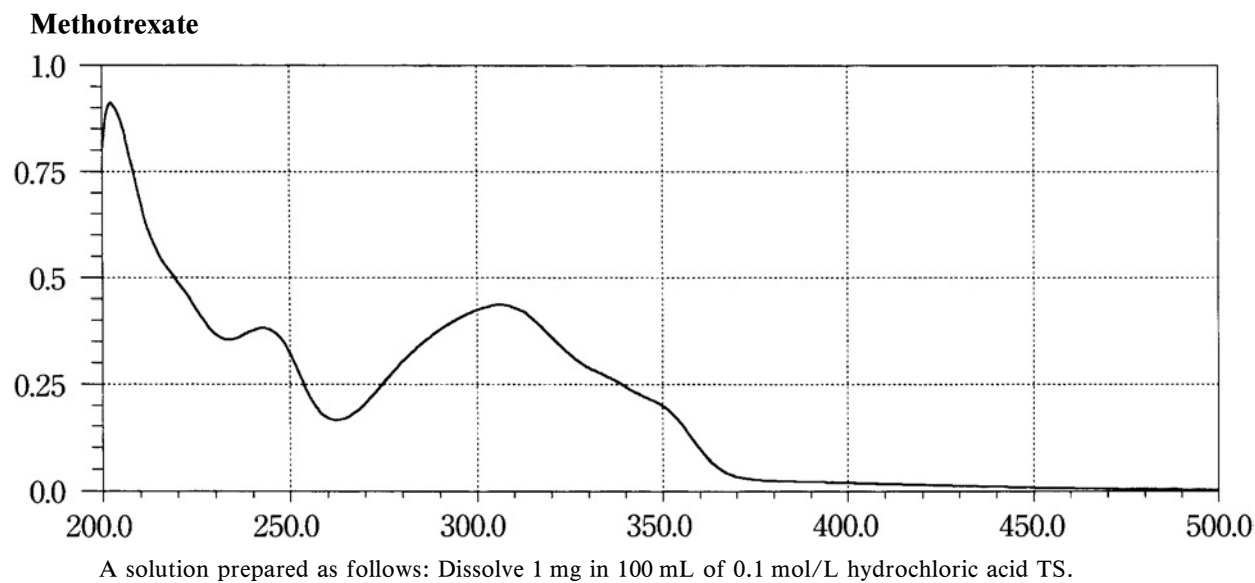
Mefenamic Acid

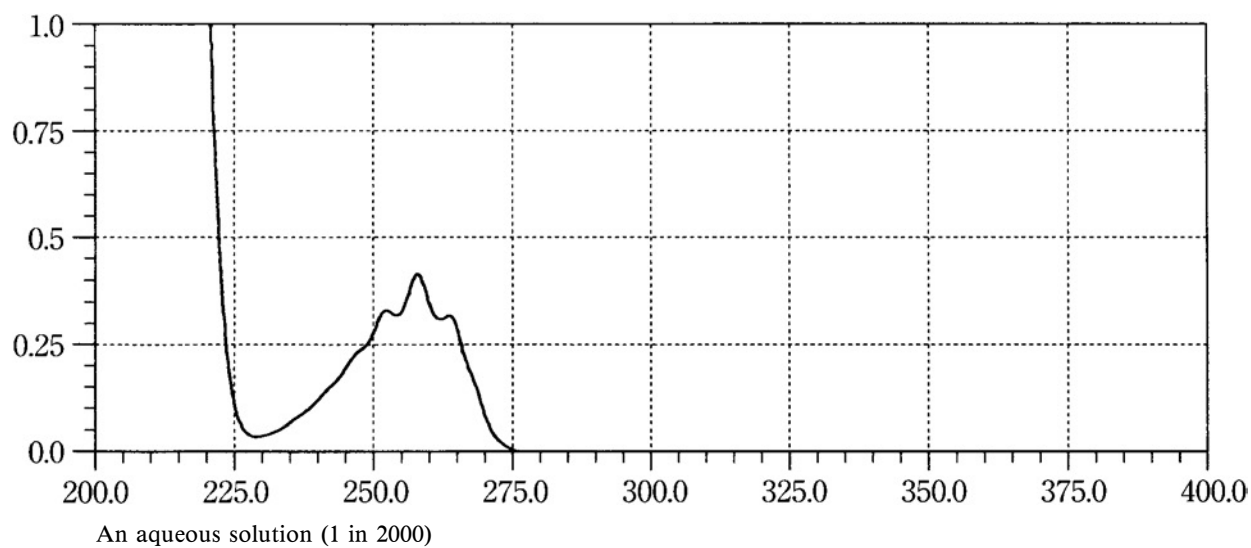
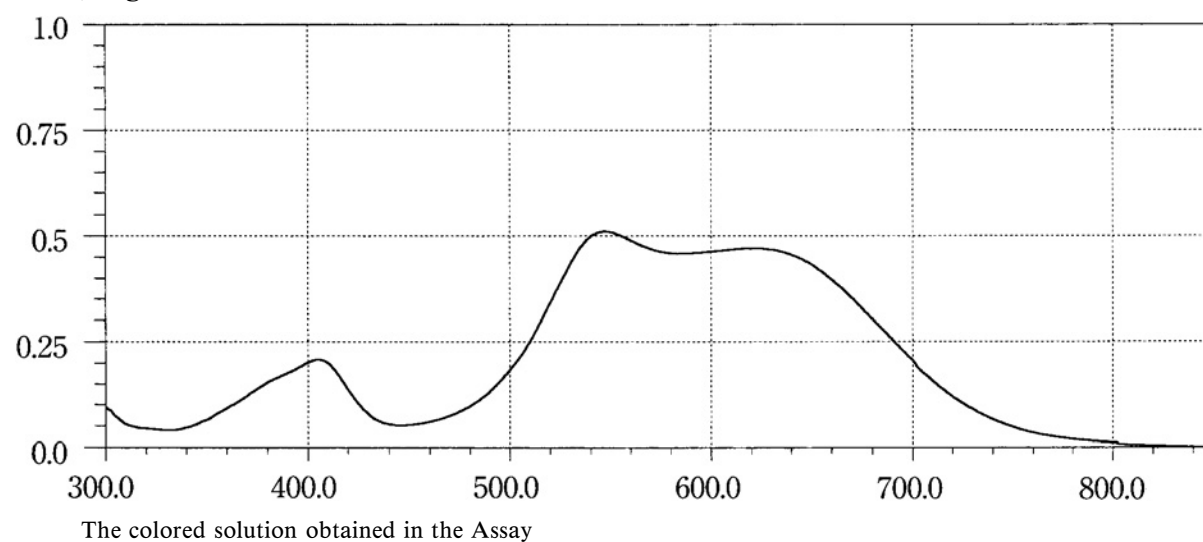
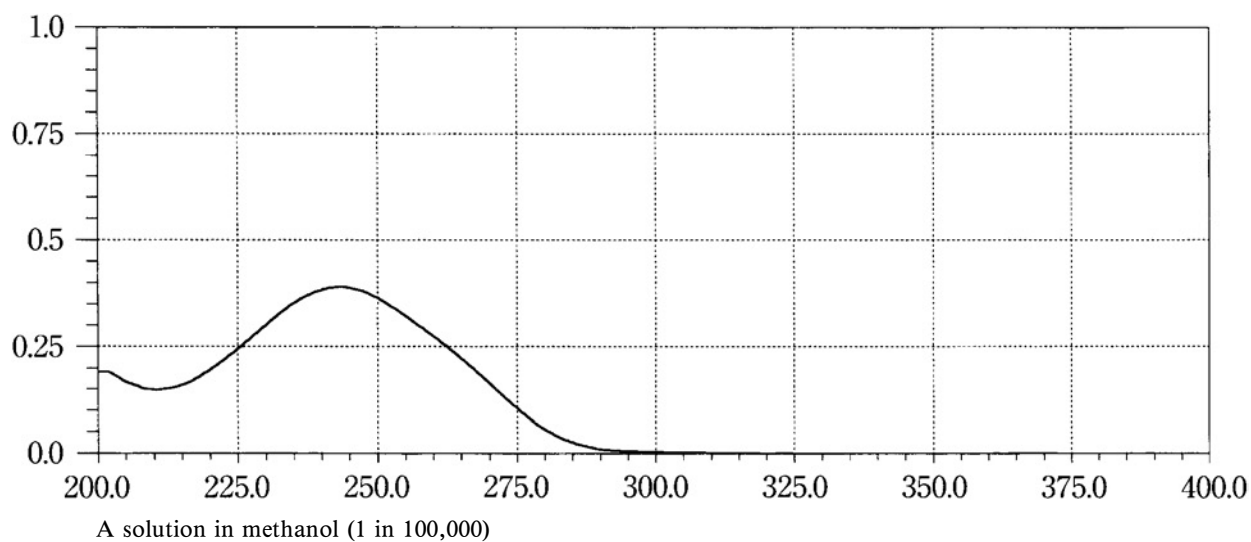
A solution prepared as follows: Dissolve 7 mg in a solution of hydrochloric acid in methanol (1 in 1000) to make 500 mL.

Mefloquine Hydrochloride**Mefruside****Melphalan**

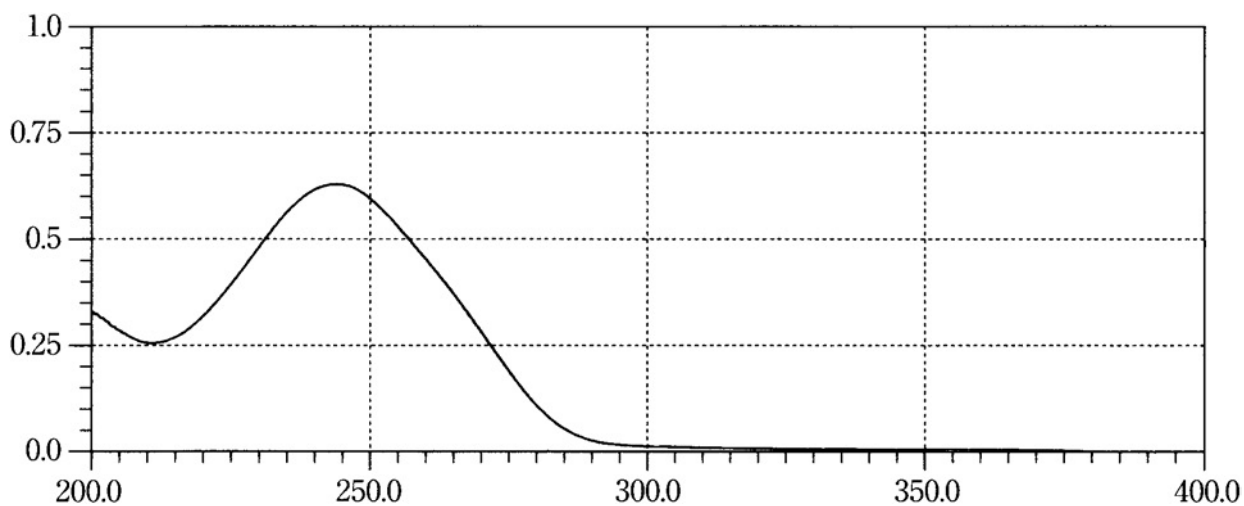
Mepenzolate Bromide**Mepivacaine Hydrochloride****Mequitazine**

Mercaptopurine Hydrate**Mestranol****Metformin Hydrochloride**



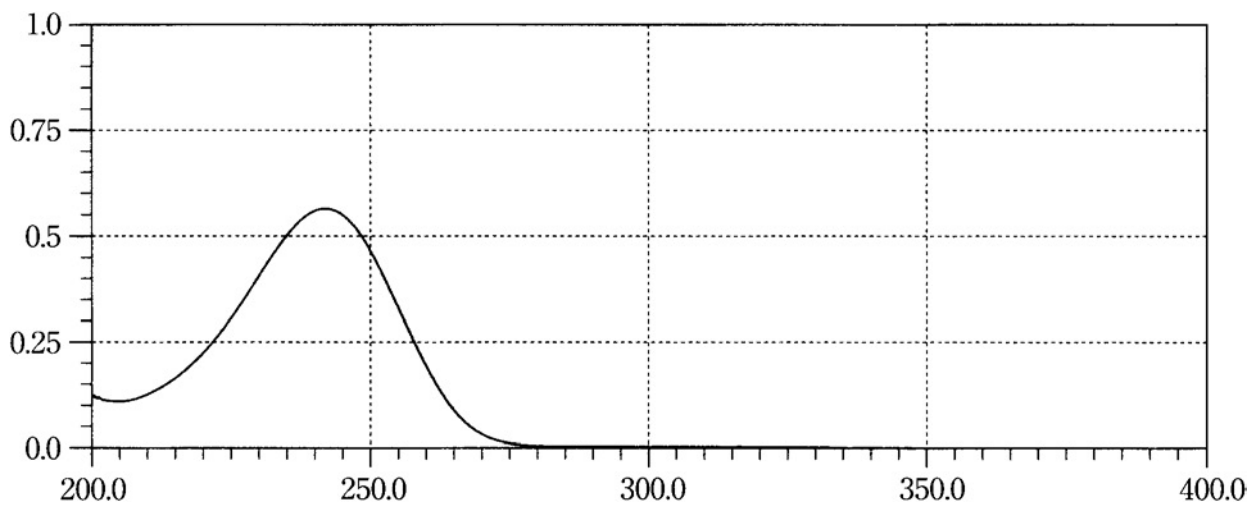
***dl*-Methylephedrine Hydrochloride****Methylephedrine Maleate****Methylprednisolone**

Methylprednisolone Succinate



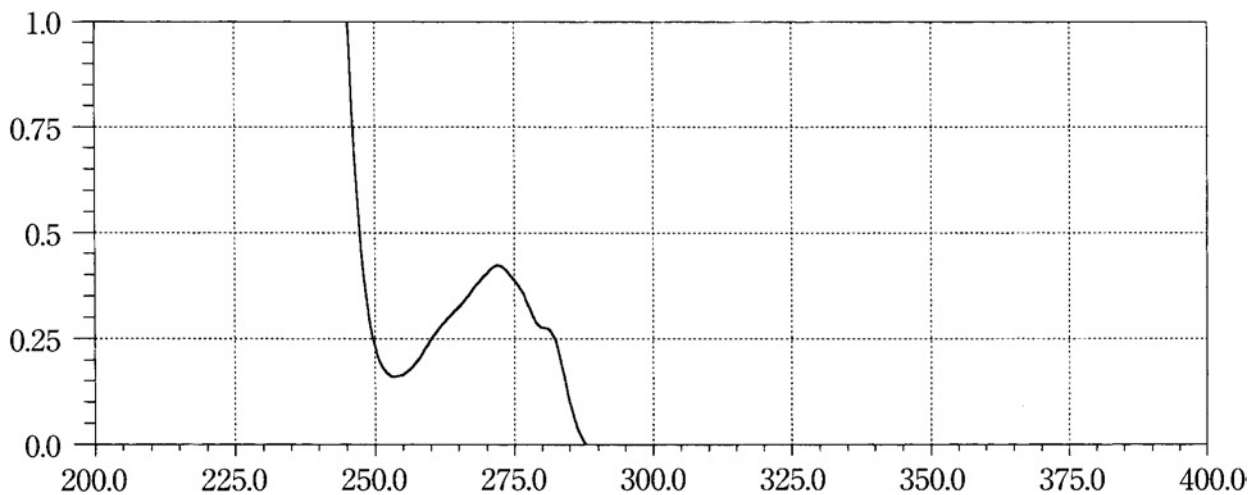
A solution in methanol (1 in 50,000)

Methyltestosterone

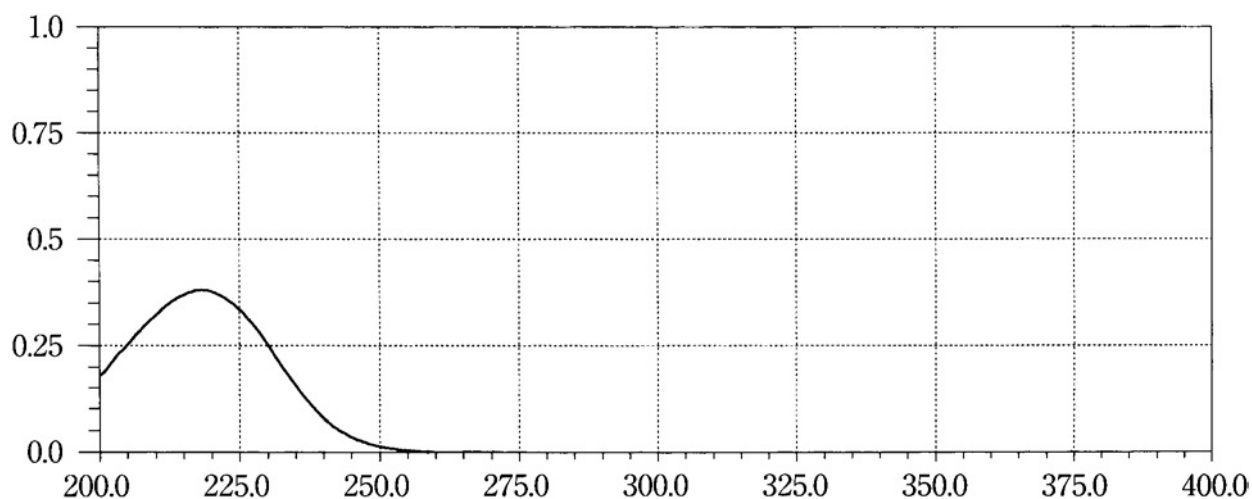


A solution in ethanol (95) (1 in 100,000)

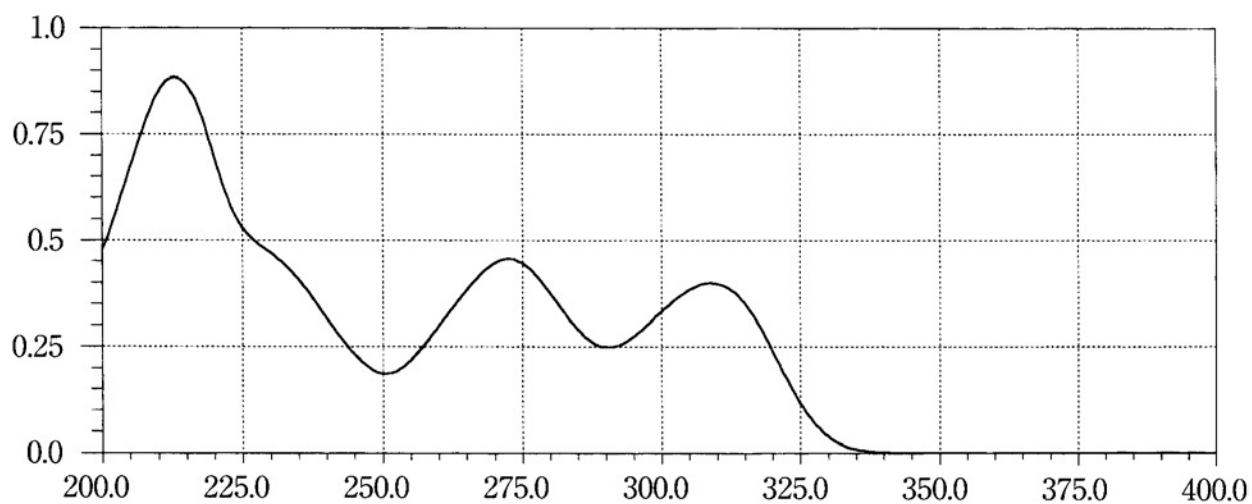
Meticrane



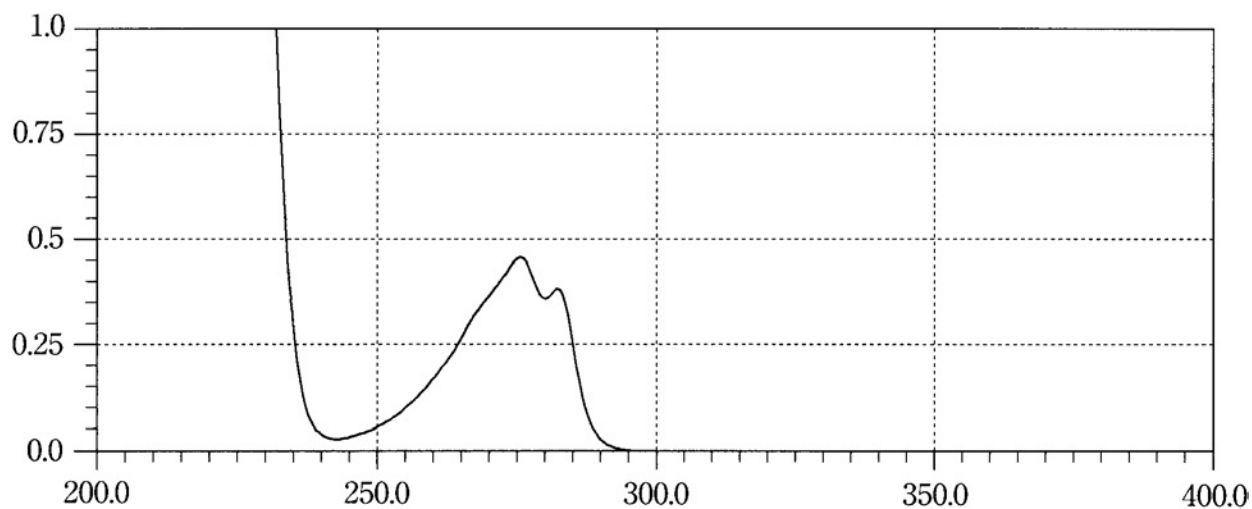
A solution in methanol (3 in 10,000)

Metildigoxin

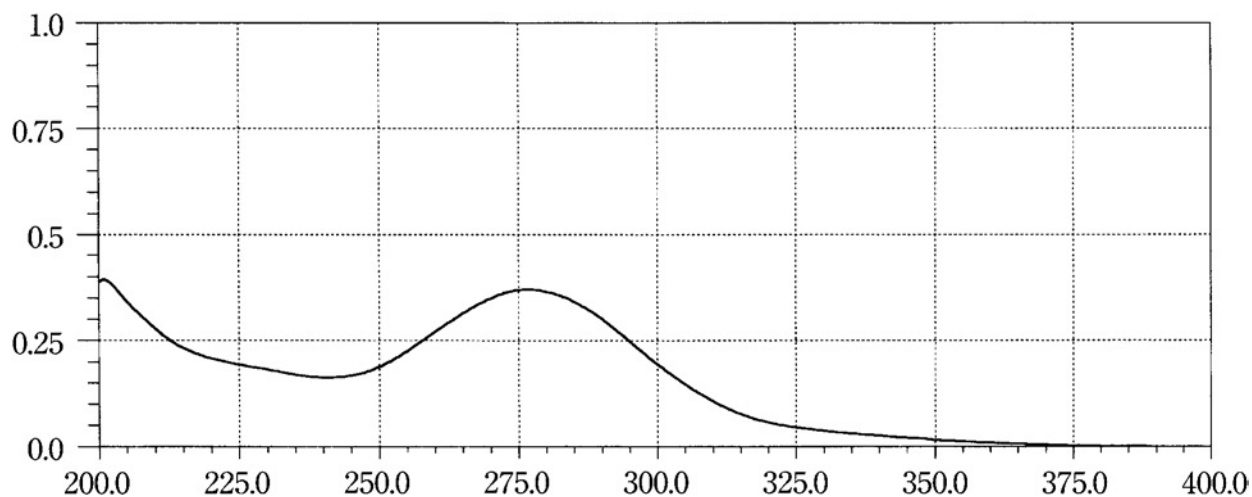
A solution in methanol (1 in 50,000)

Metoclopramide

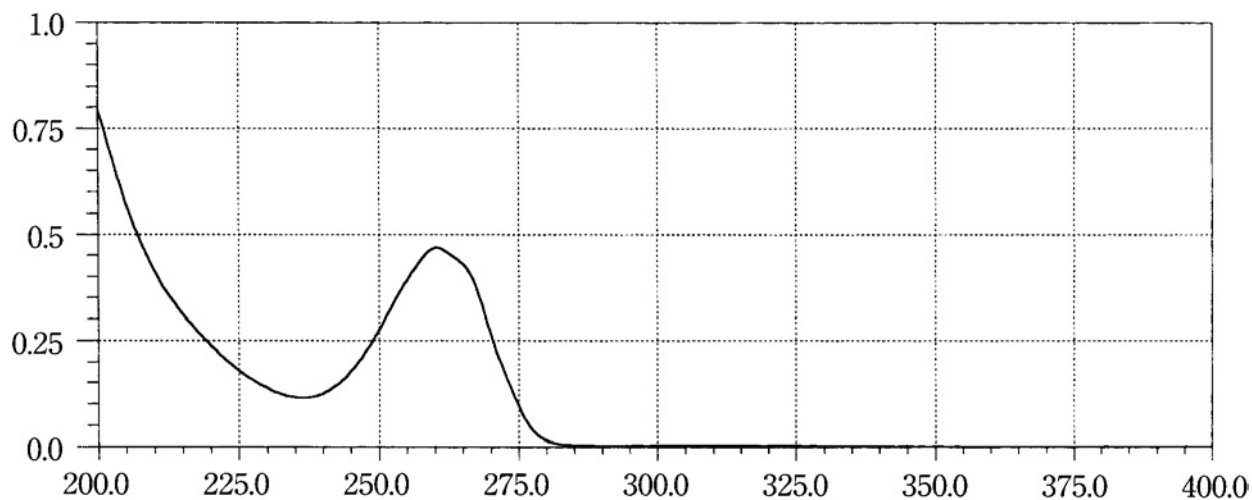
A solution prepared as follows: Dissolve 0.1 g in 1 mL of 1 mol/L hydrochloric acid TS, and add water to make 100 mL. To 1 mL of this solution add water to make 100 mL.

Metoprolol Tartrate

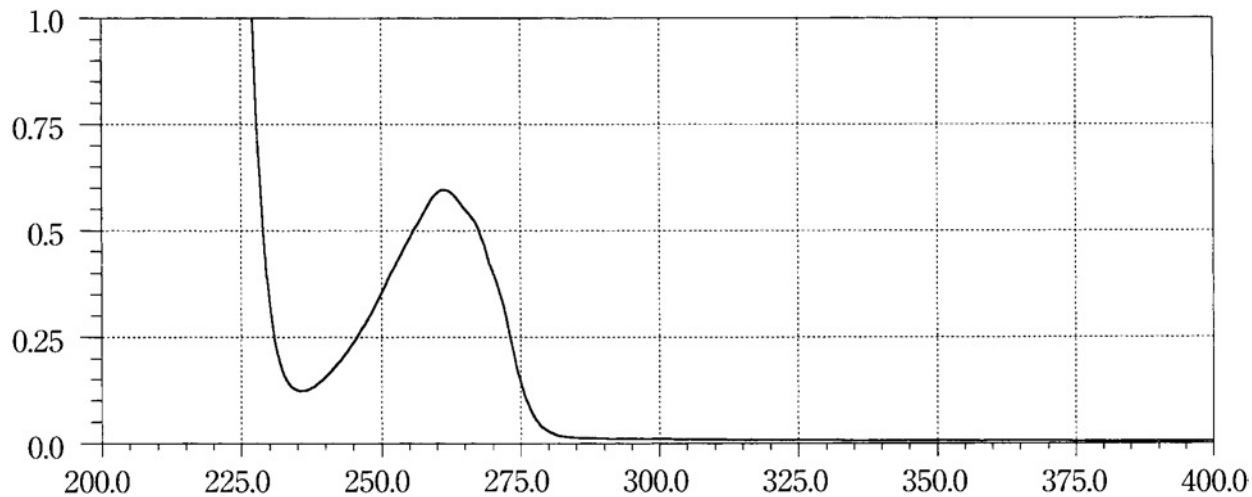
A solution in ethanol (95) (1 in 10,000)

Metronidazole

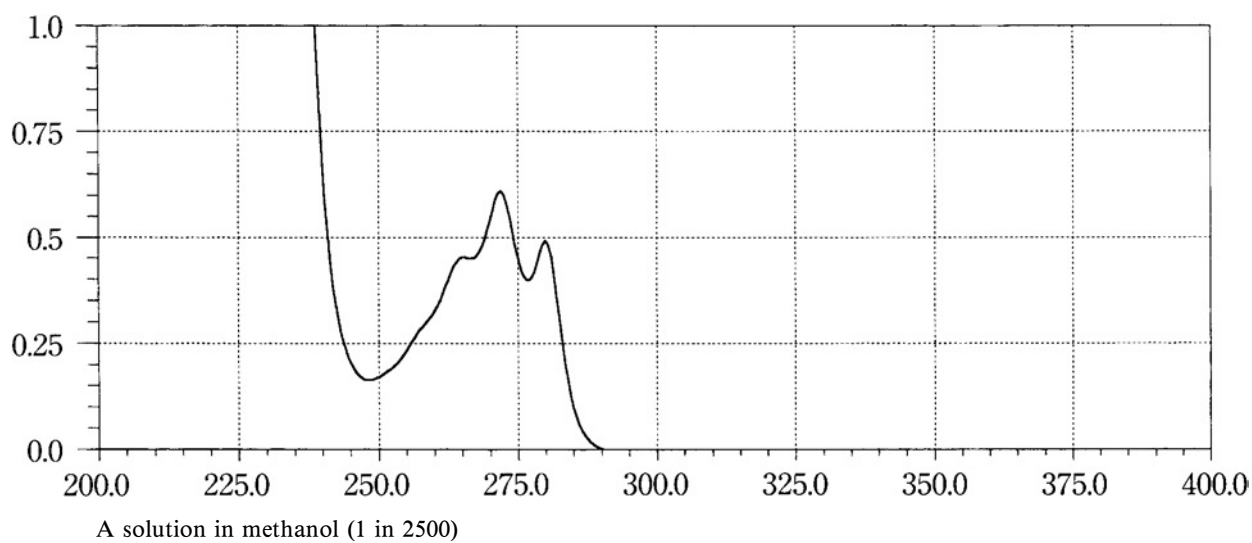
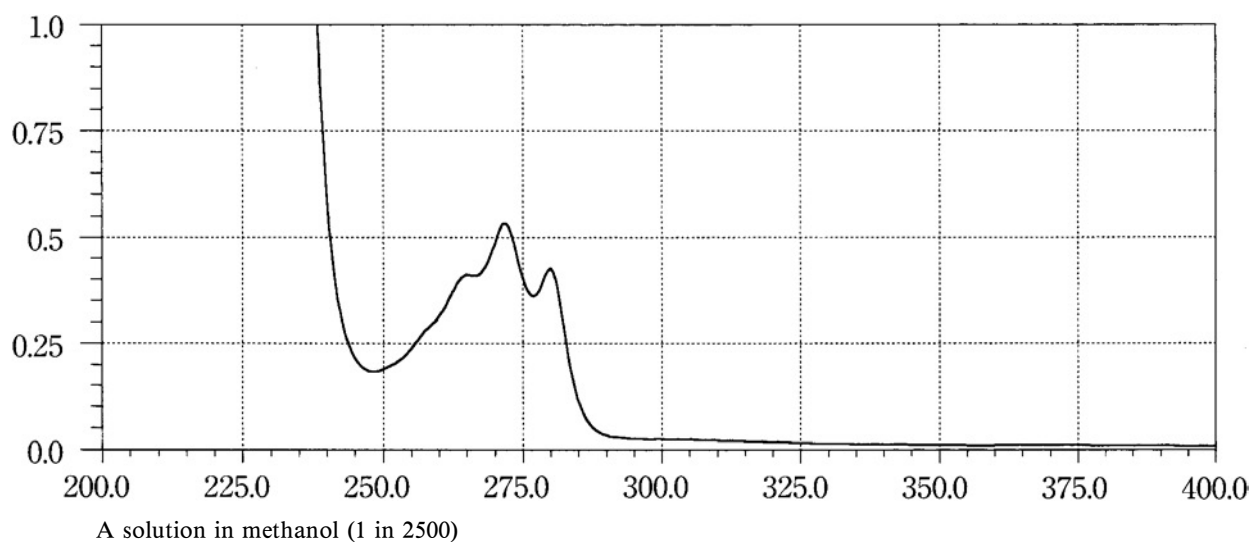
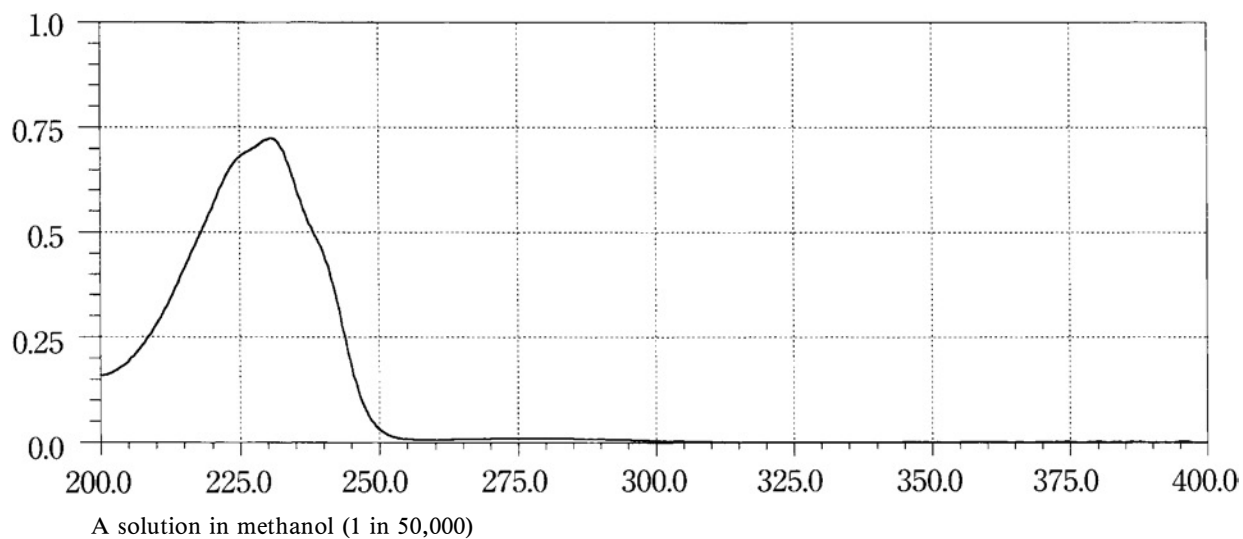
A solution in 0.1 mol/L hydrochloric acid TS (1 in 100,000)

Metyrapone

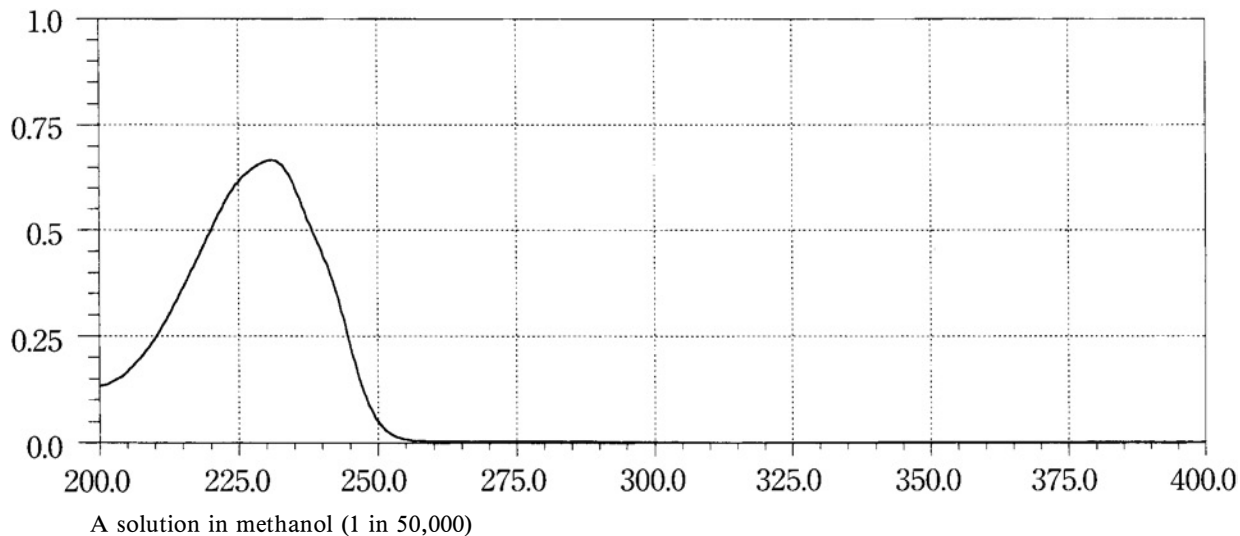
A solution in 0.5 mol/L sulfuric acid TS (1 in 100,000)

Mexiletine Hydrochloride

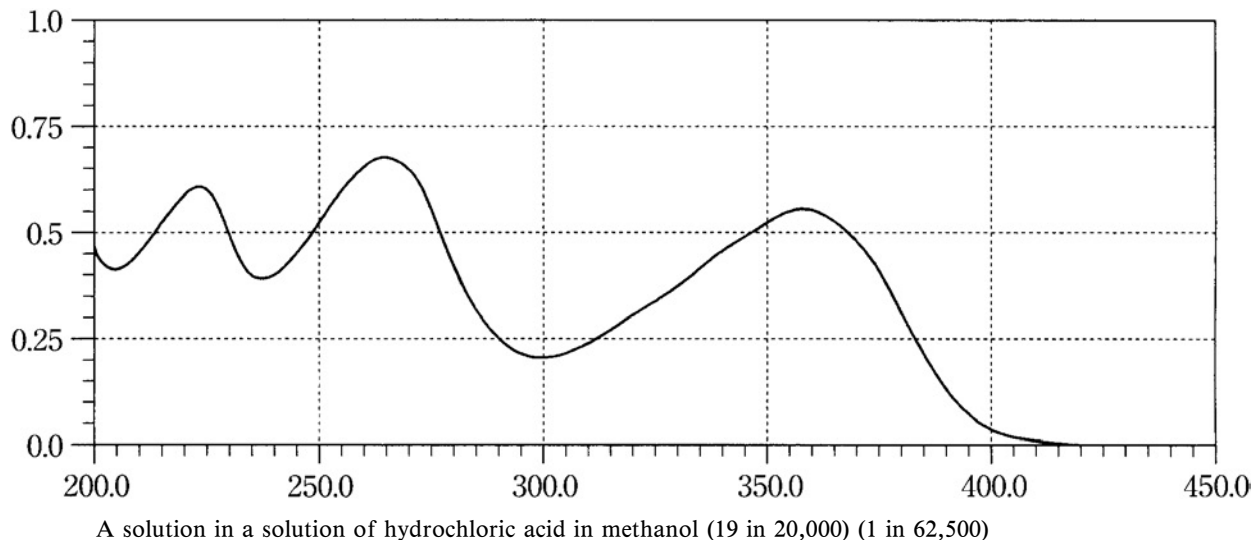
A solution in 0.01 mol/L hydrochloric acid TS (1 in 2000)

Miconazole**Miconazole Nitrate****Midecamycin**

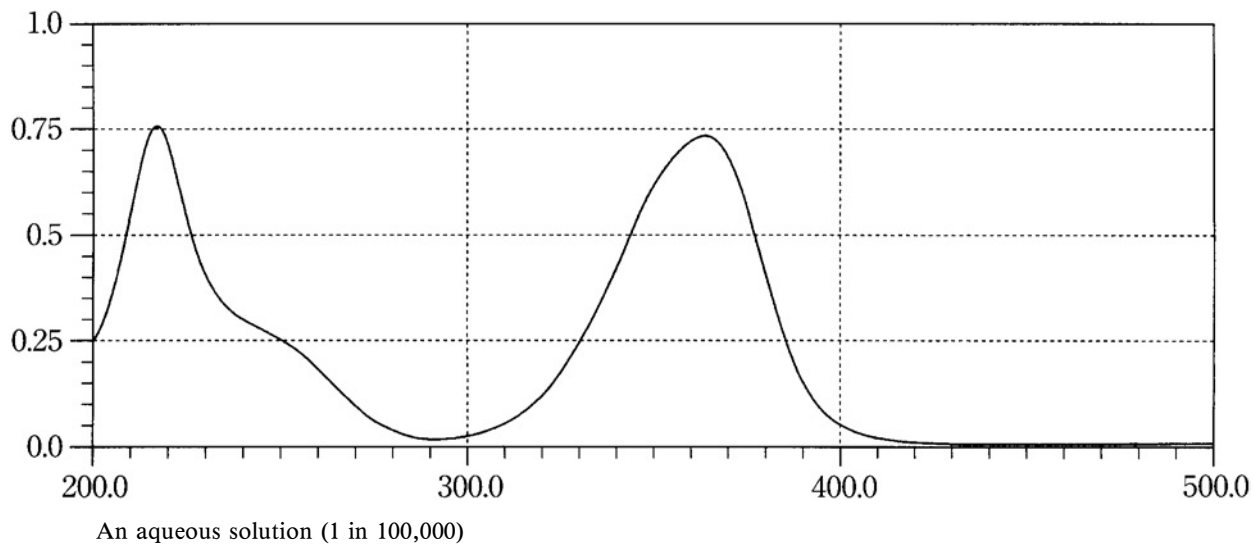
Midecamycin Acetate

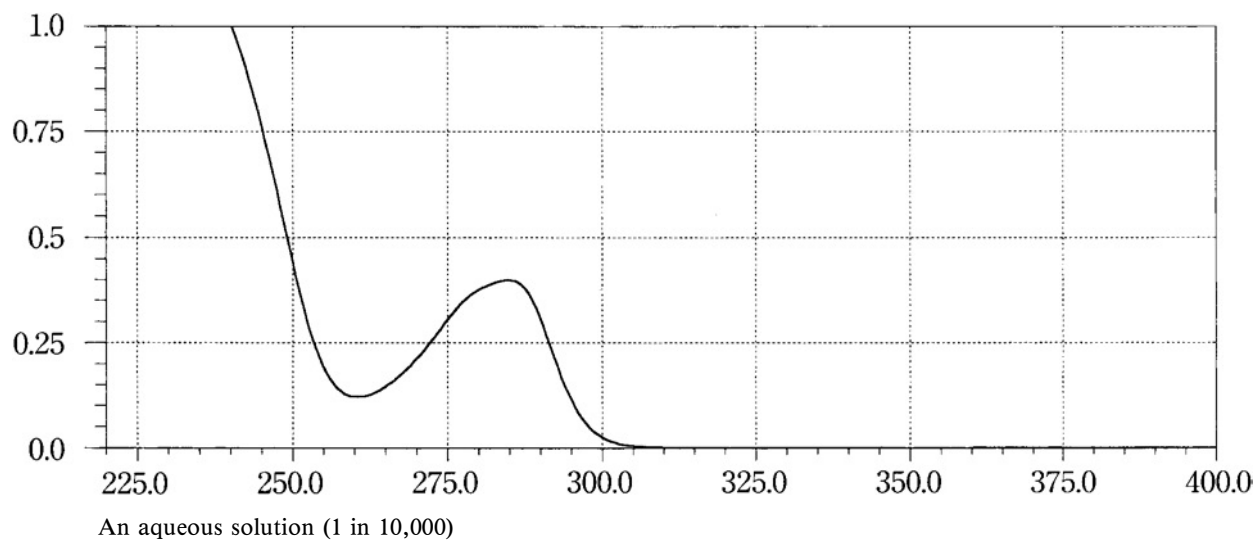
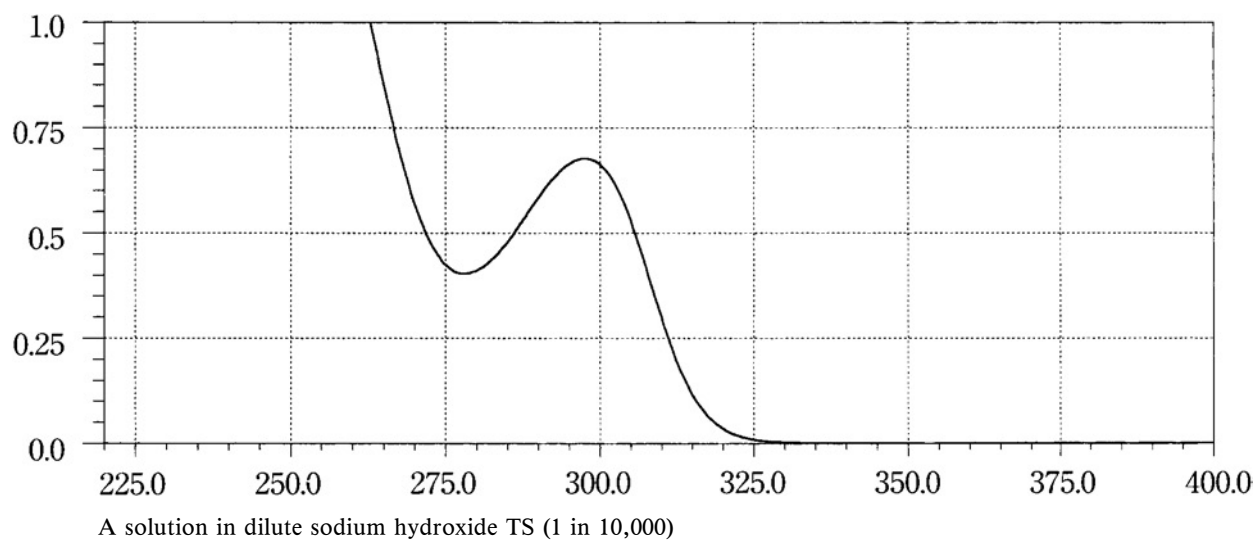
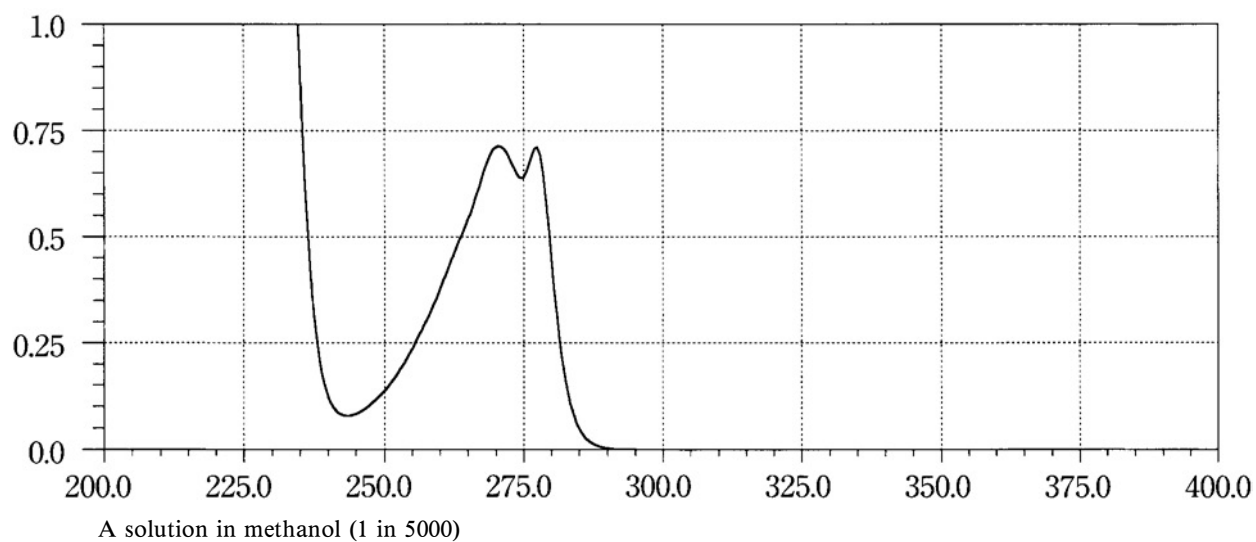


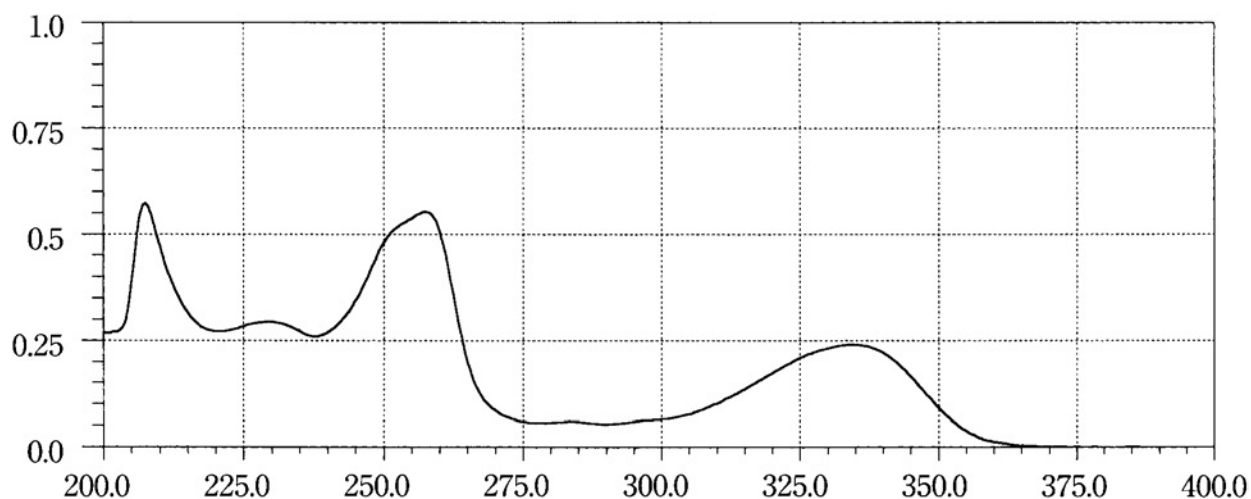
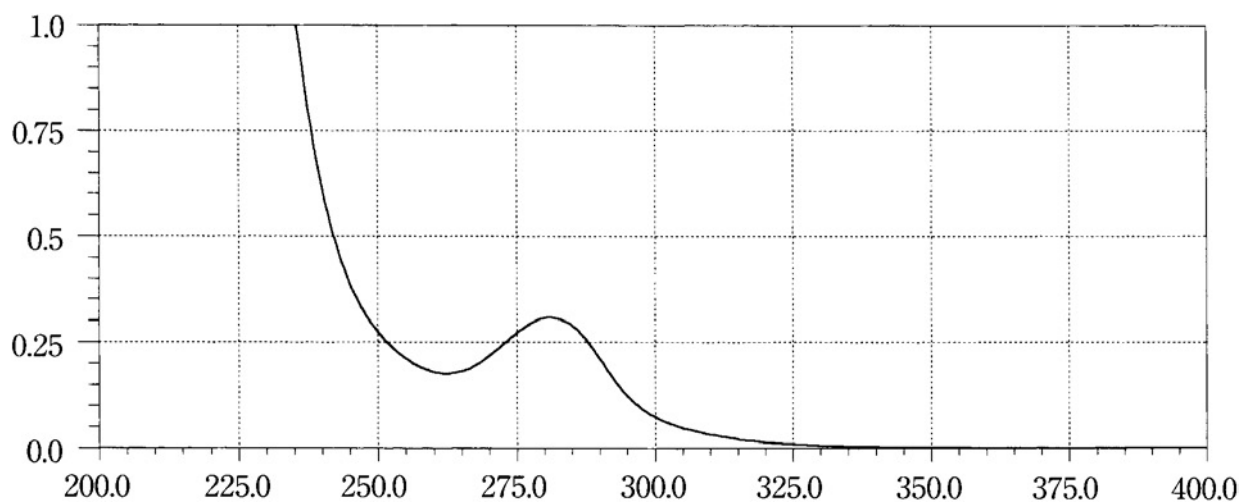
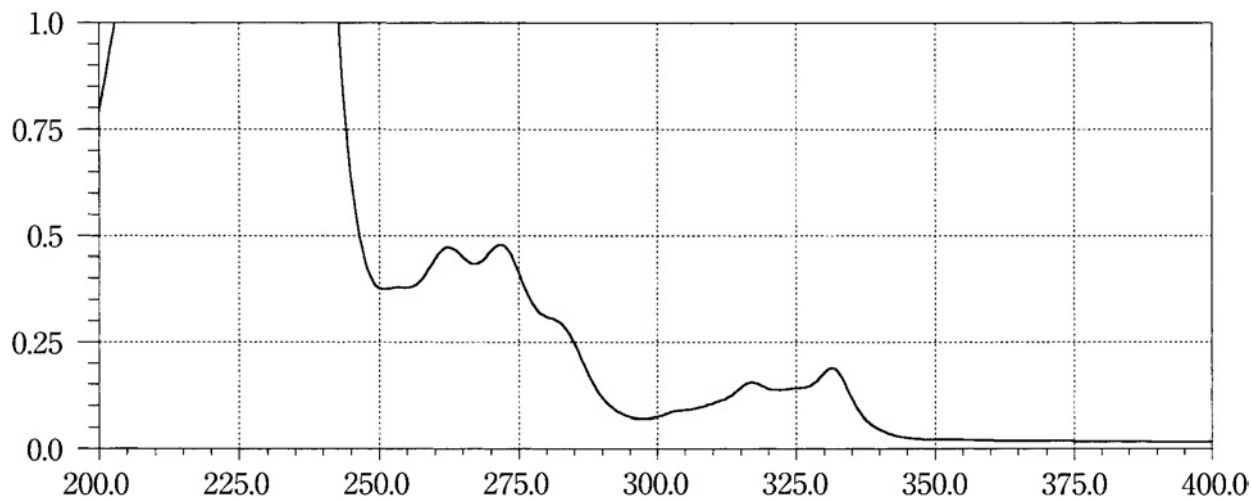
Minocycline Hydrochloride

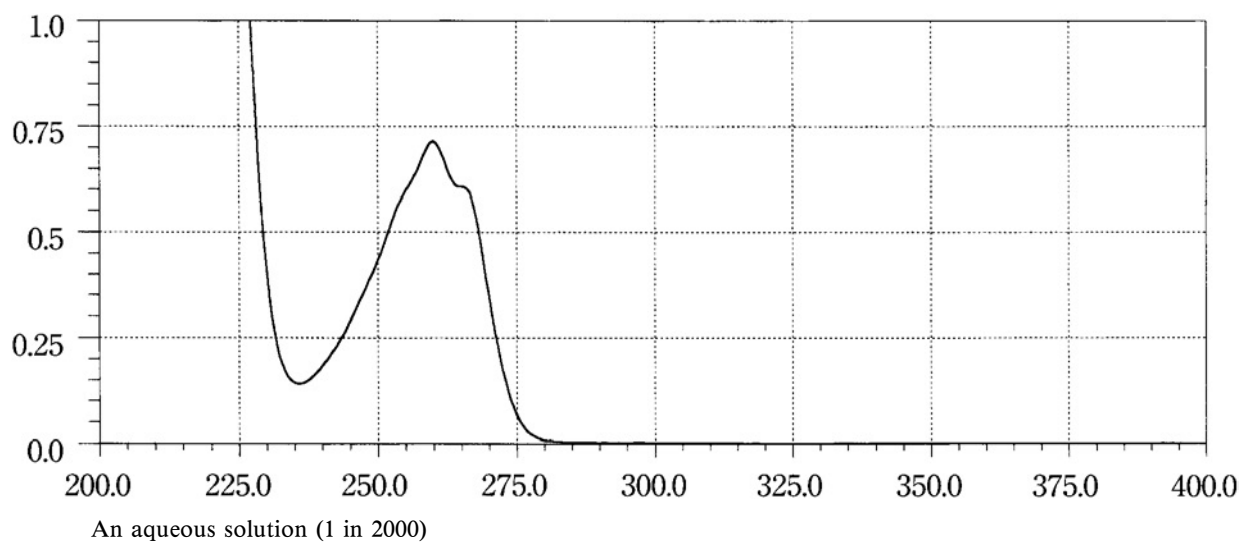
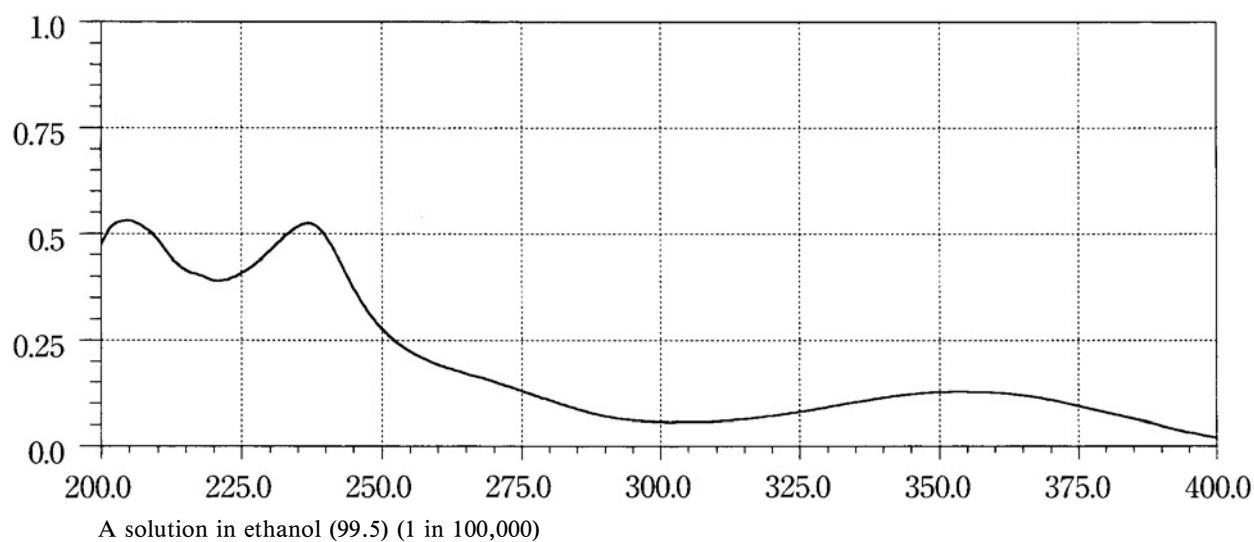
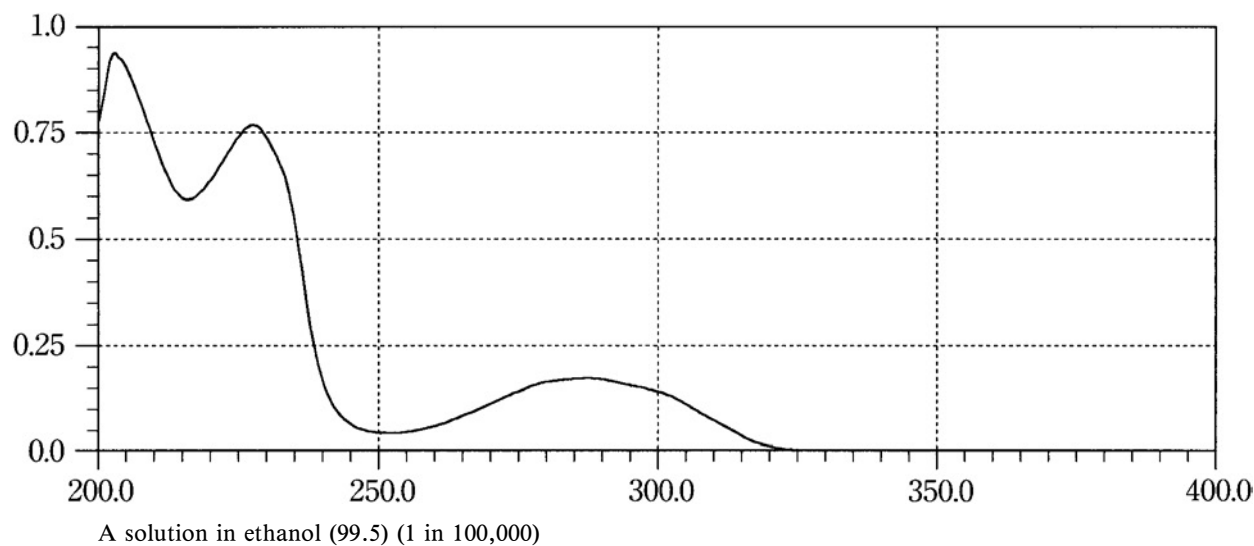


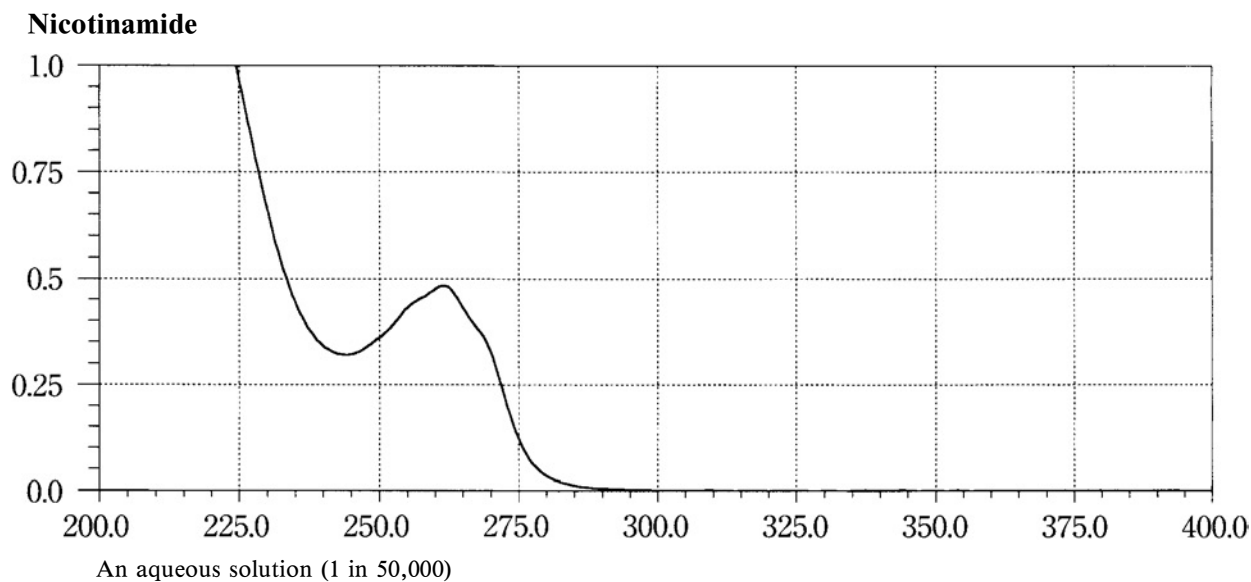
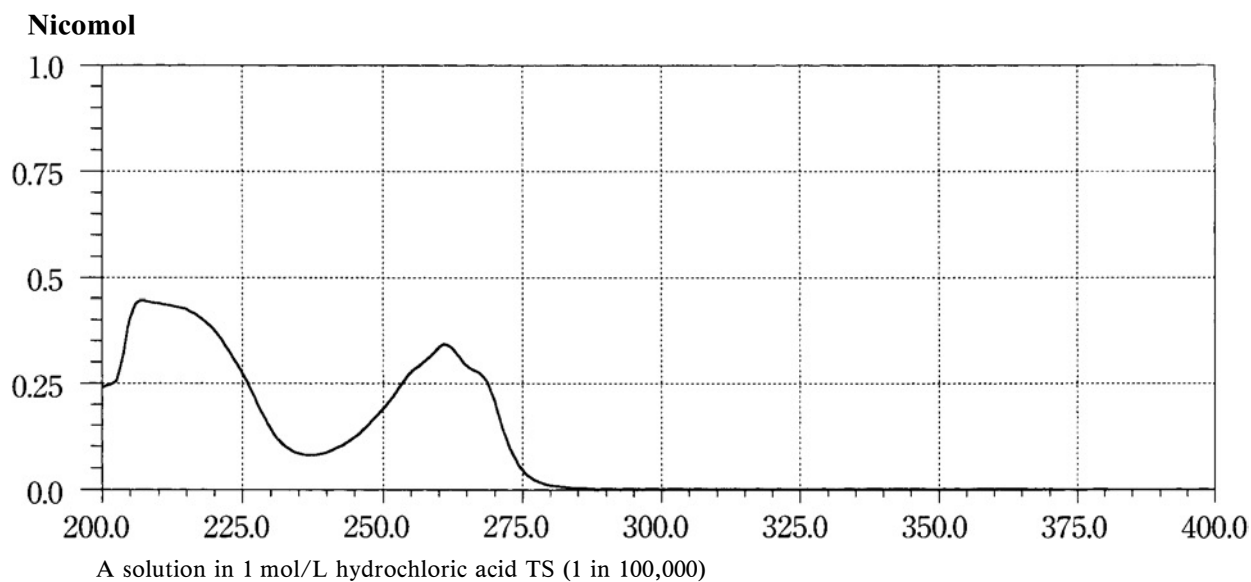
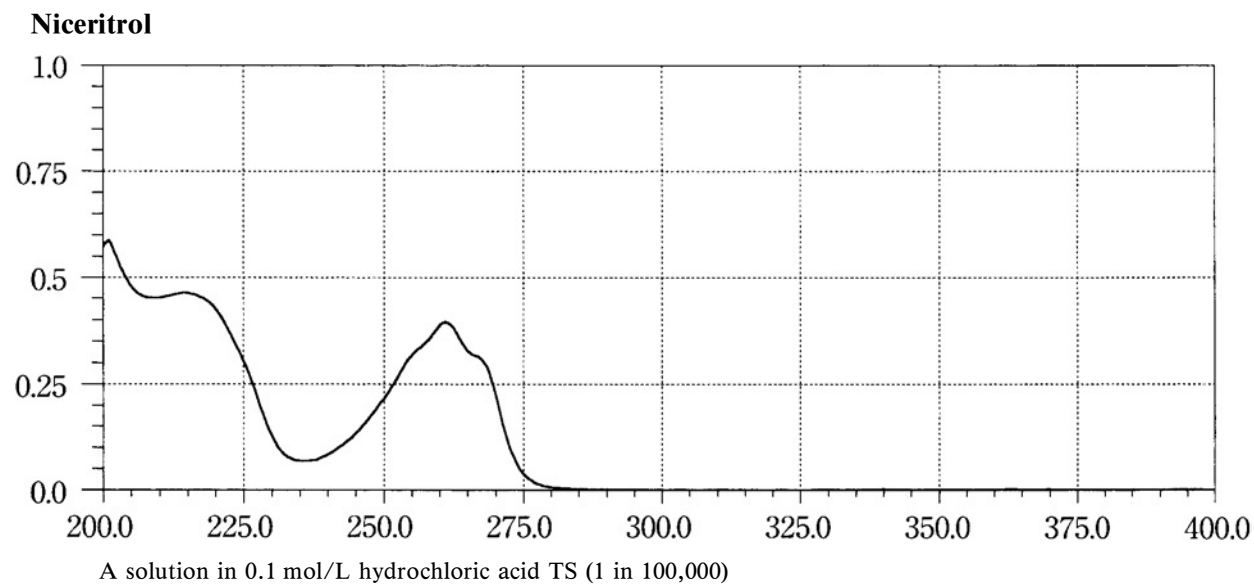
Mitomycin C

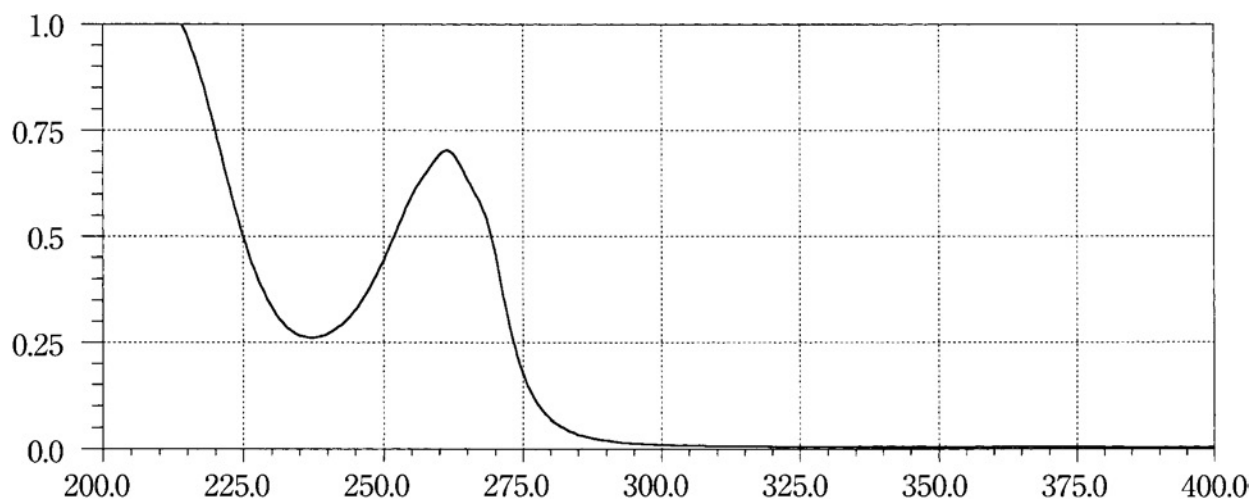
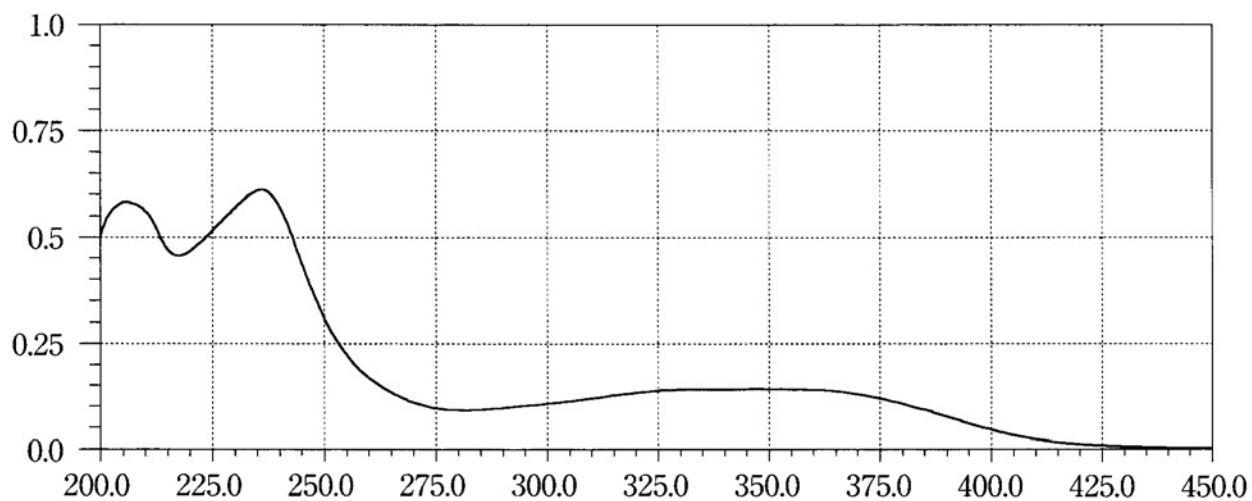
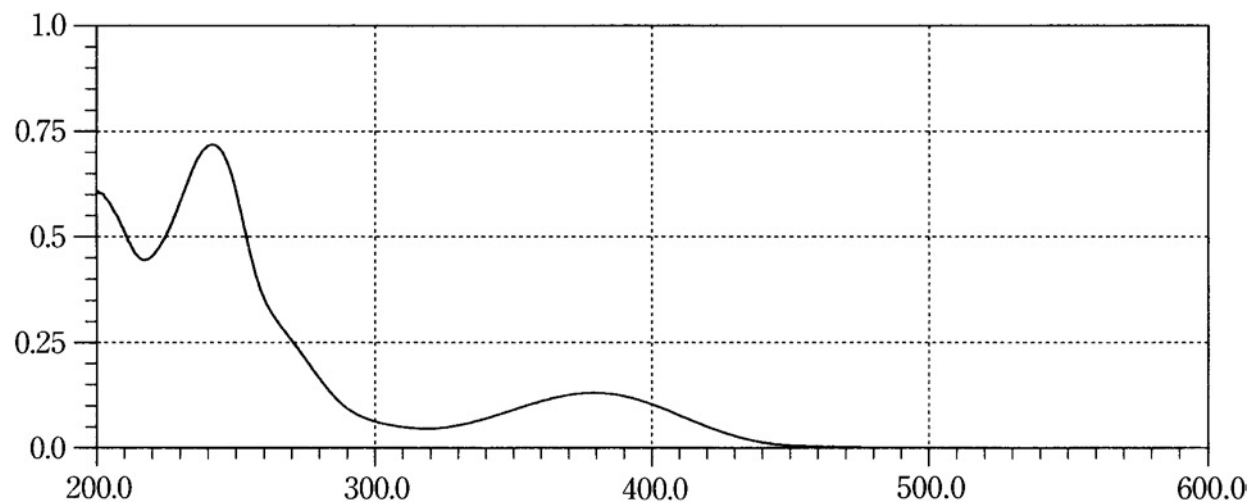


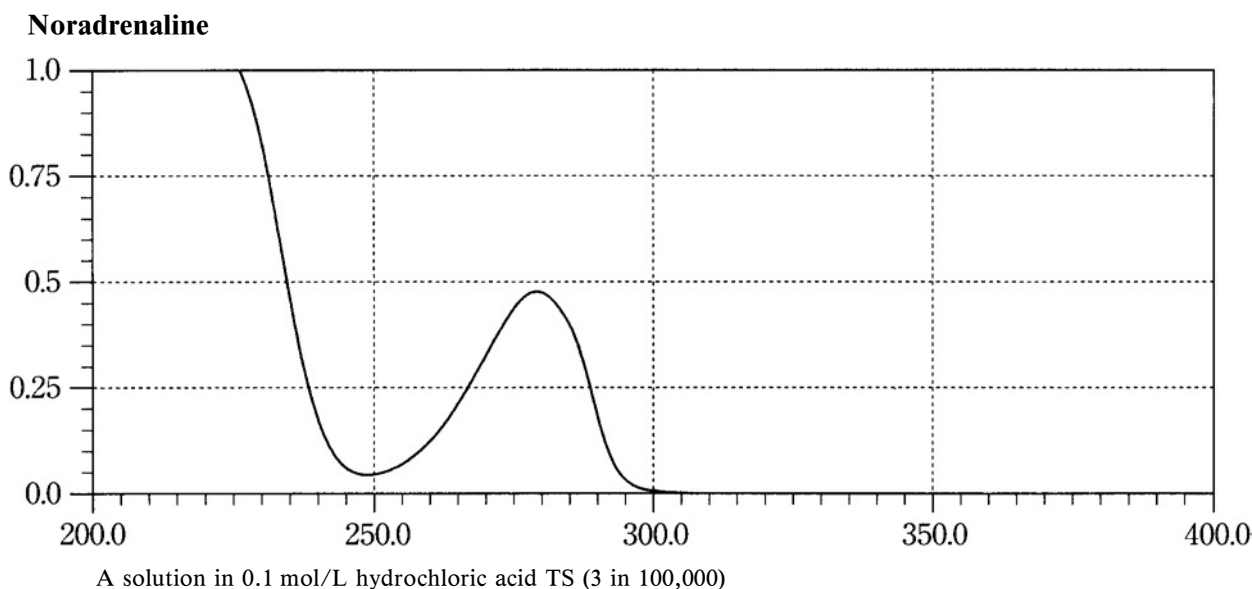
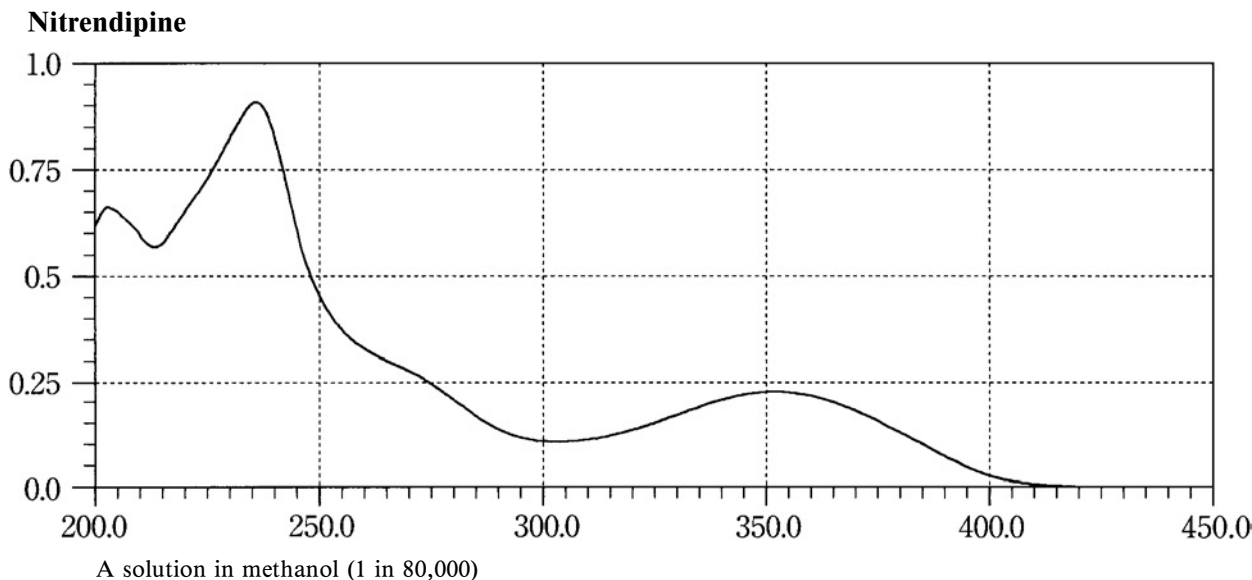
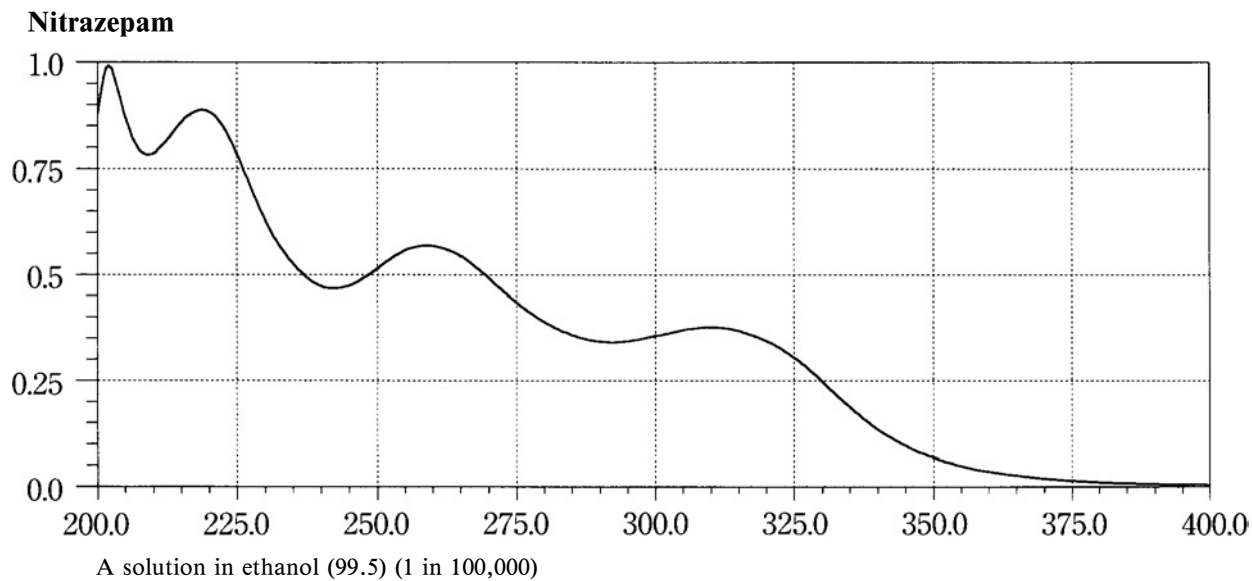
Morphine Hydrochloride Hydrate 1**Morphine Hydrochloride Hydrate 2****Nadolol**

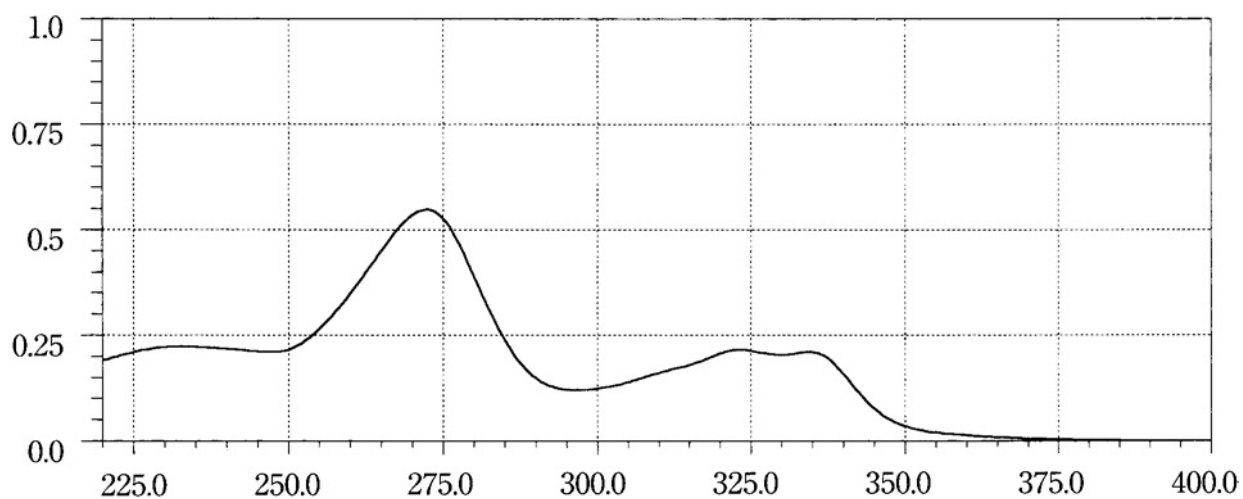
Nalidixic Acid**Naloxone Hydrochloride****Naproxen**

Neostigmine Methylsulfate**Nicardipine Hydrochloride****Nicergoline**

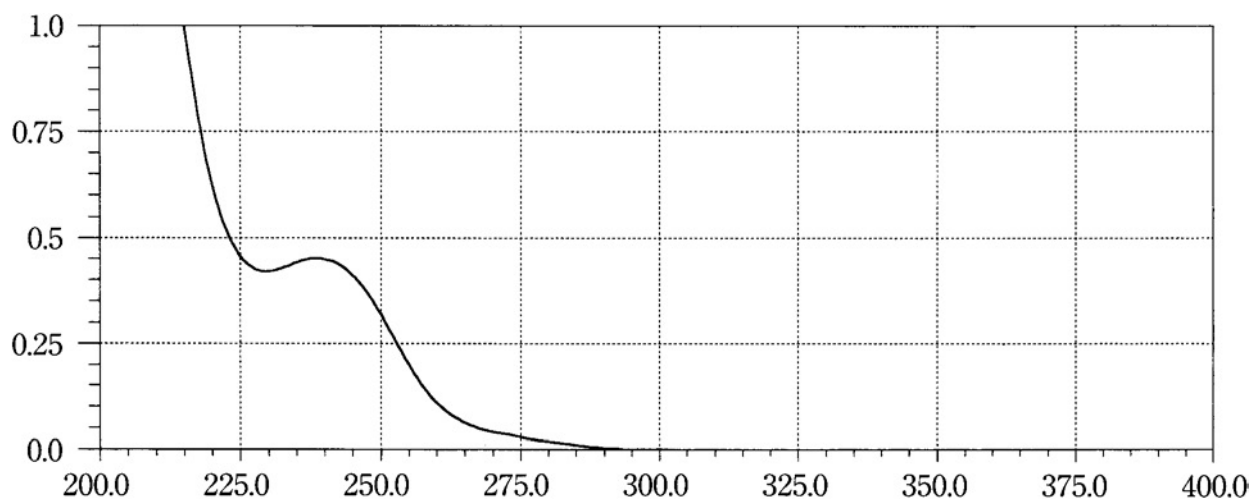


Nicotinic Acid**Nifedipine****Nilvadipine**

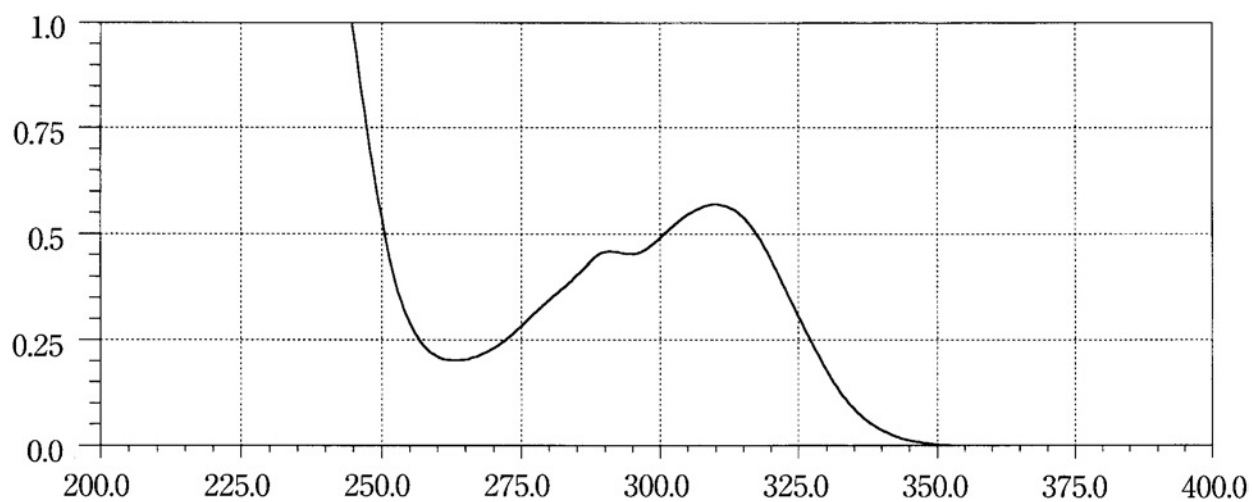


Norfloxacin

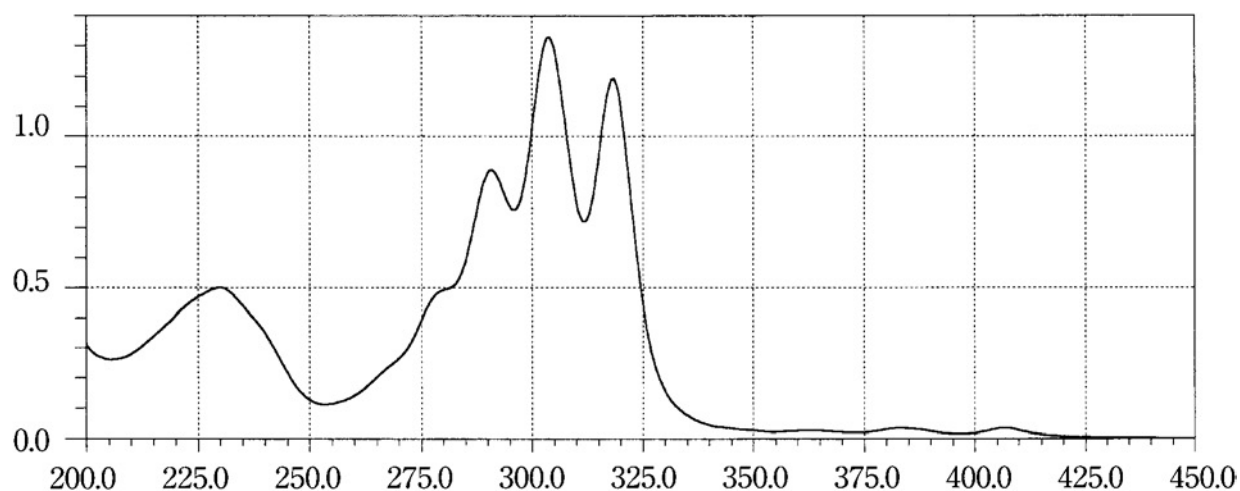
A solution in an aqueous solution of sodium hydroxide (1 in 250) (1 in 200,000)

Nortriptyline Hydrochloride

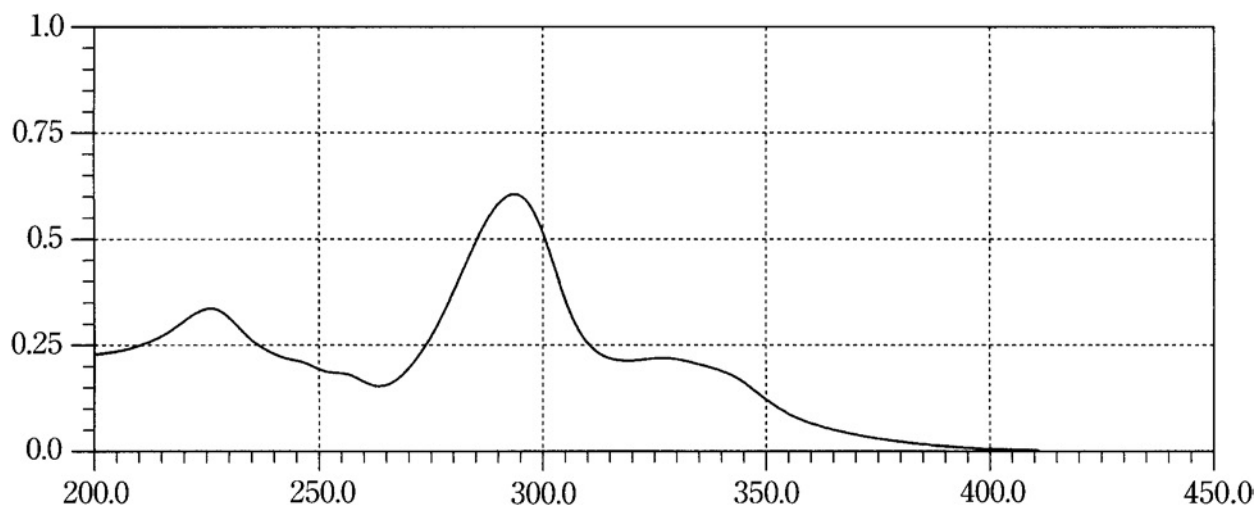
An aqueous solution (1 in 100,000)

Noscapine

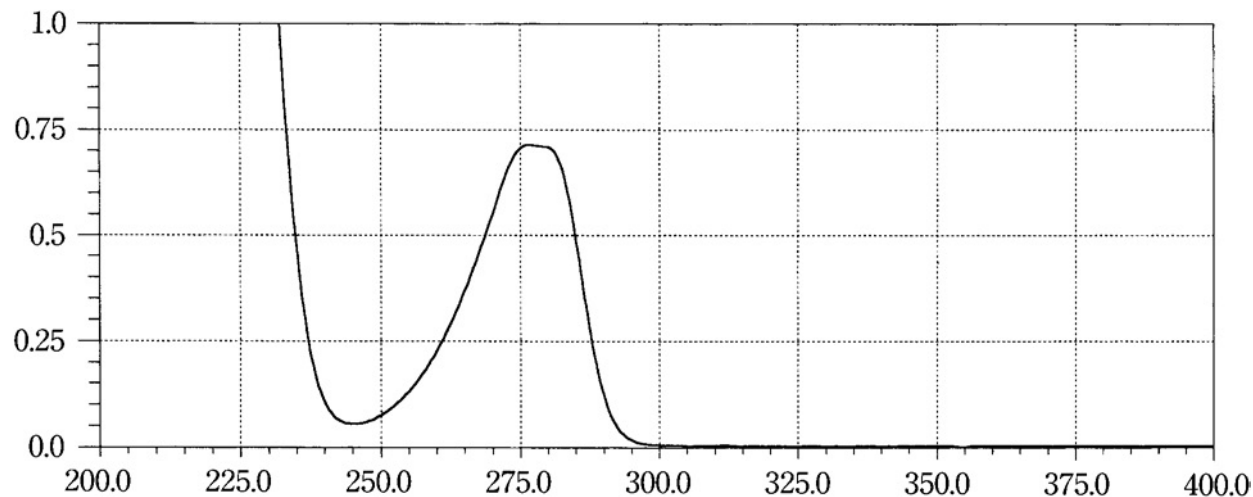
A solution in methanol (1 in 20,000)

Nystatin

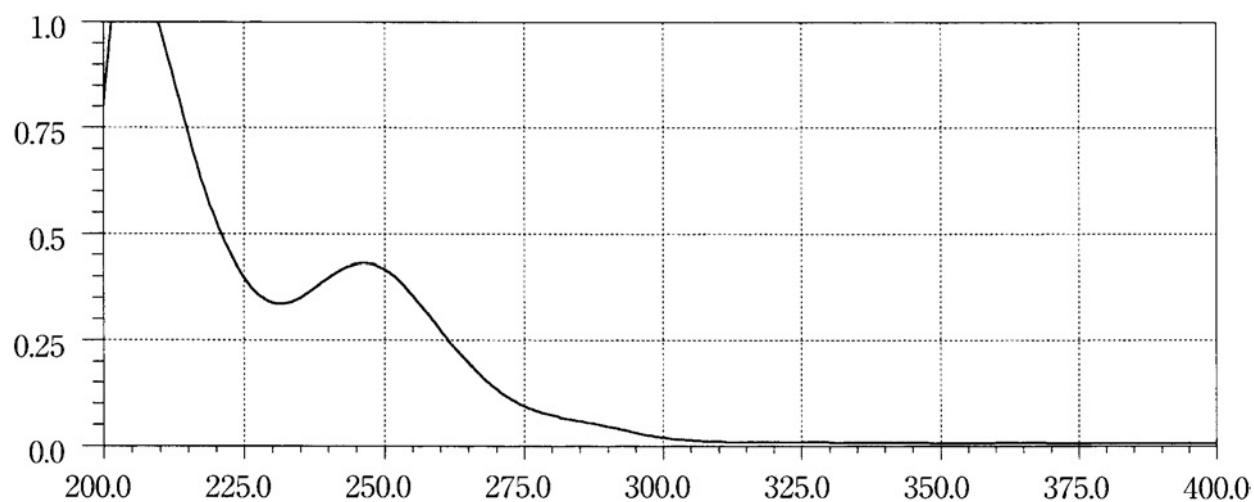
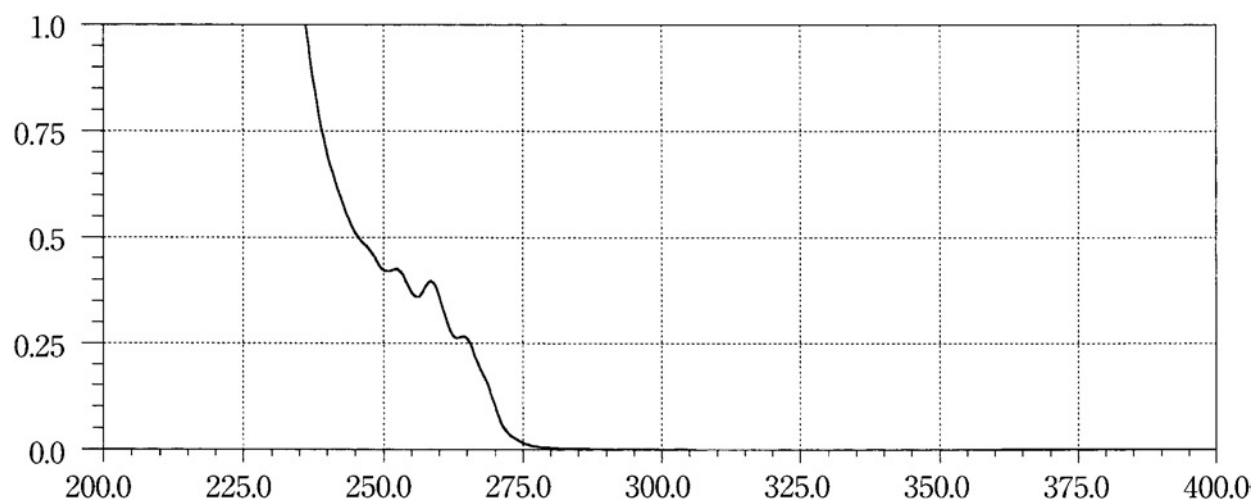
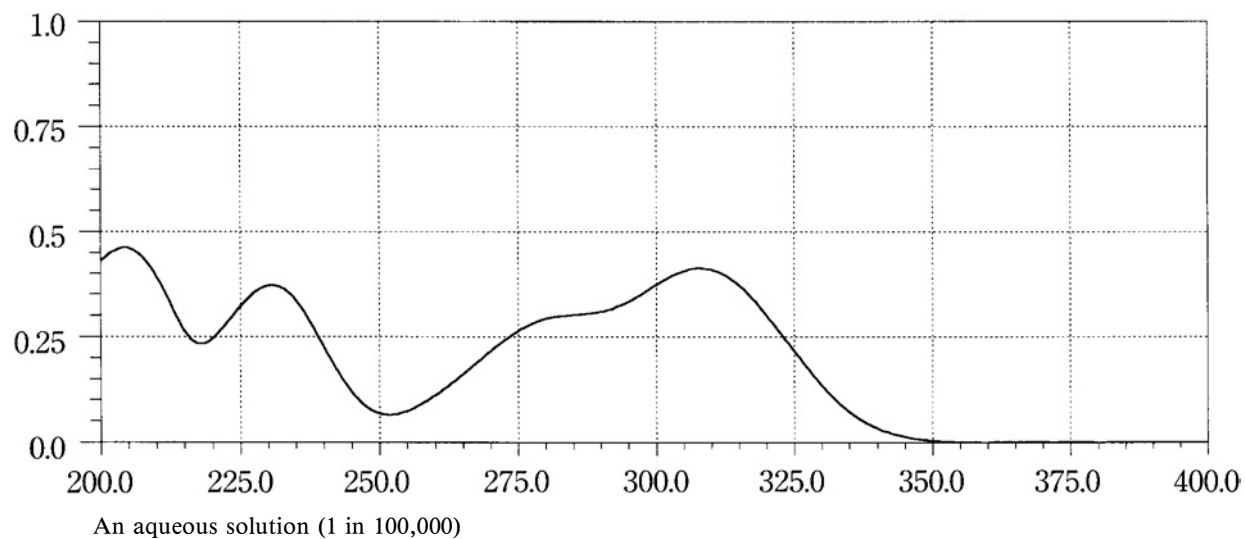
A solution prepared as follows: To 10 mg add 50.25 mL of a mixture of diluted methanol (4 in 5) and sodium hydroxide TS (200:1), dissolve by warming at not exceeding 50°C, and add diluted methanol (4 in 5) to make 500 mL.

Ofloxacin

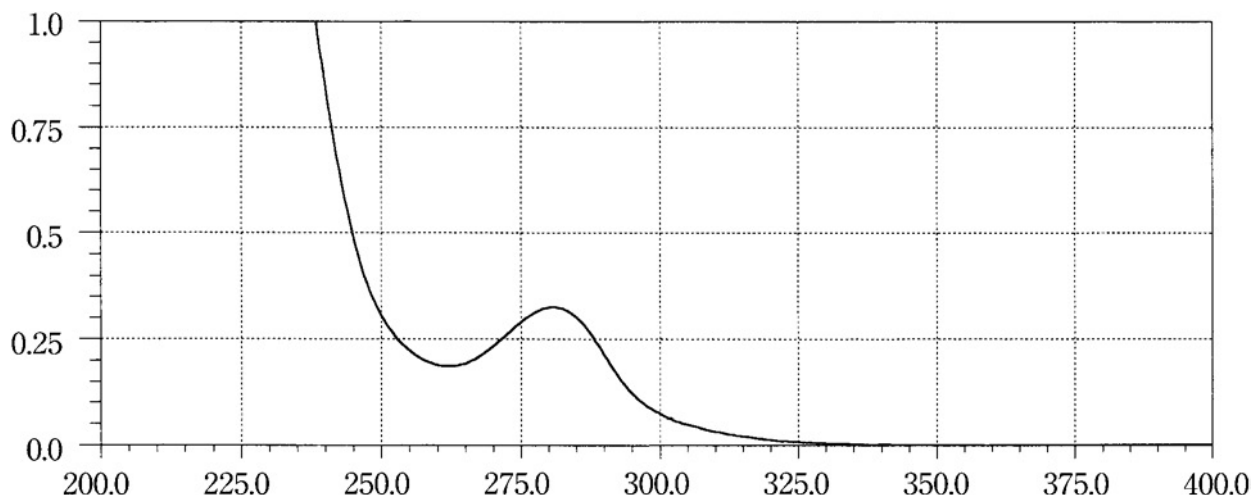
A solution in 0.1 mol/L hydrochloric acid TS (1 in 150,000)

Orciprenaline Sulfate

A solution in 0.01 mol/L hydrochloric acid TS (1 in 10,000)

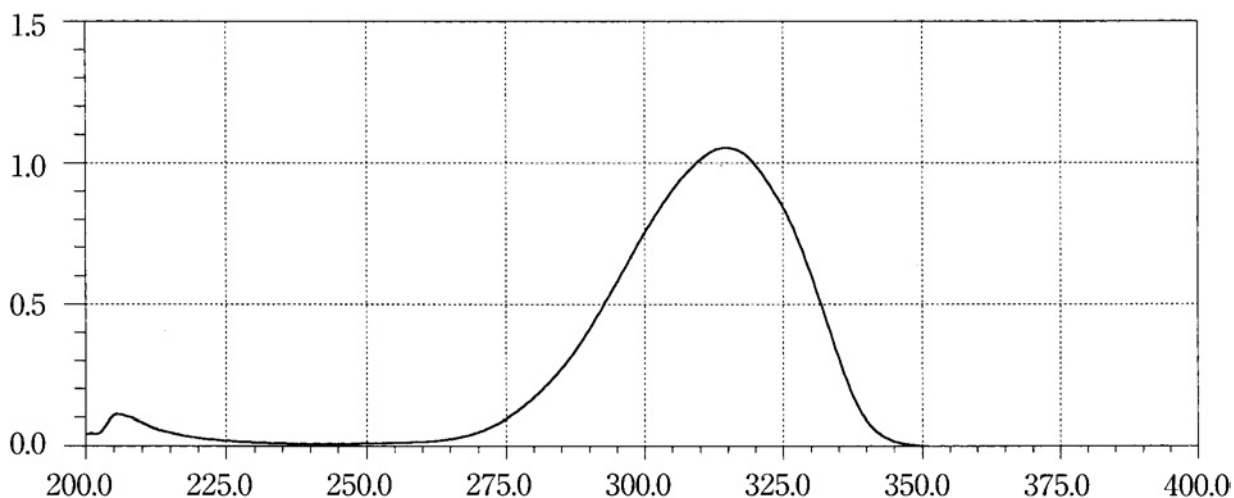
Oxazolam**Oxethazaine****Oxybuprocaine Hydrochloride**

Oxycodone Hydrochloride Hydrate



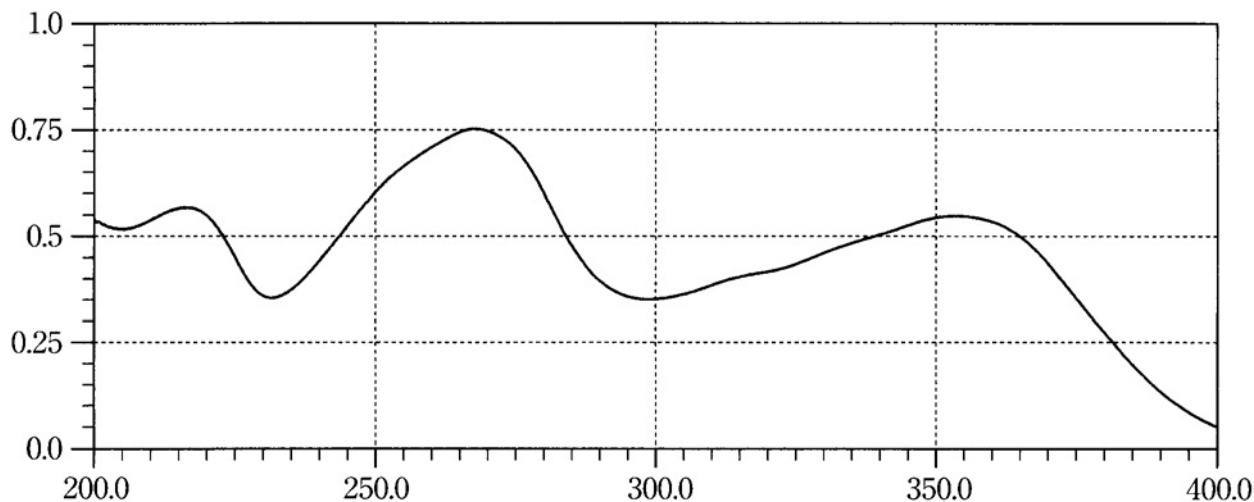
An aqueous solution (1 in 10,000)

Oxymetholone

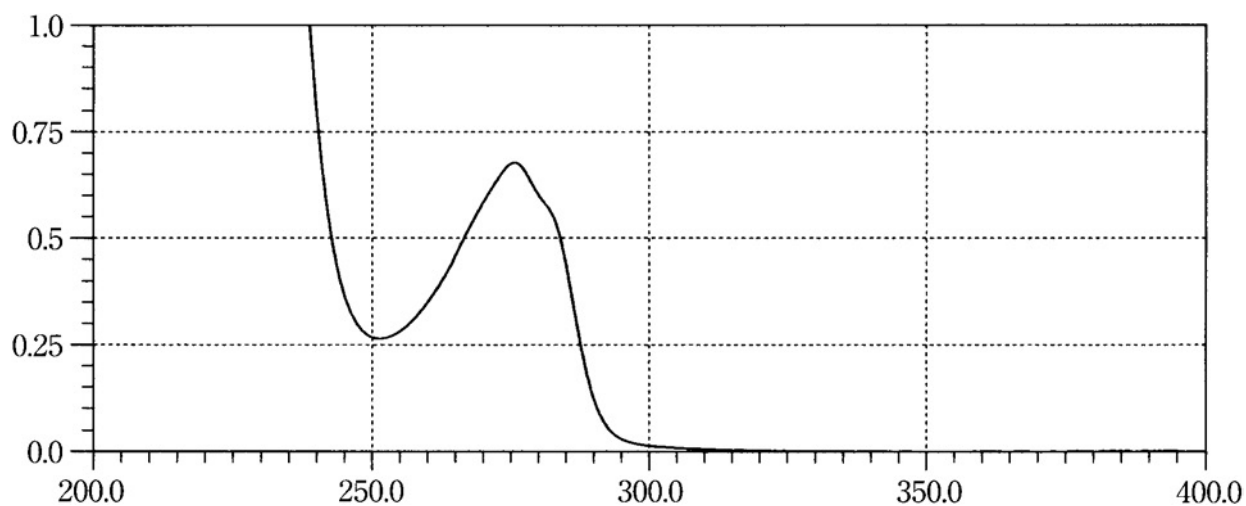
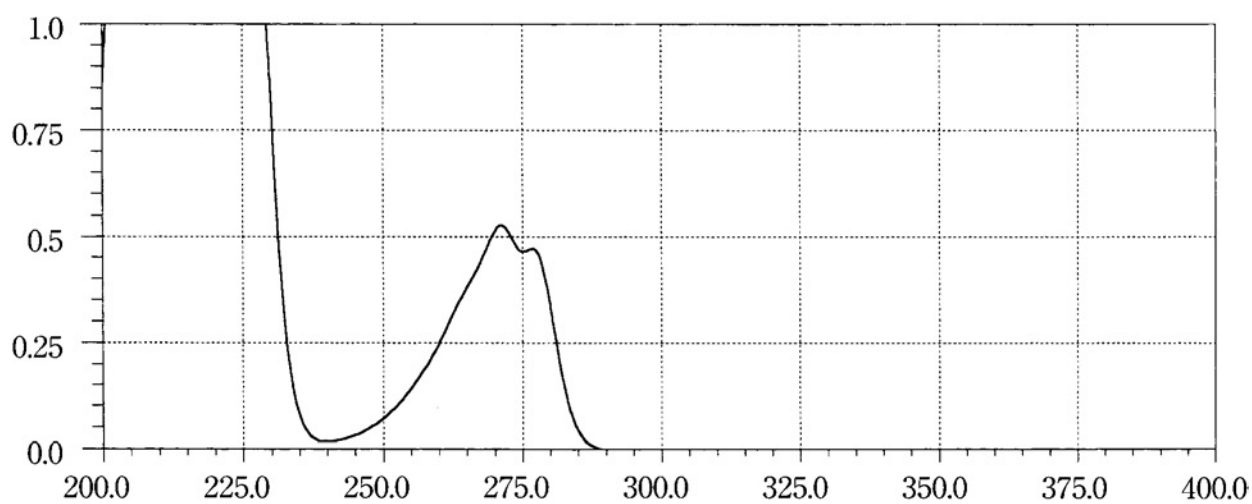
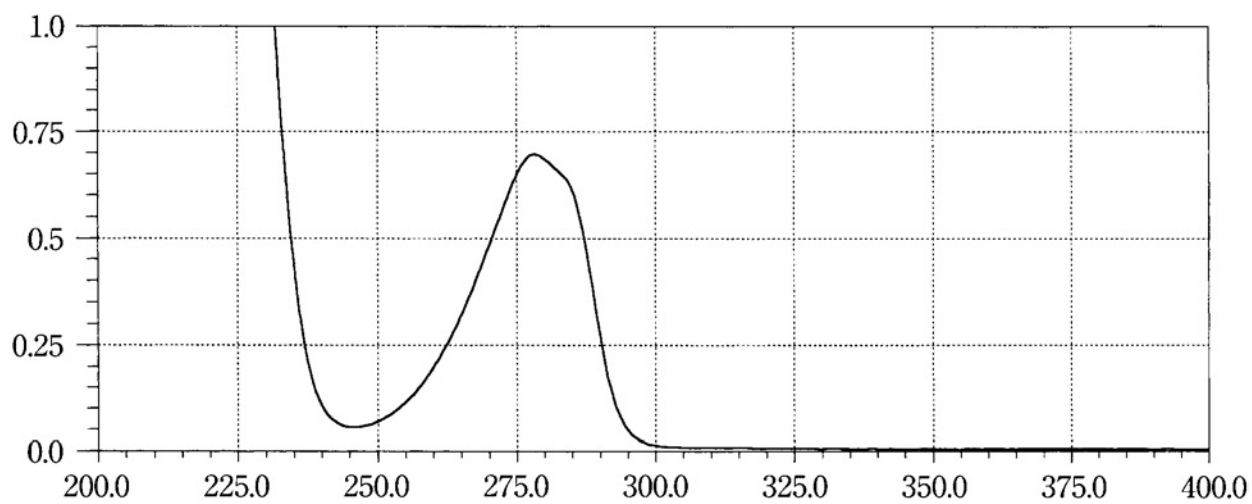


A solution prepared as follows: To 5 mL of a solution in methanol (1 in 5000) add 5 mL of sodium hydroxide-methanol TS and methanol to make 50 mL.

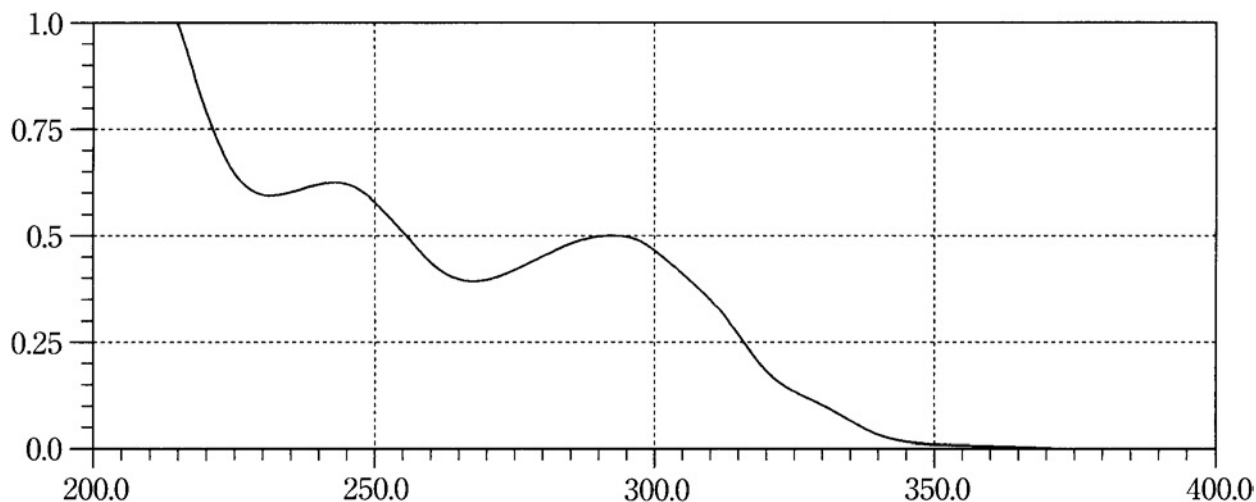
Oxytetracycline Hydrochloride



A solution in 0.1 mol/L hydrochloric acid TS (1 in 50,000)

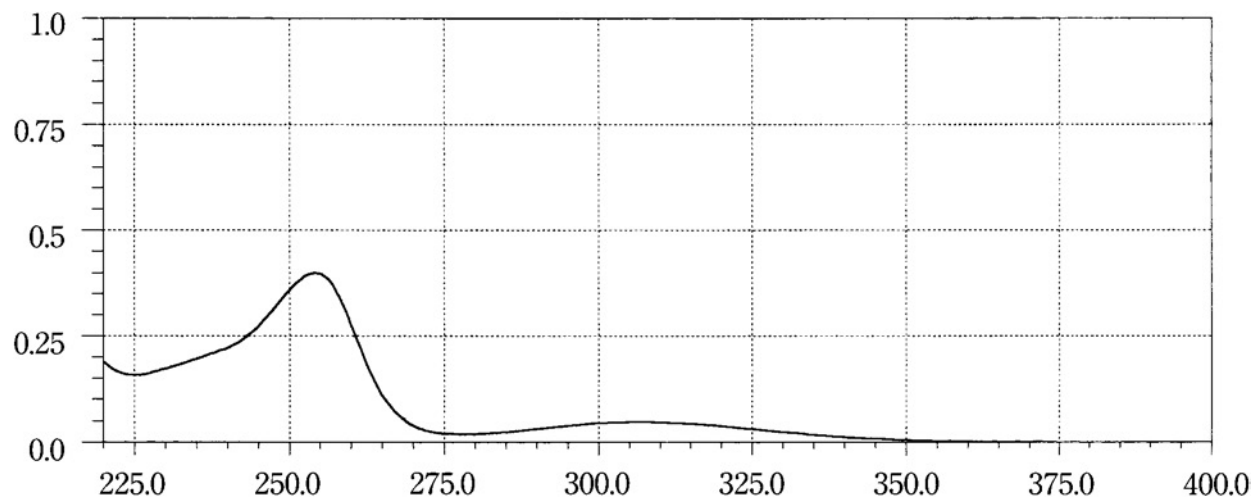
Oxytocin**Penbutolol Sulfate****Pentazocine**

Peplomycin Sulfate



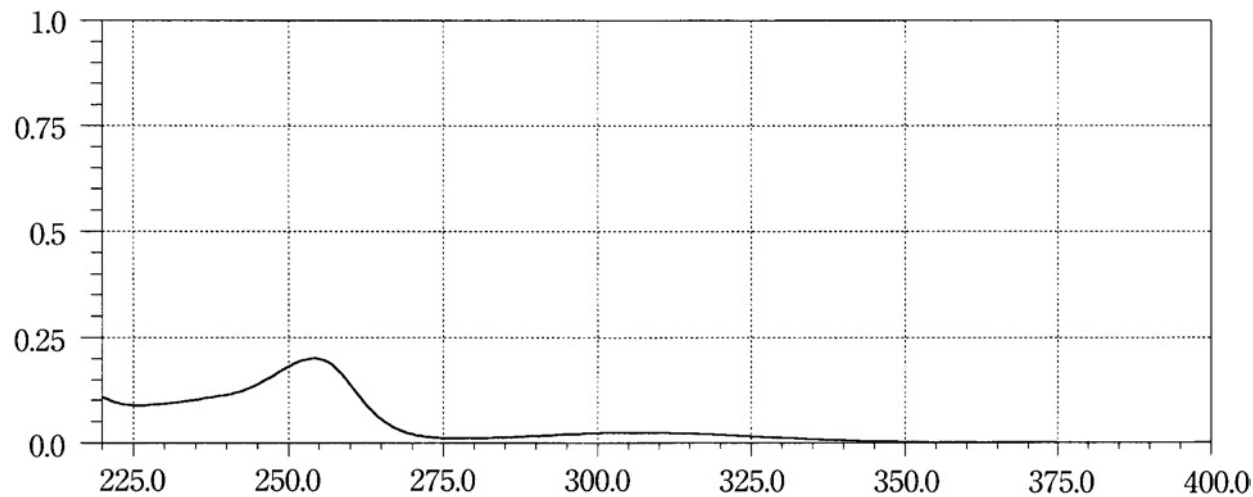
A solution prepared as follows: To 4 mg add 5 μ L of copper (II) sulfate TS, and dissolve in water to make 100 mL.

Perphenazine 1

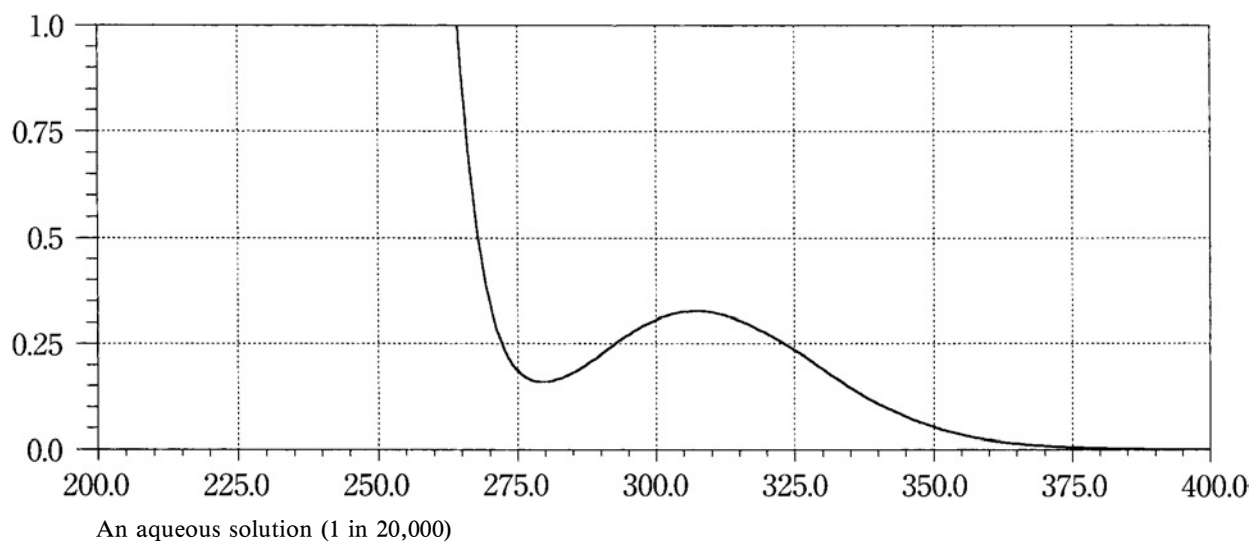
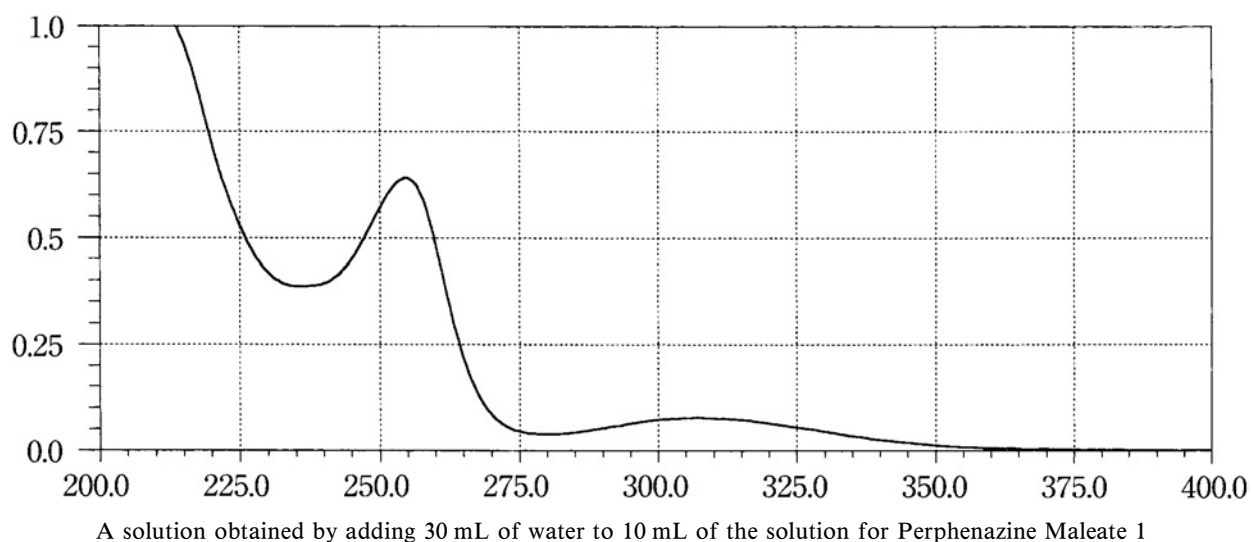
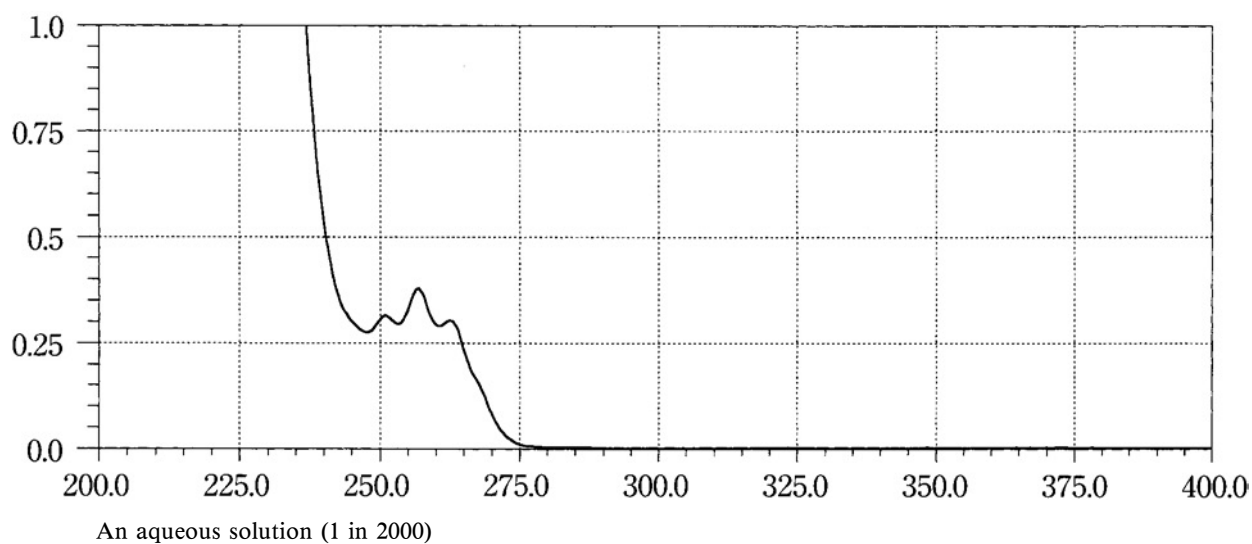


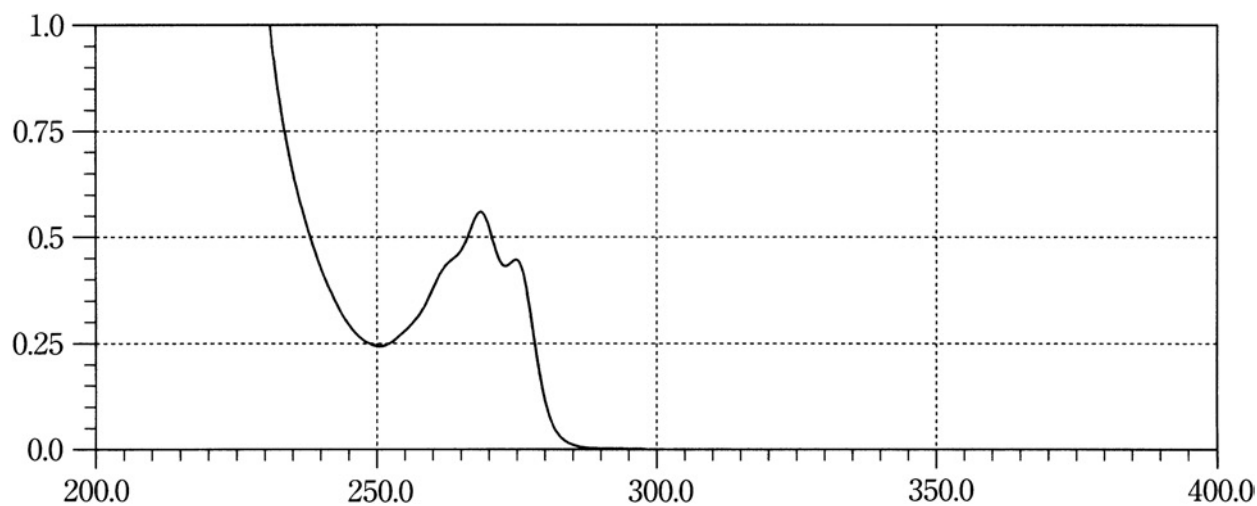
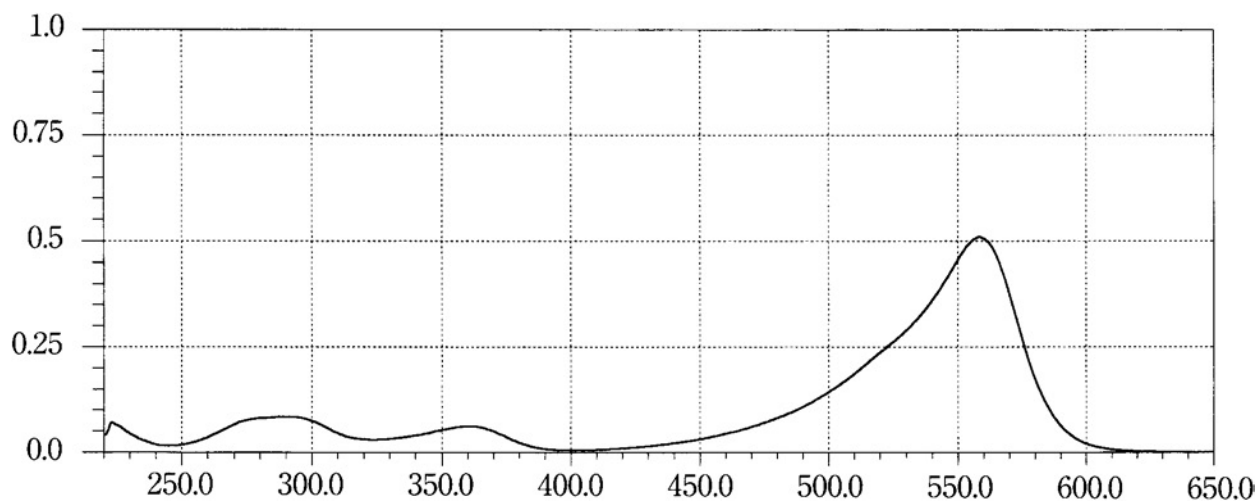
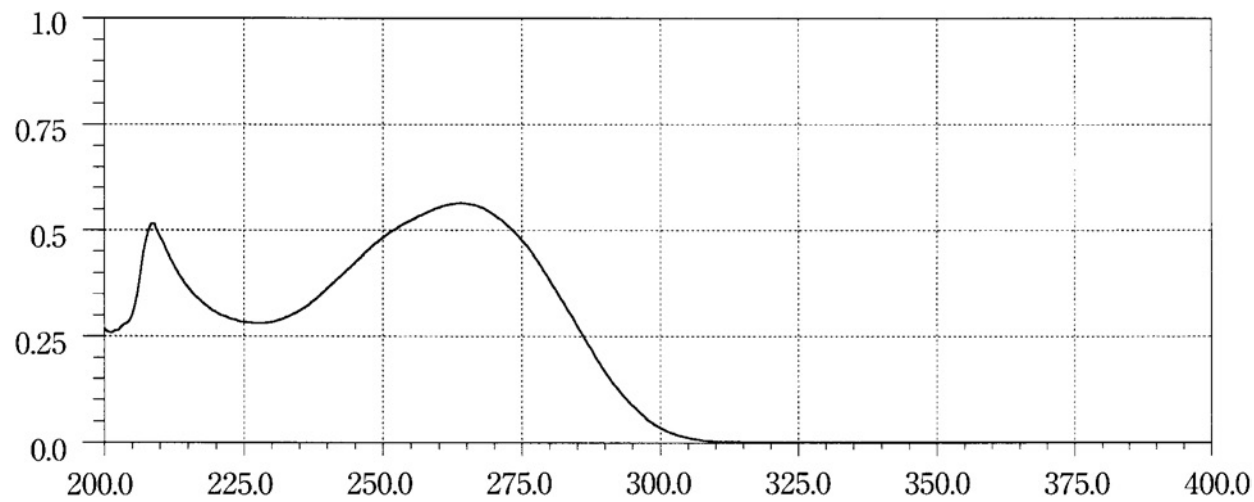
A solution in 0.1 mol/L hydrochloric acid TS (1 in 200,000)

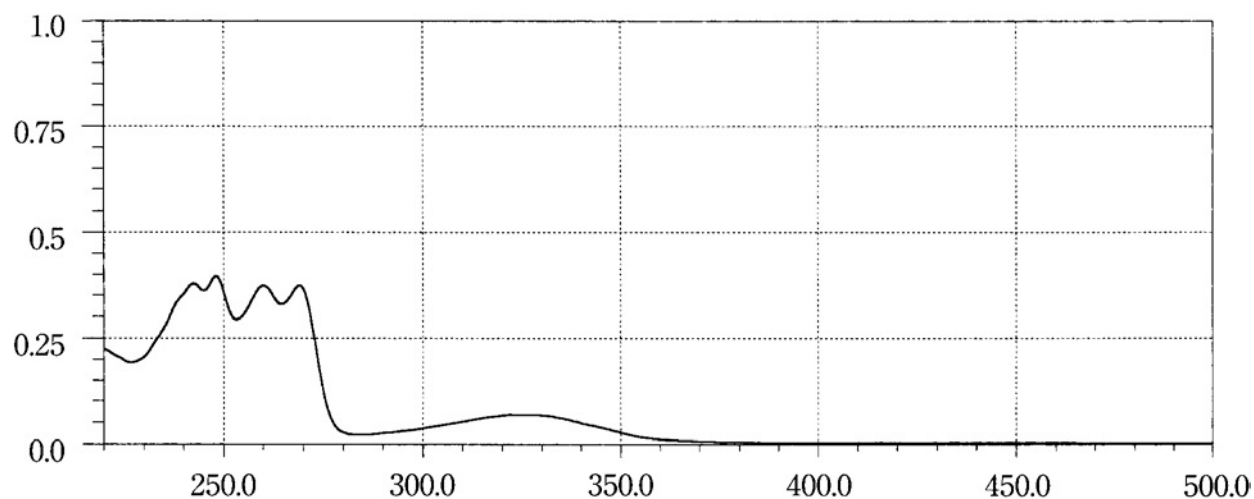
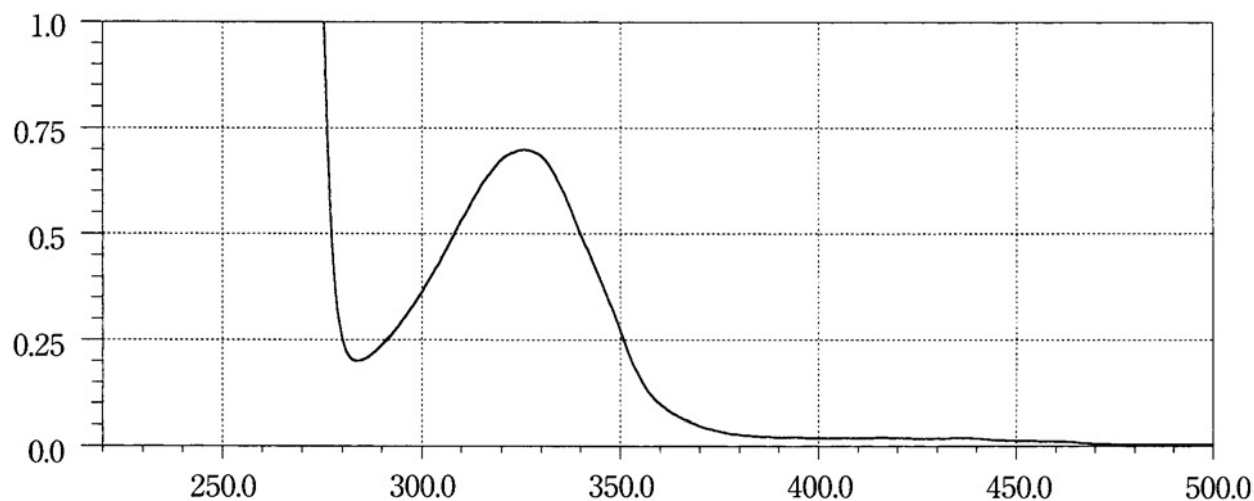
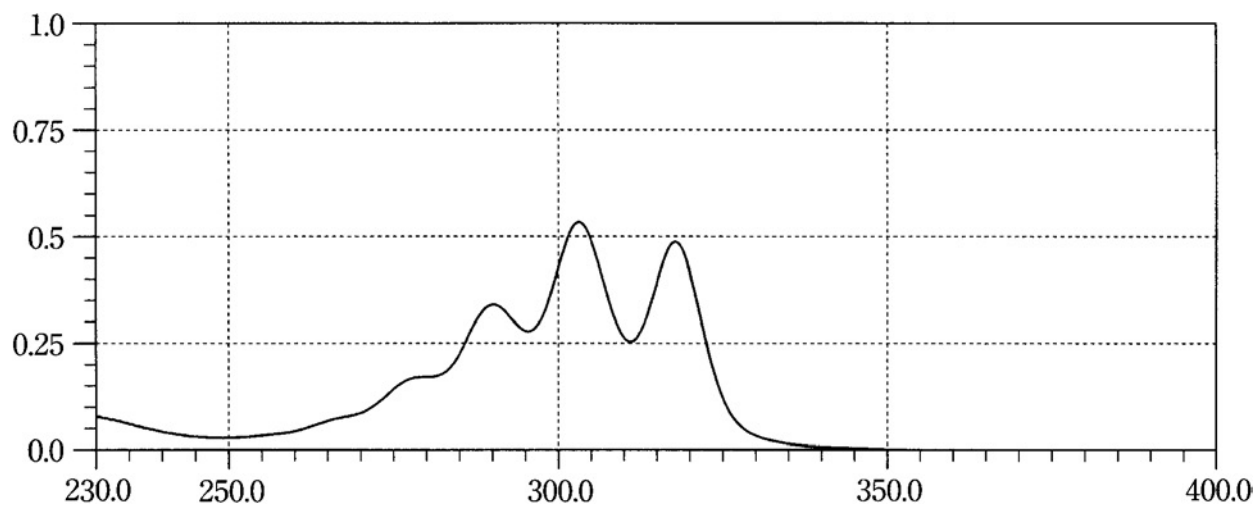
Perphenazine 2

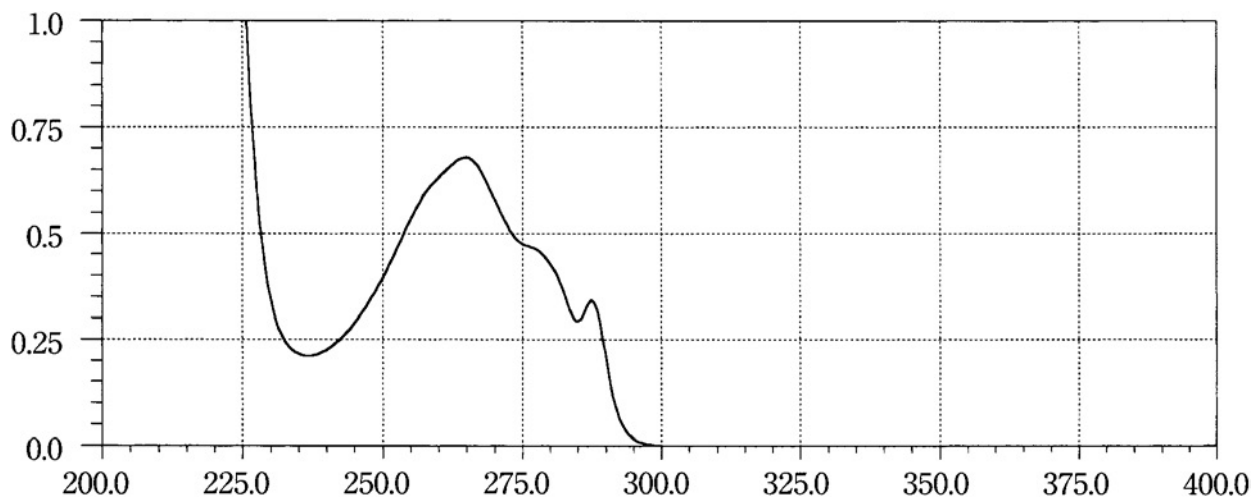
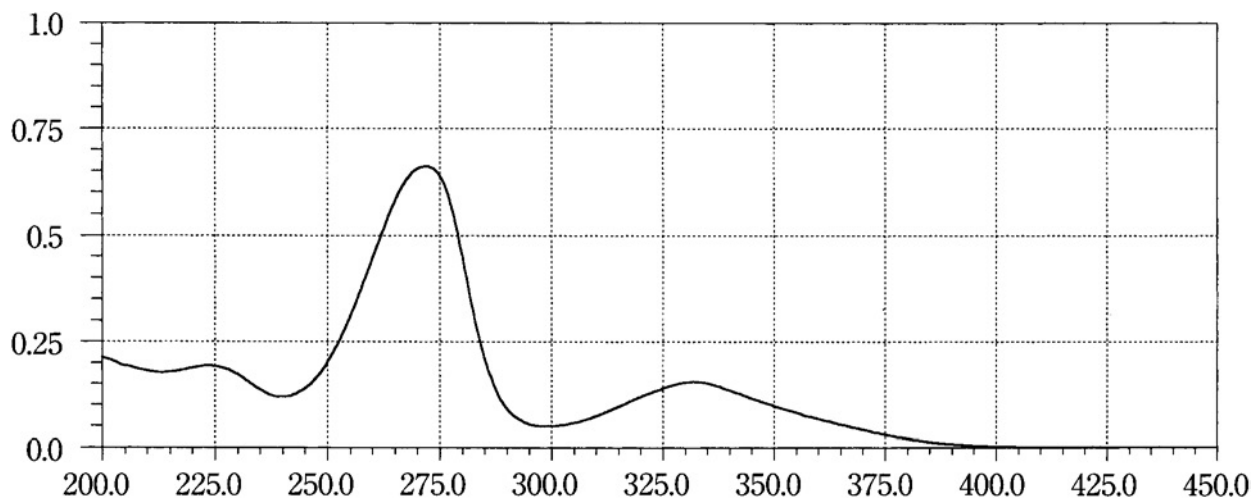
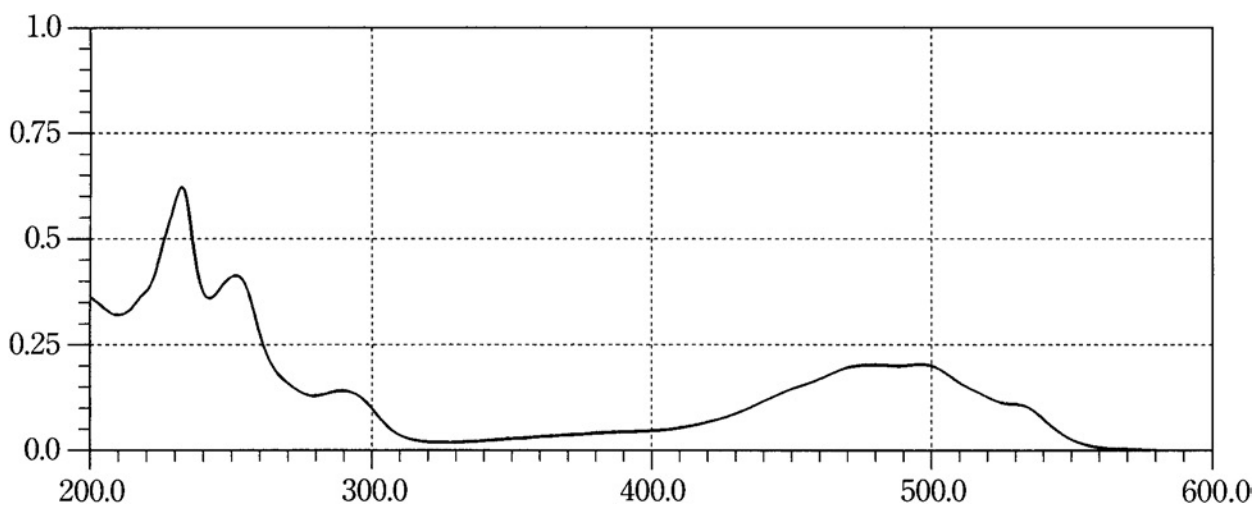


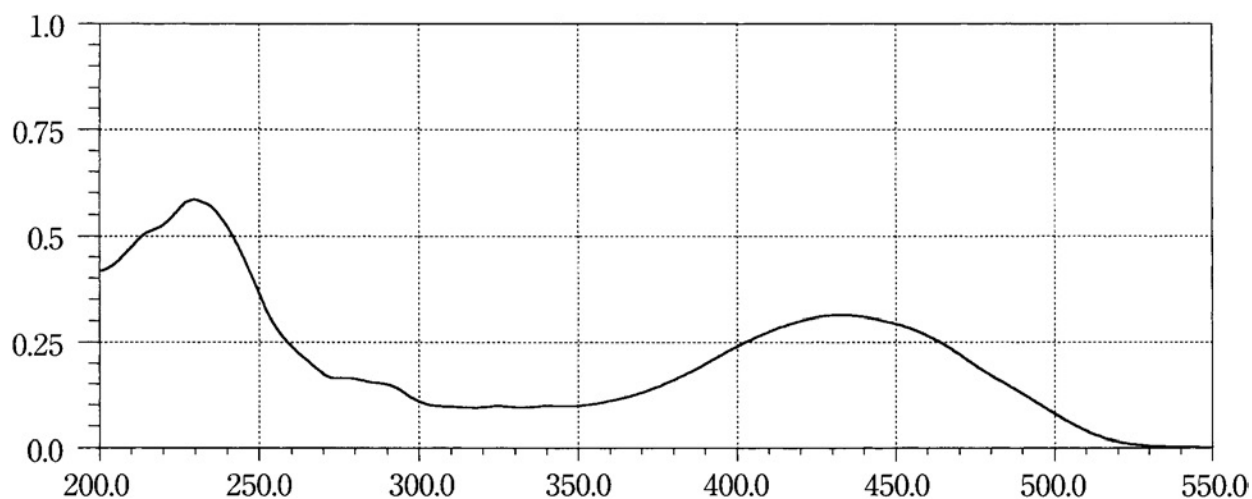
A solution obtained by adding 10 mL of water to 10 mL of the solution for Perphenazine 1

Perphenazine Maleate 1**Perphenazine Maleate 2****Pethidine Hydrochloride**

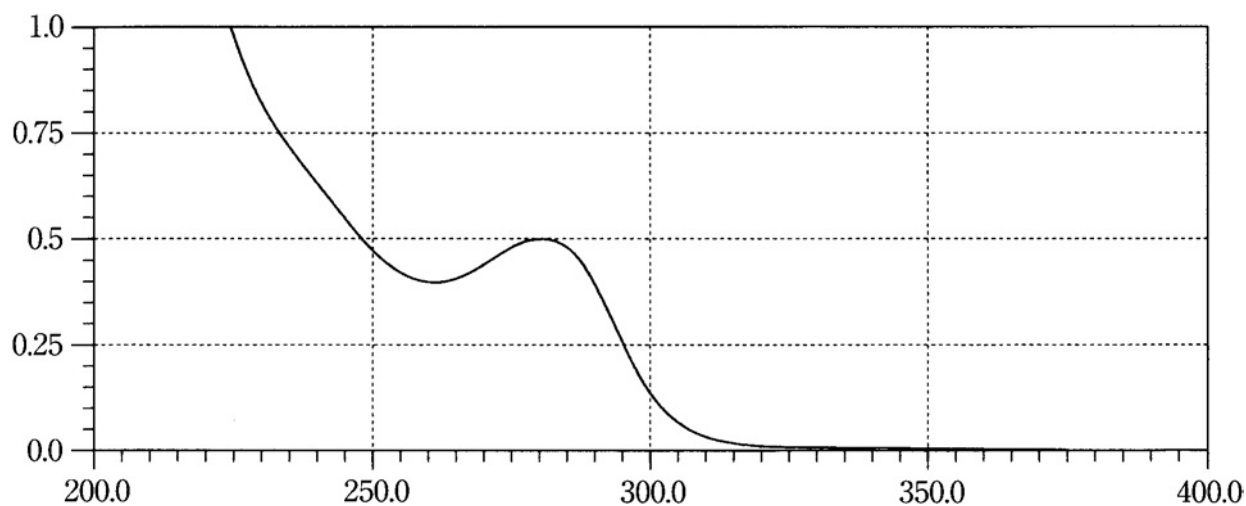
Phenethicillin Potassium**Phenolsulfonphthalein****Phenylbutazone**

Phytonadione 1**Phytonadione 2****Pimaricin**

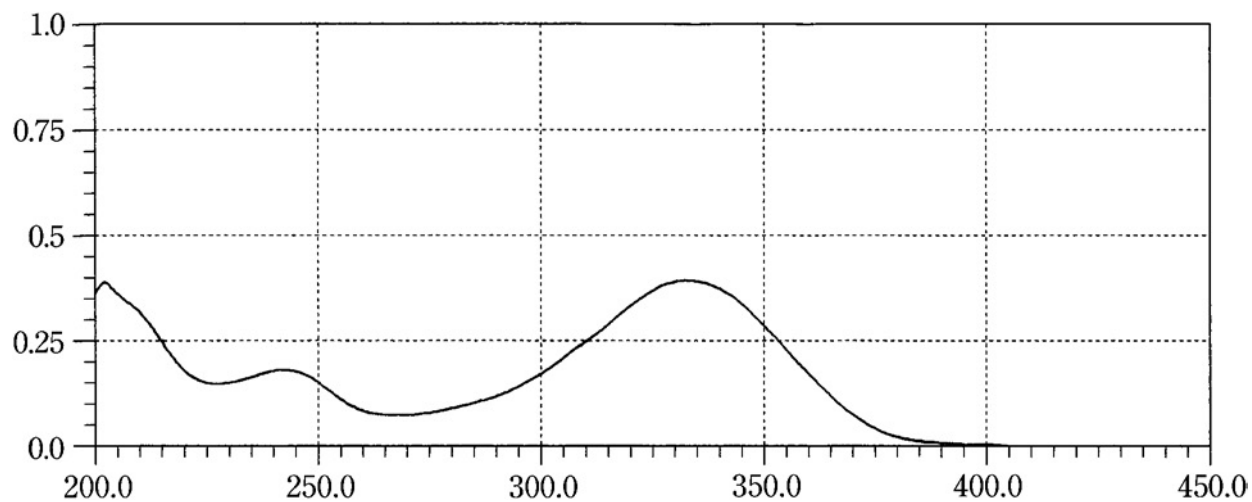
Pindolol**Pipemidic Acid Hydrate****Pirarubicin**

Pirenoxine

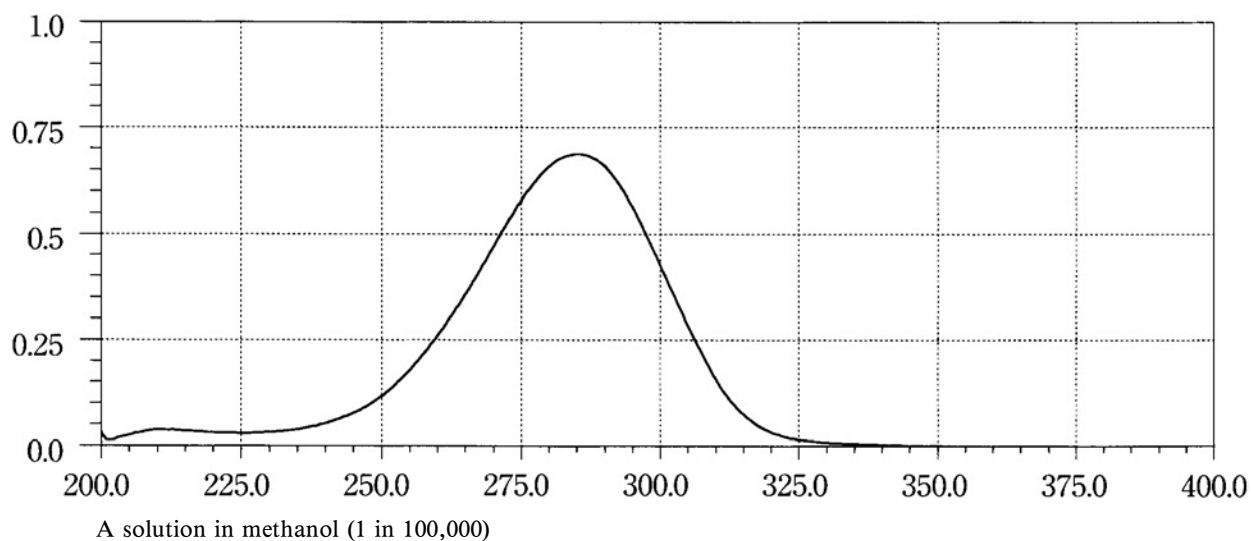
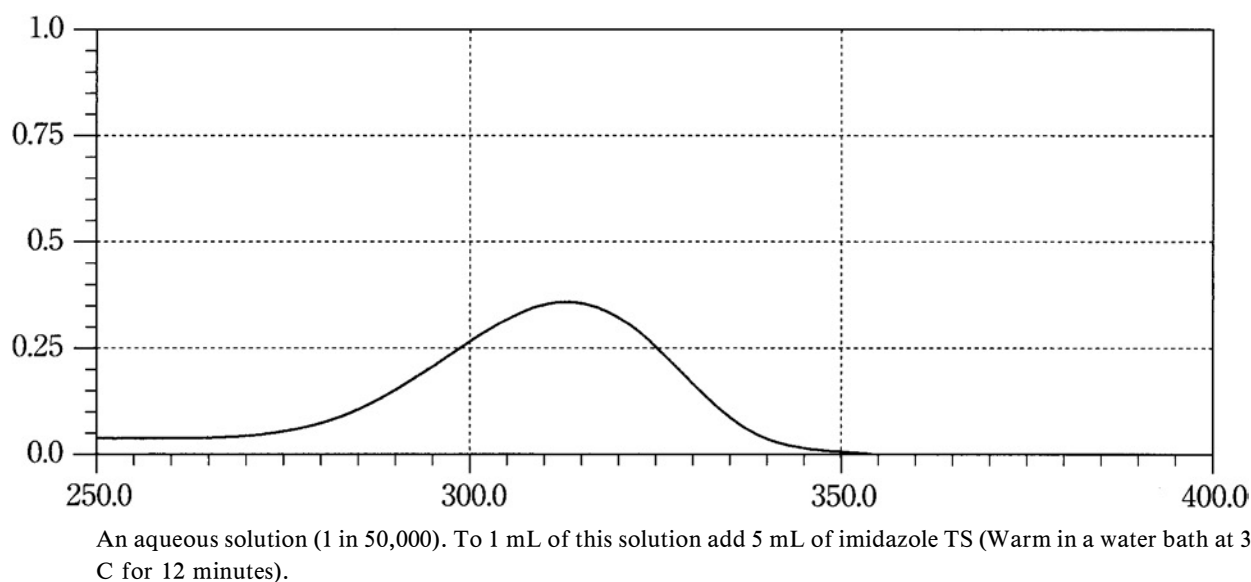
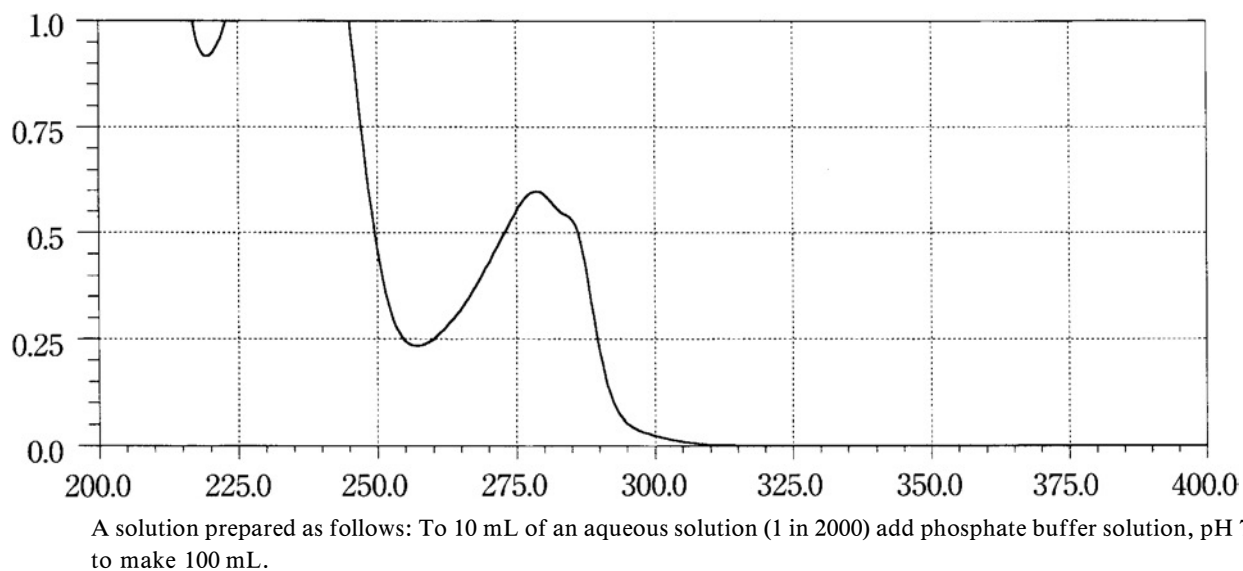
A solution in phosphate buffer solution, pH 6.5 (1 in 200,000)

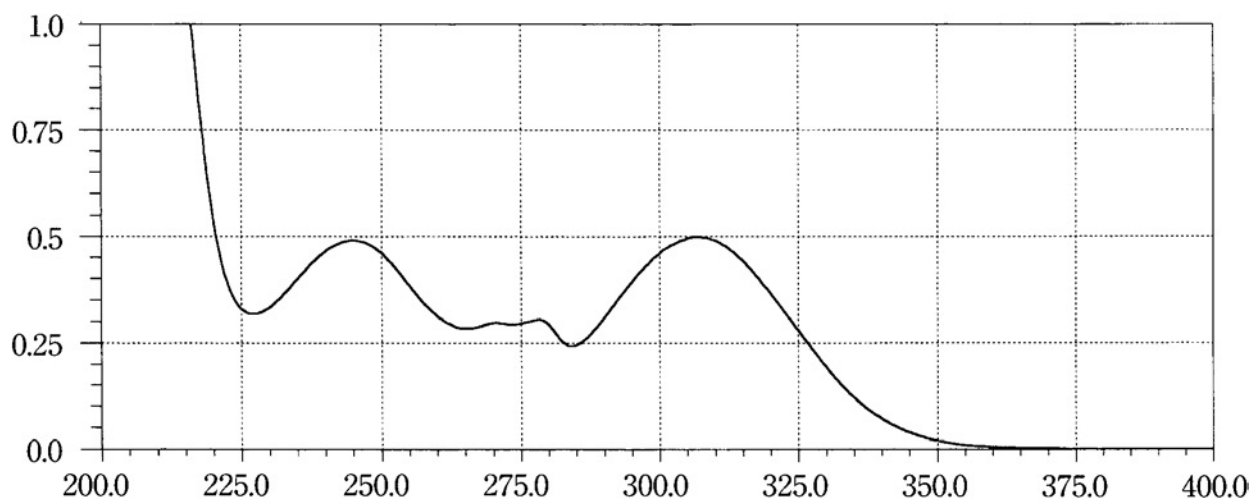
Pirenzepine Hydrochloride Hydrate

An aqueous solution (1 in 40,000)

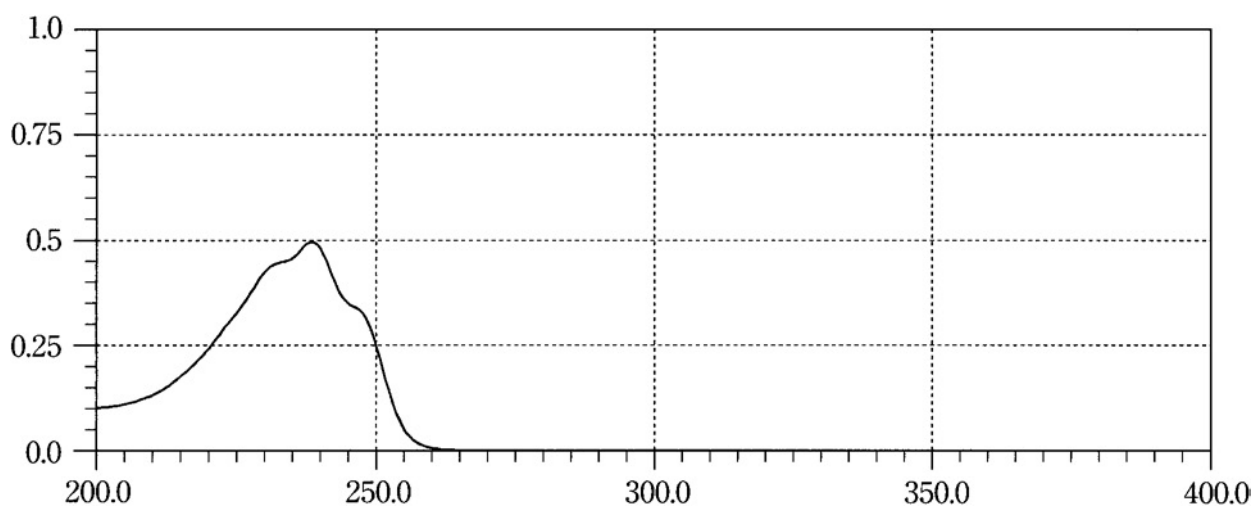
Piroxicam

A solution in a mixture of methanol and 0.5 mol/L hydrochloric acid TS (490:1) (1 in 200,000)

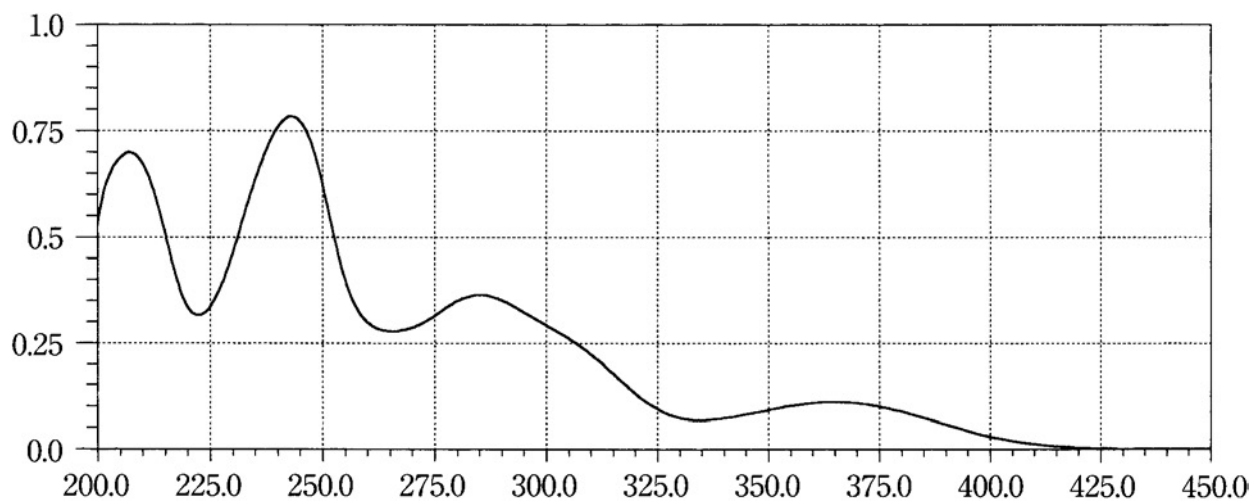
Potassium Canrenoate**Potassium Clavulanate****Potassium Guaiacolsulfonate**

Pranoprofen

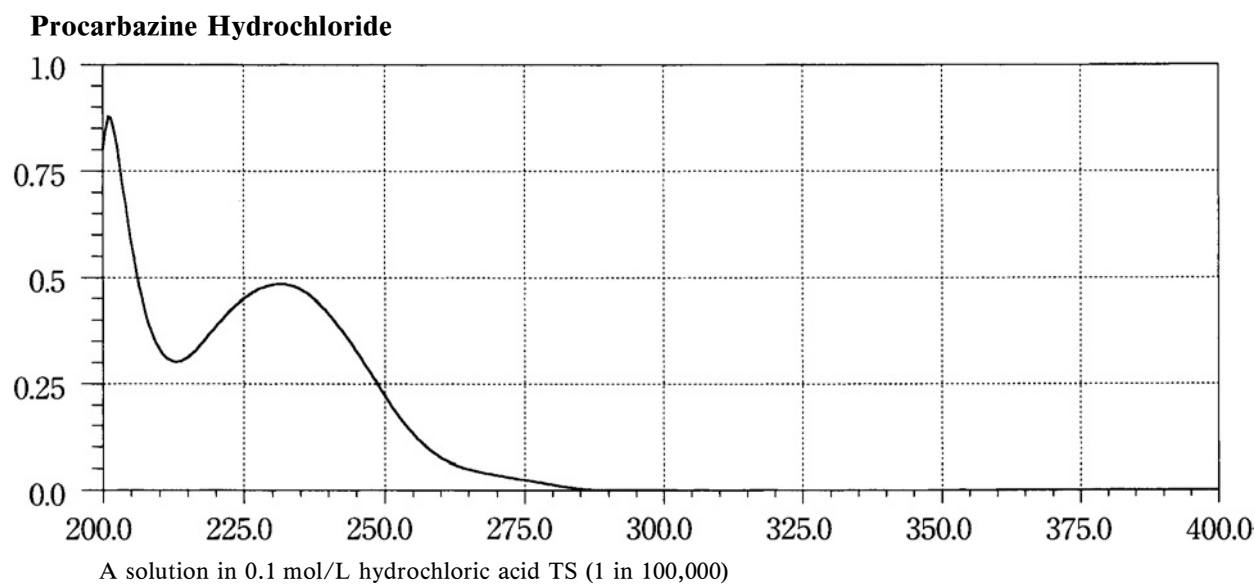
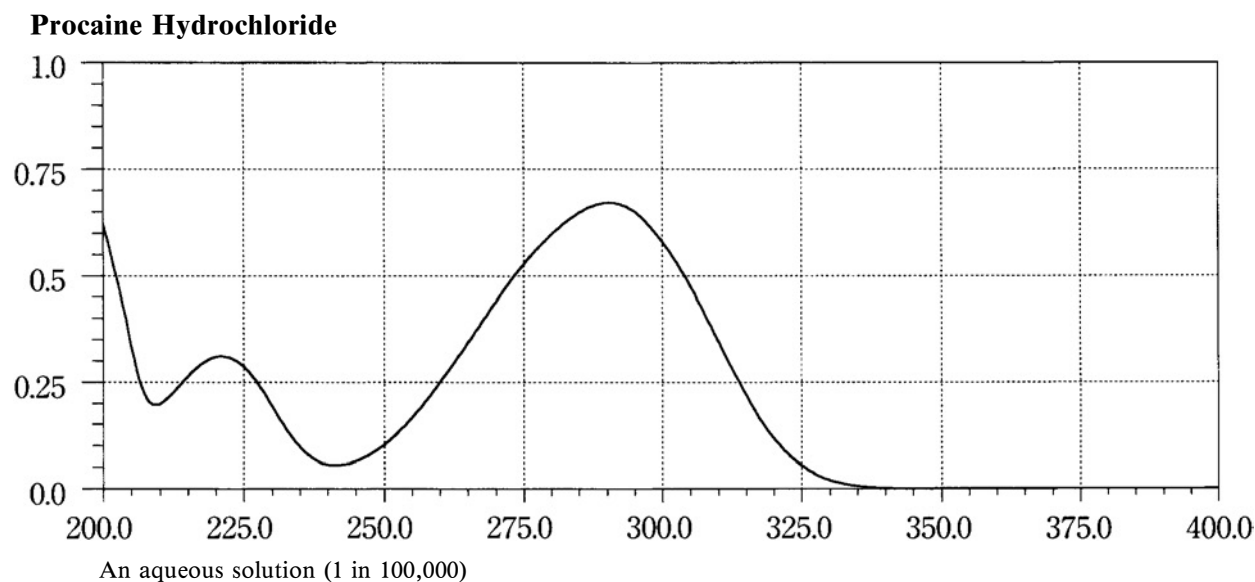
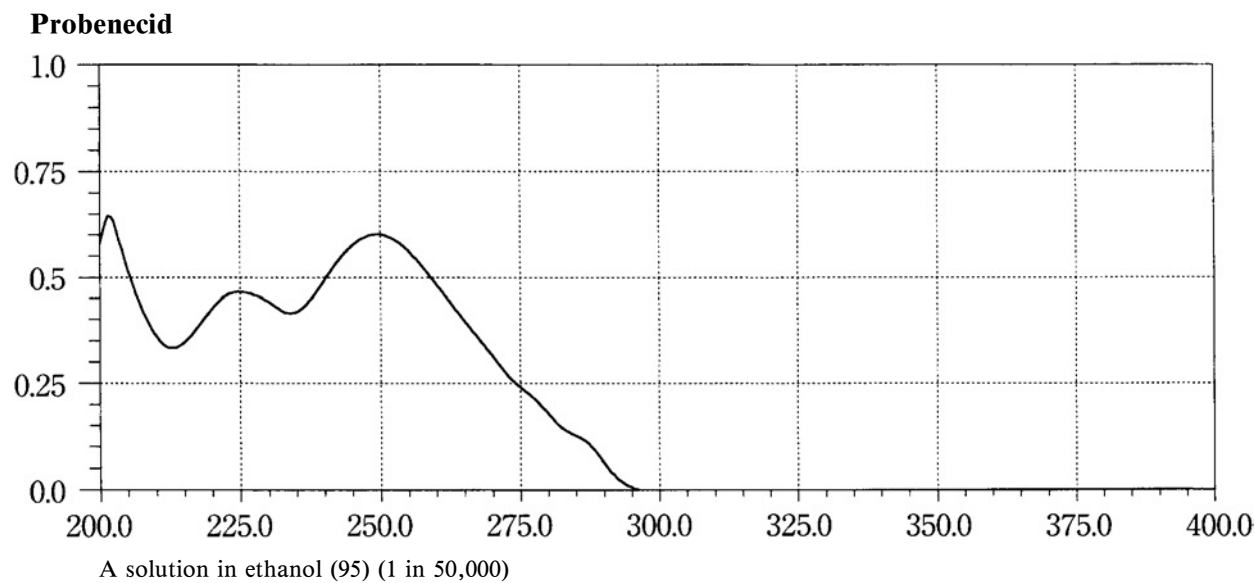
A solution prepared as follows: To 10 mL of a solution in 1 mol/L hydrochloric acid TS (1 in 5000) add water to make 100 mL.

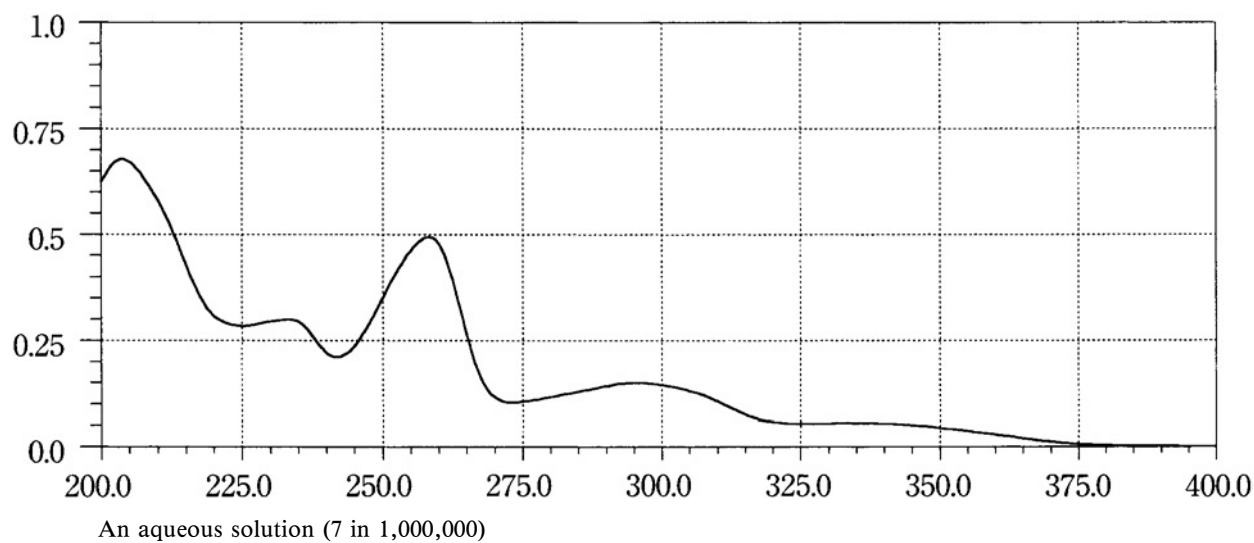
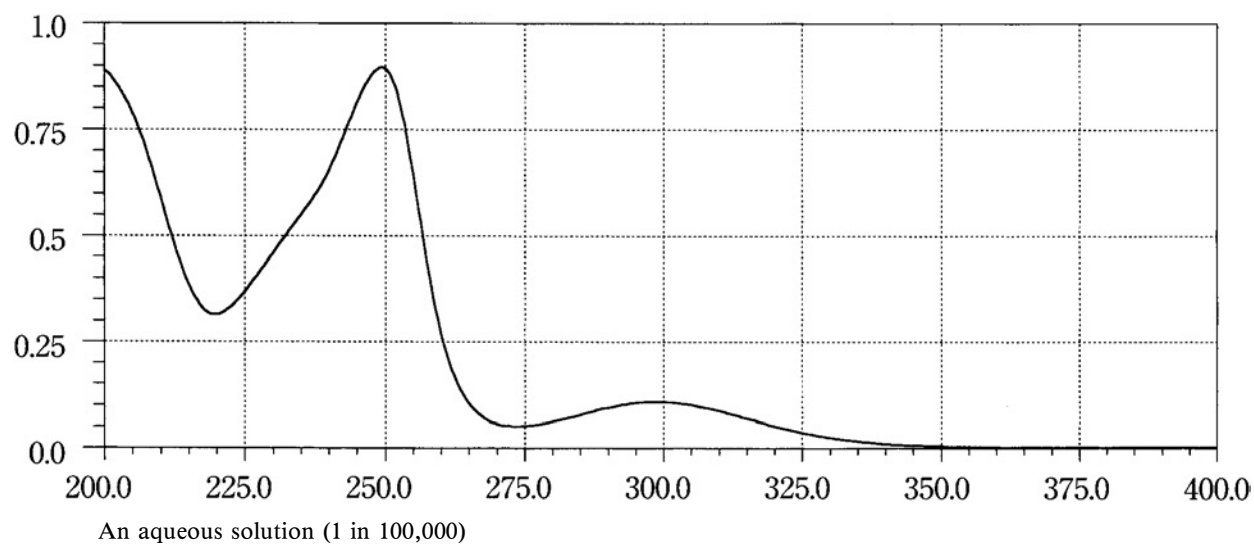
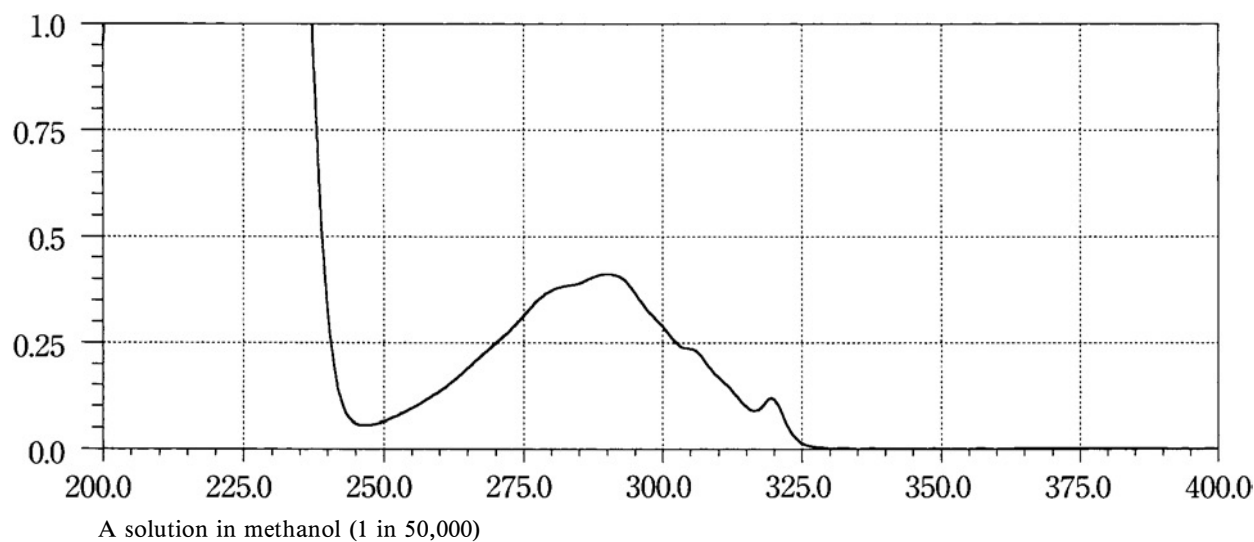
Pravastatin Sodium

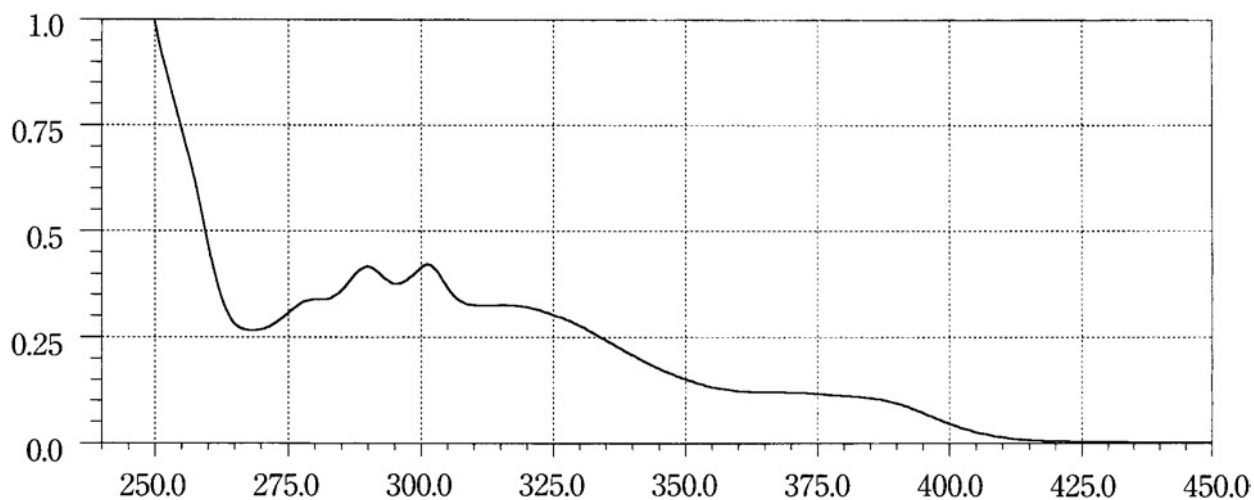
An aqueous solution (1 in 100,000)

Prazepam

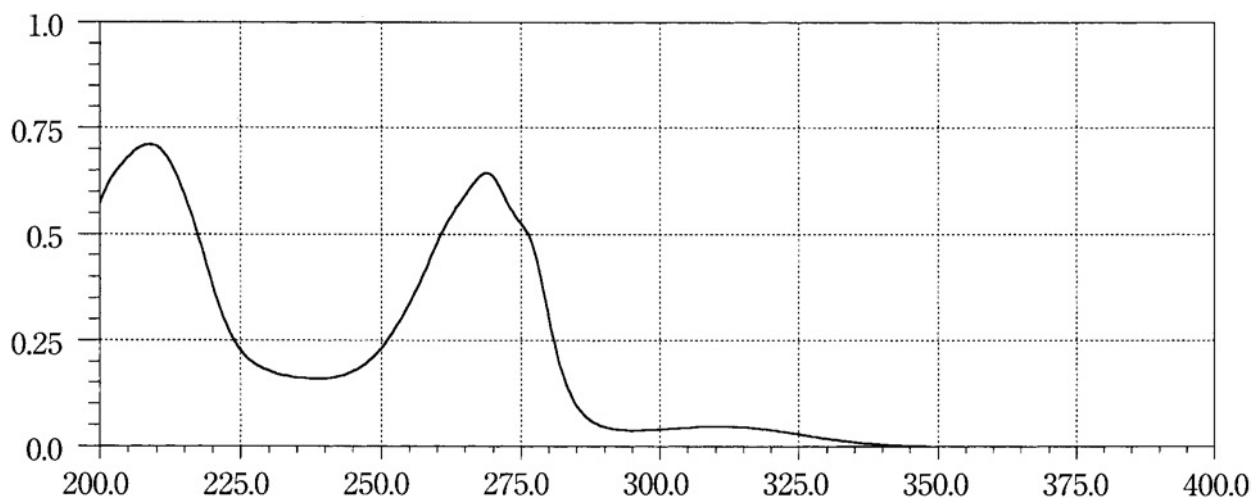
A solution prepared as follows: Dissolve 0.01 g in 1000 mL of a solution of sulfuric acid in ethanol (99.5) (3 in 1000).



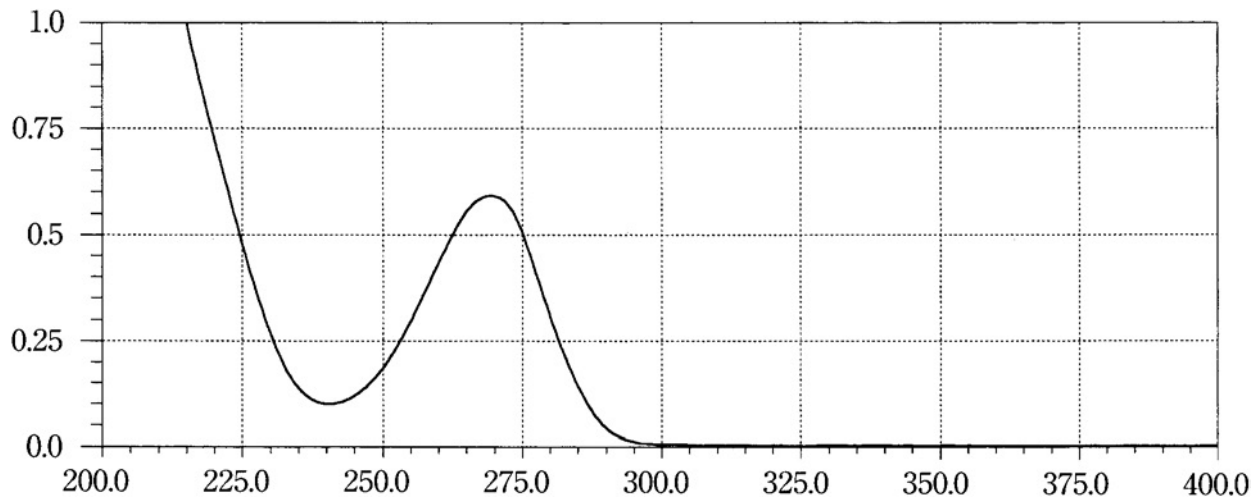
Procaterol Hydrochloride Hydrate**Promethazine Hydrochloride****Propranolol Hydrochloride**

Pyrantel Pamoate

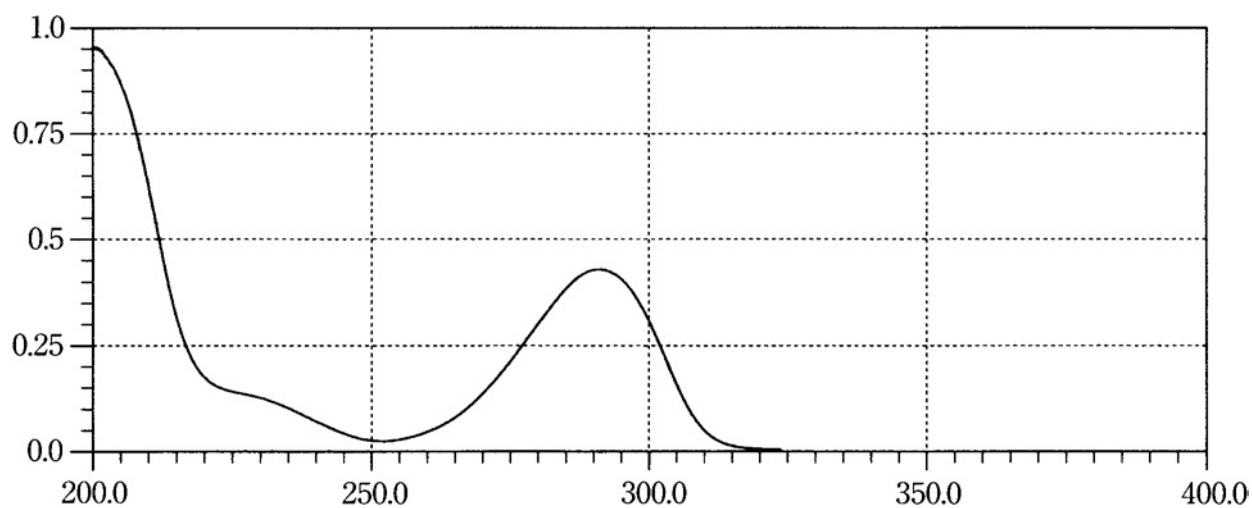
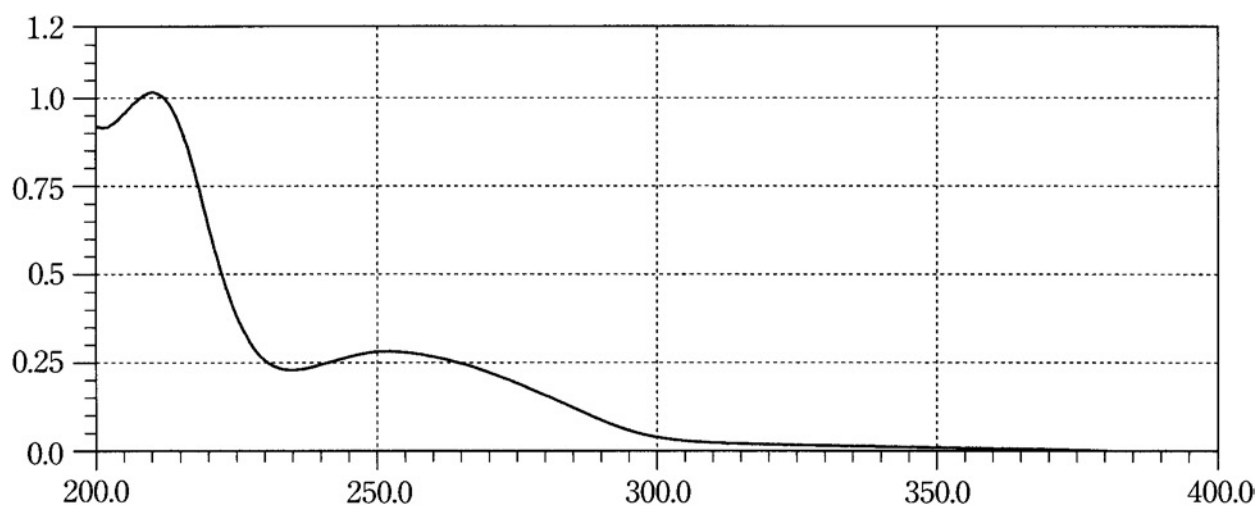
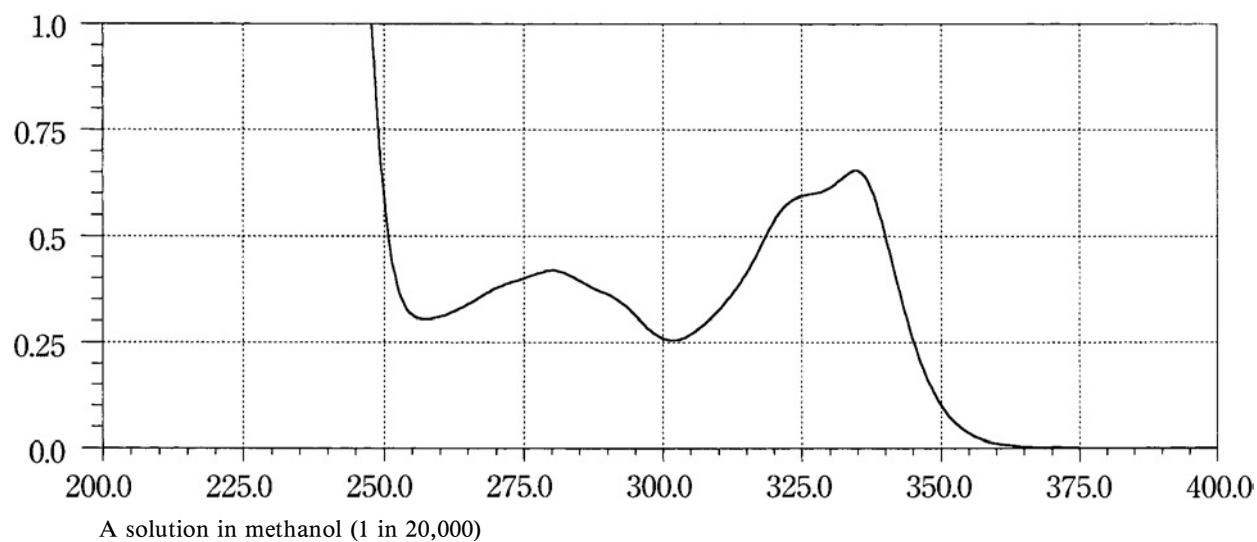
A solution prepared as follows: Dissolve 0.1 g in 50 mL of dimethylformamide, and add methanol to make 200 mL. To 2 mL of this solution add a solution of hydrochloric acid in methanol (9 in 1000) to make 100 mL.

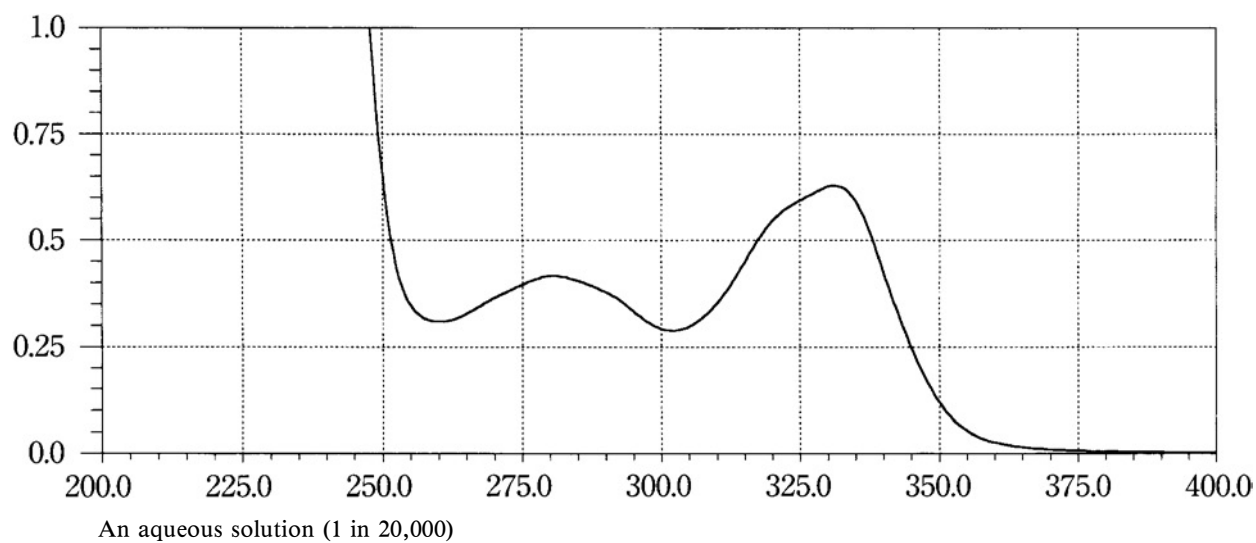
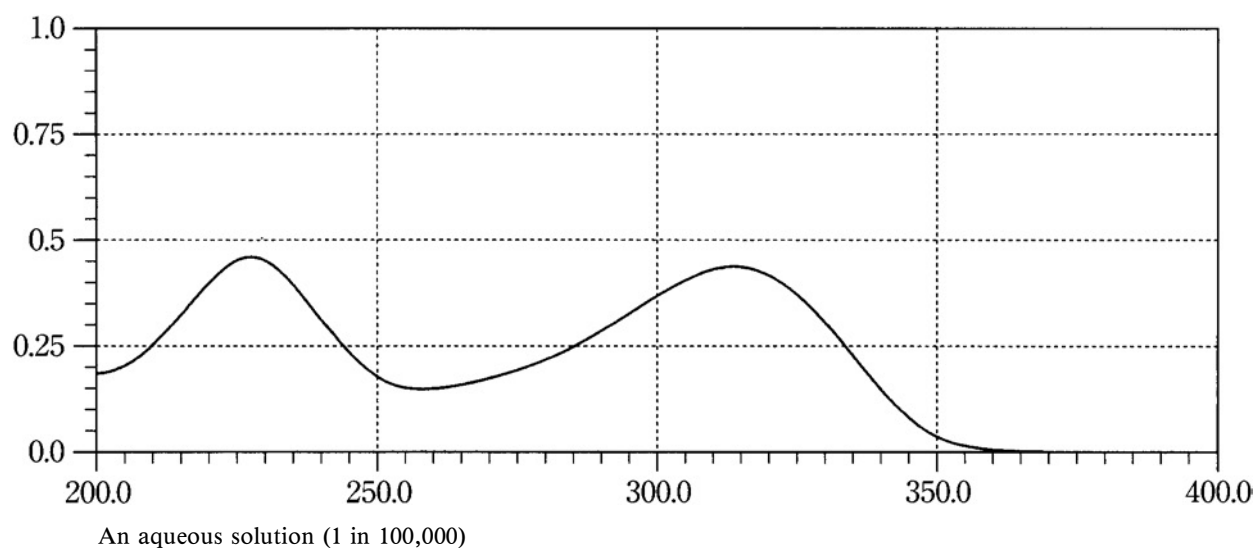
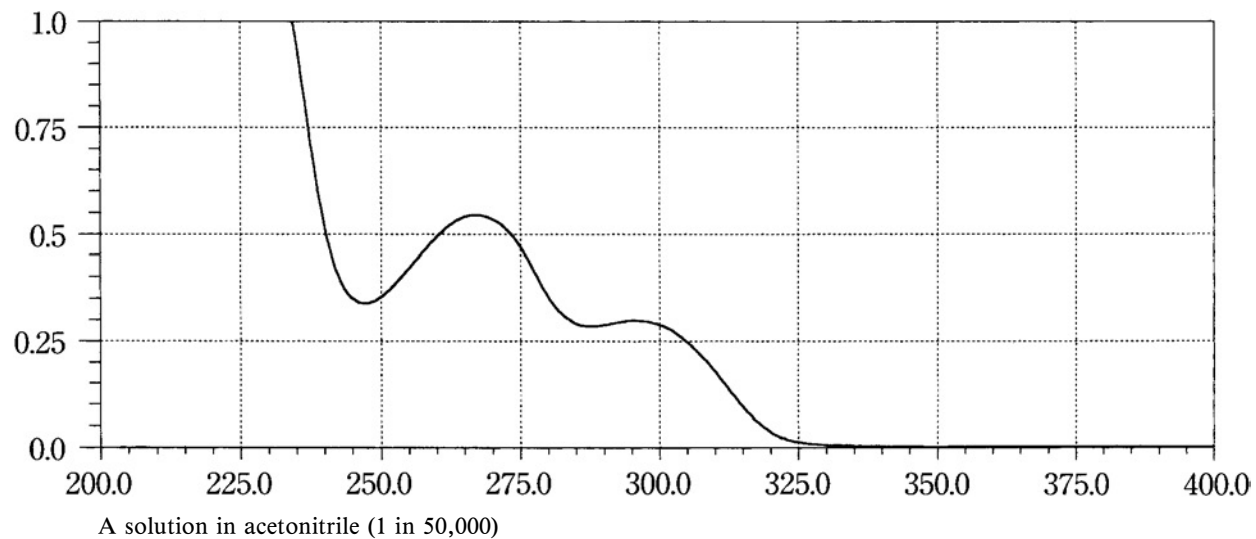
Pyrazinamide

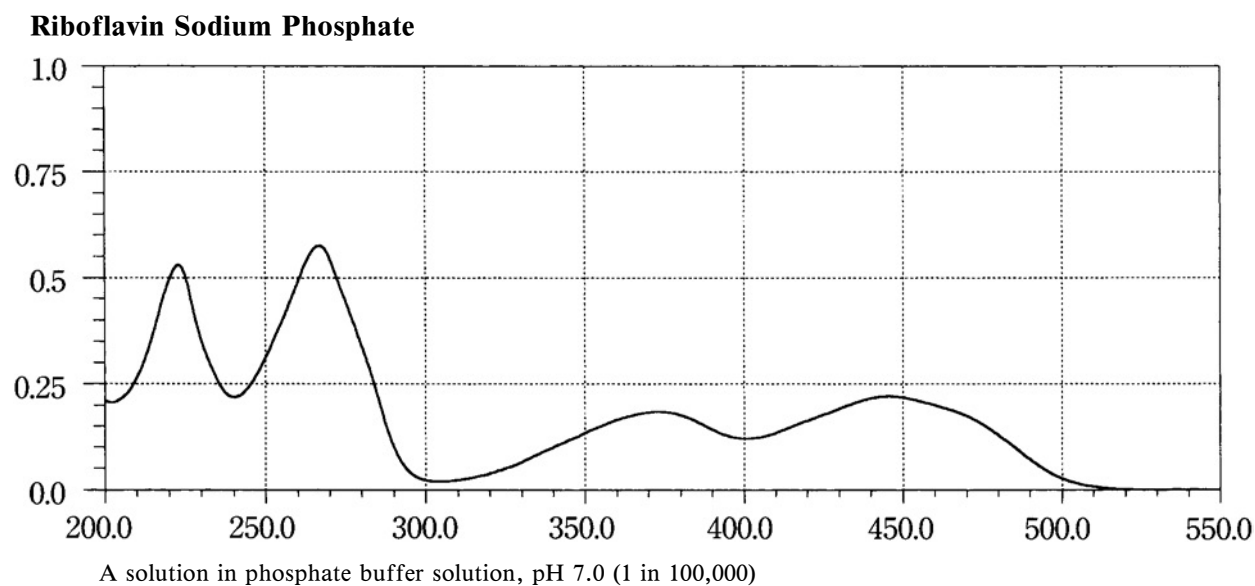
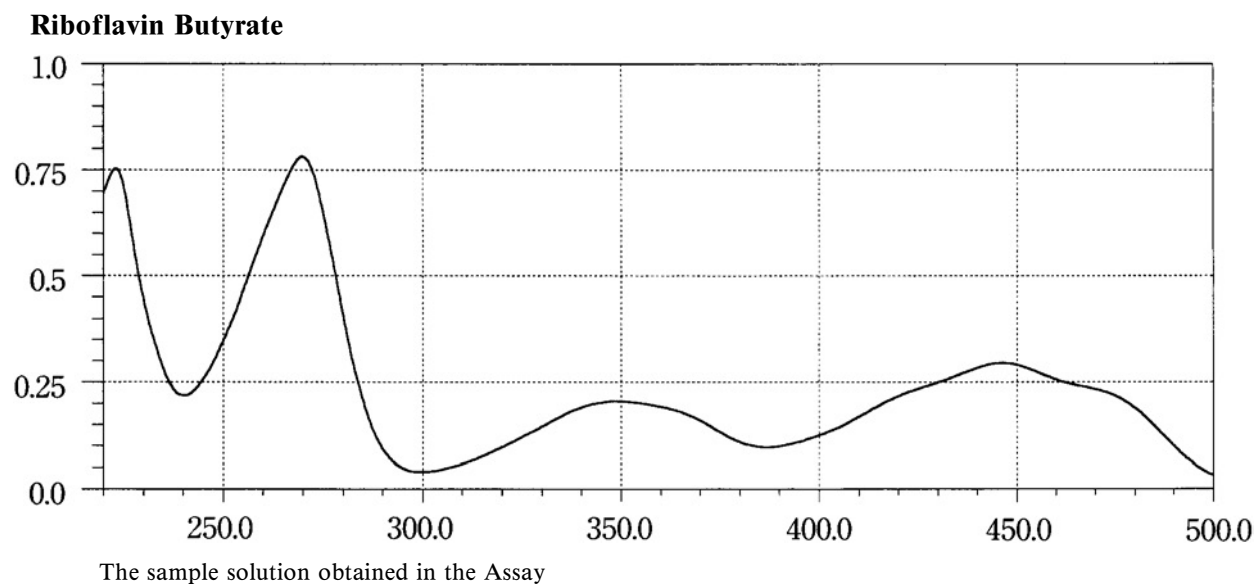
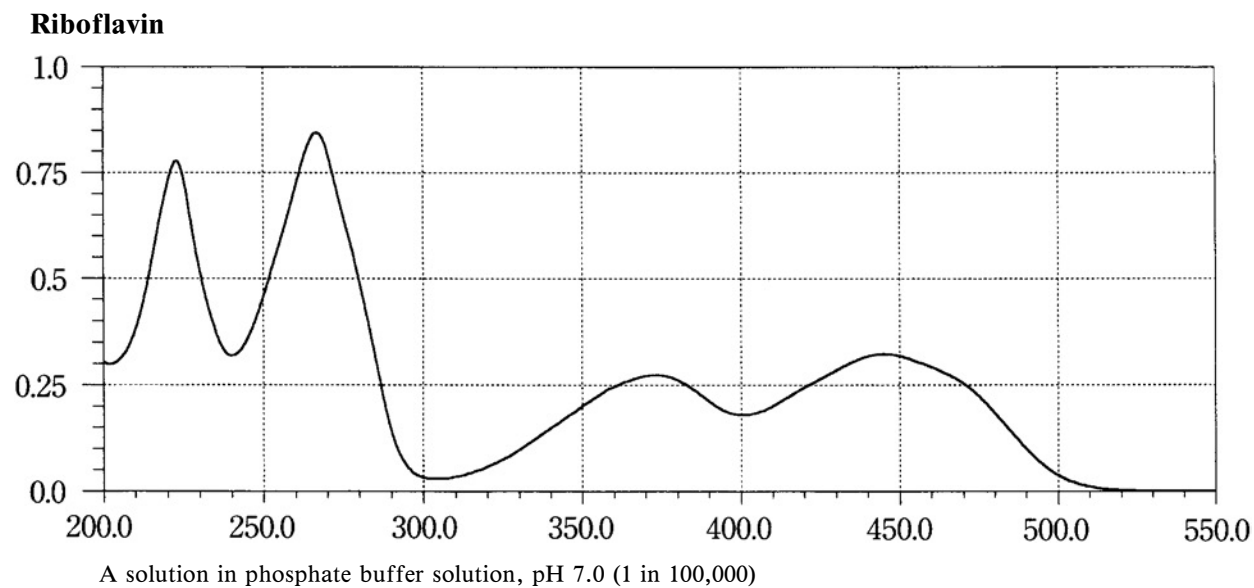
A solution in 0.1 mol/L hydrochloric acid TS (1 in 100,000)

Pyridostigmine Bromide

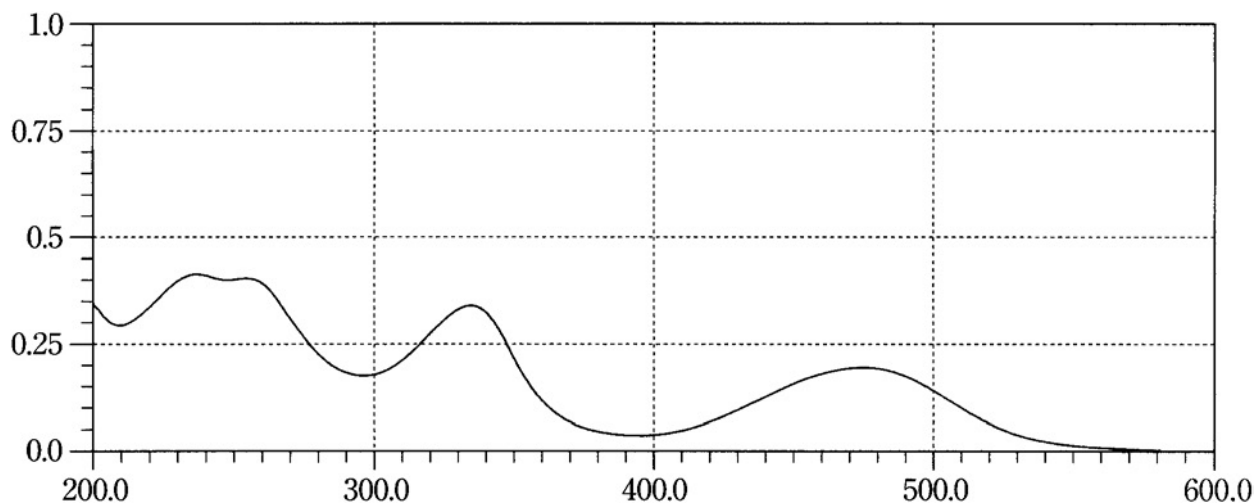
A solution in 0.1 mol/L hydrochloric acid TS (1 in 30,000)

Pyridoxine Hydrochloride**Pyrrolnitrin****Quinine Ethyl Carbonate**

Quinine Sulfate Hydrate**Ranitidine Hydrochloride****Reserpine**

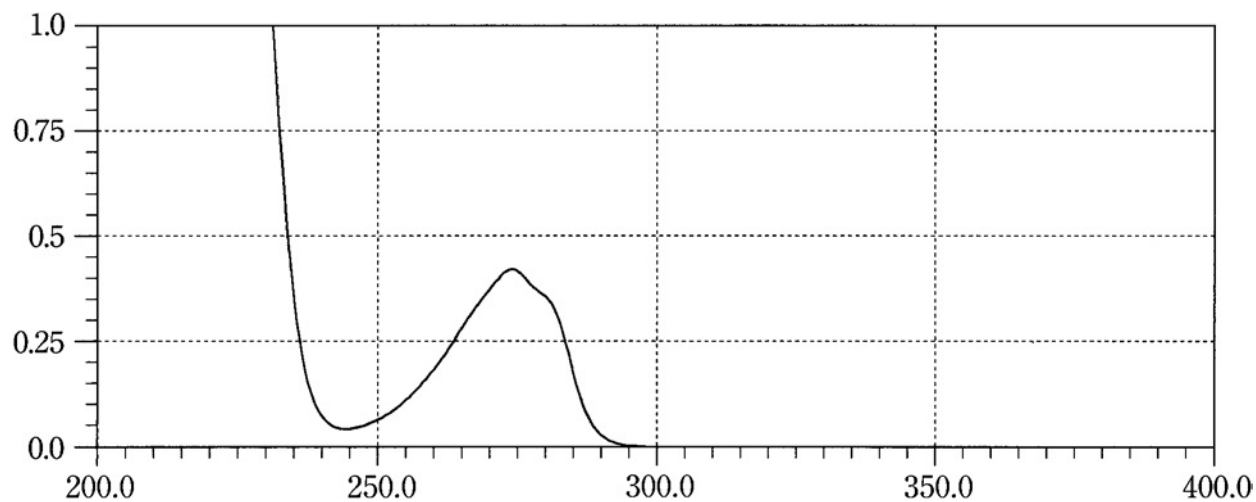


Rifampicin



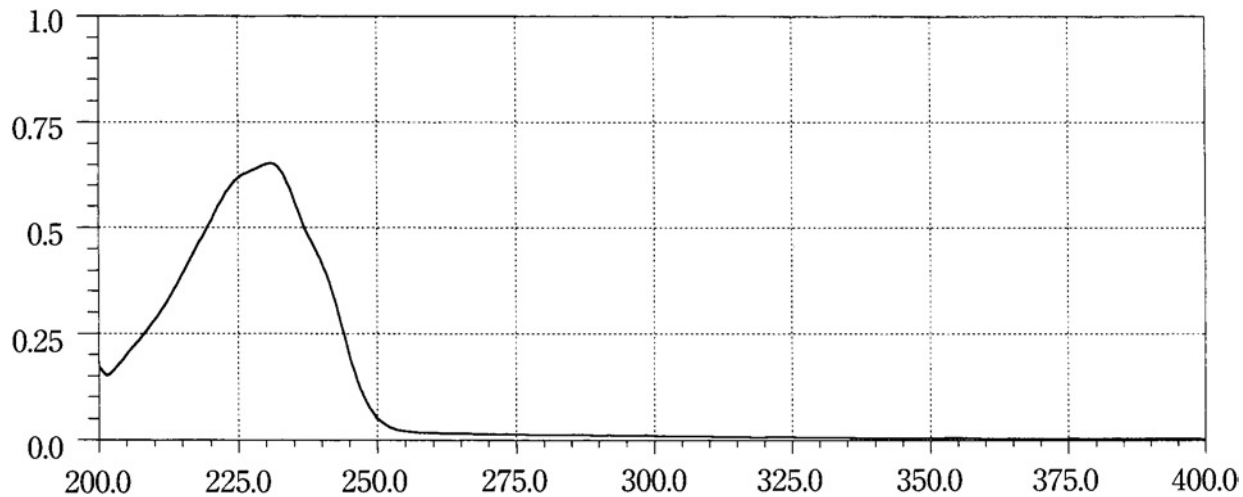
A solution prepared as follows: To 5 mL of a solution in methanol (1 in 5000) add 0.05 mol/L phosphate buffer solution, pH 7.0 to make 100 mL.

Ritodrine Hydrochloride

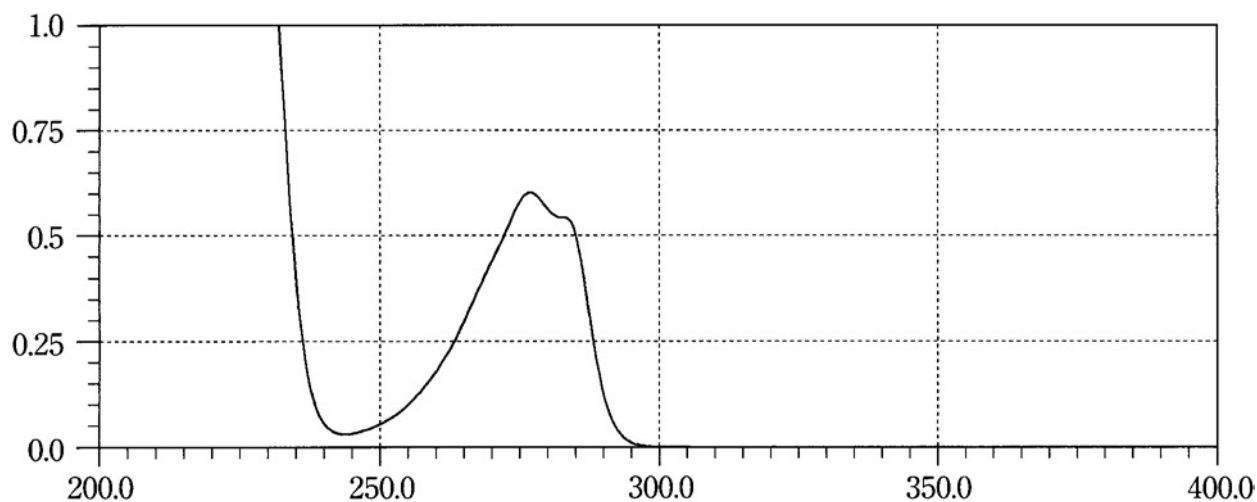


A solution in 0.01 mol/L hydrochloric acid TS (1 in 20,000)

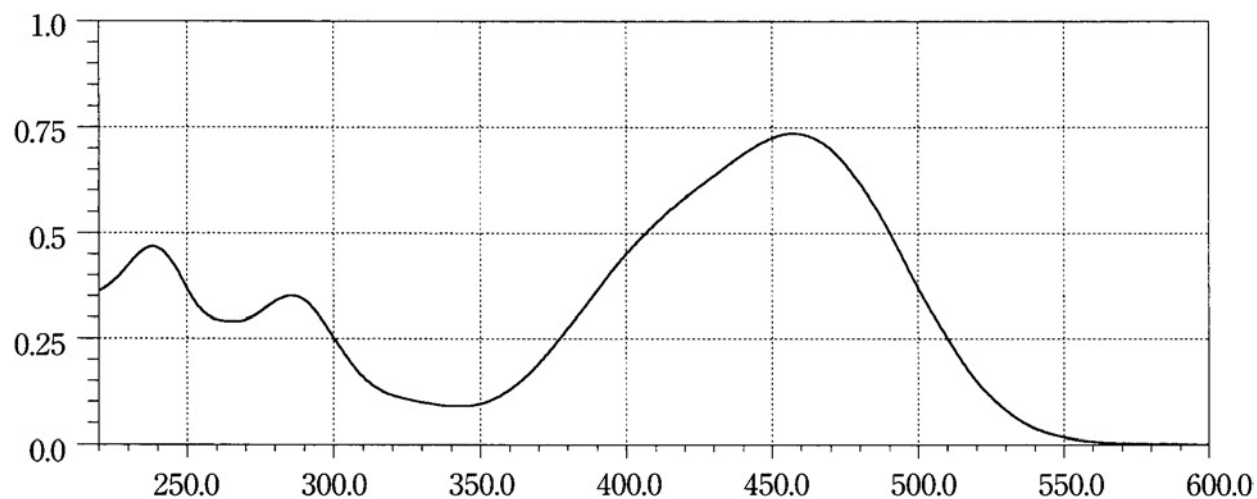
Rokitamycin



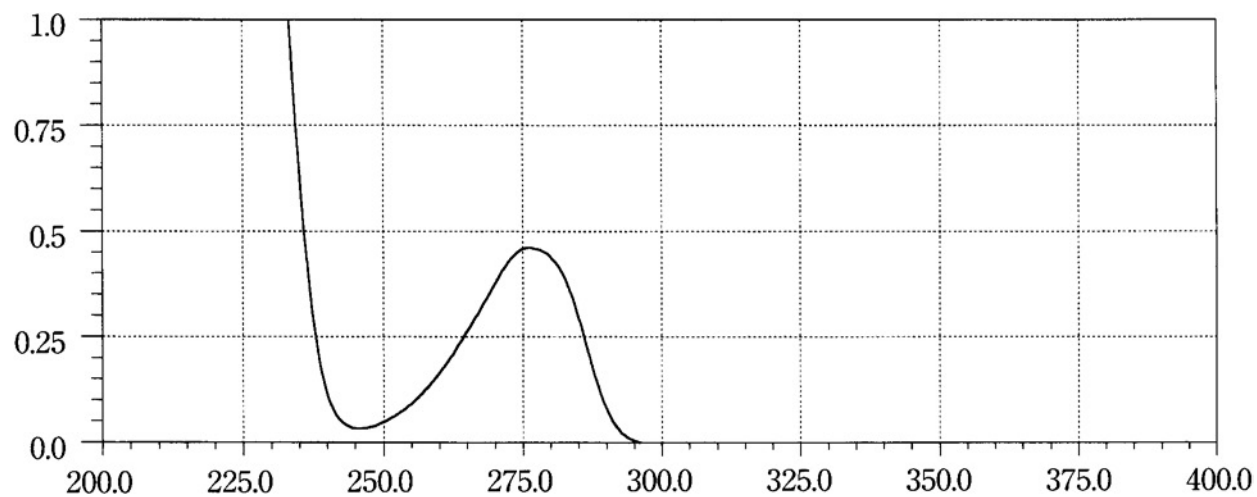
A solution in methanol (1 in 50,000)

Roxatidine Acetate Hydrochloride

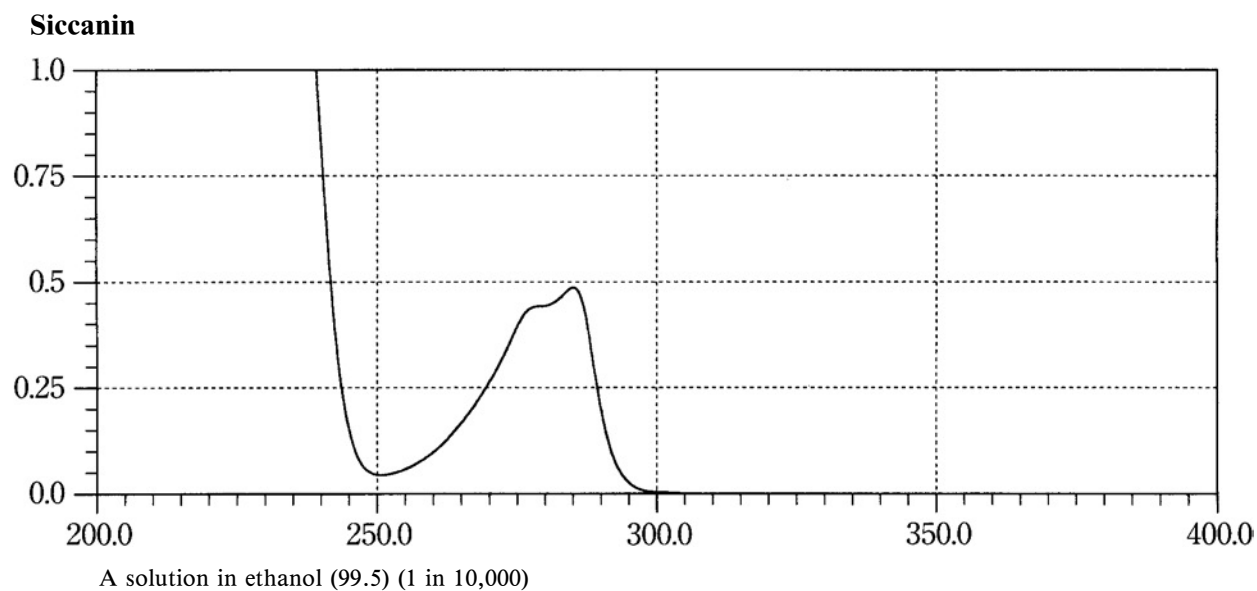
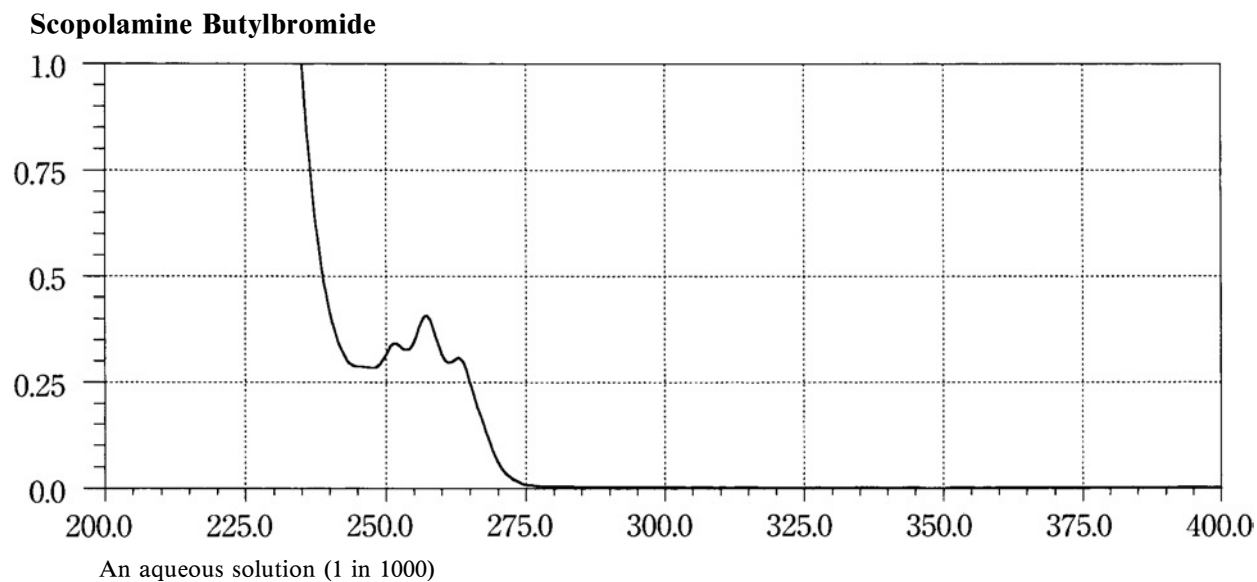
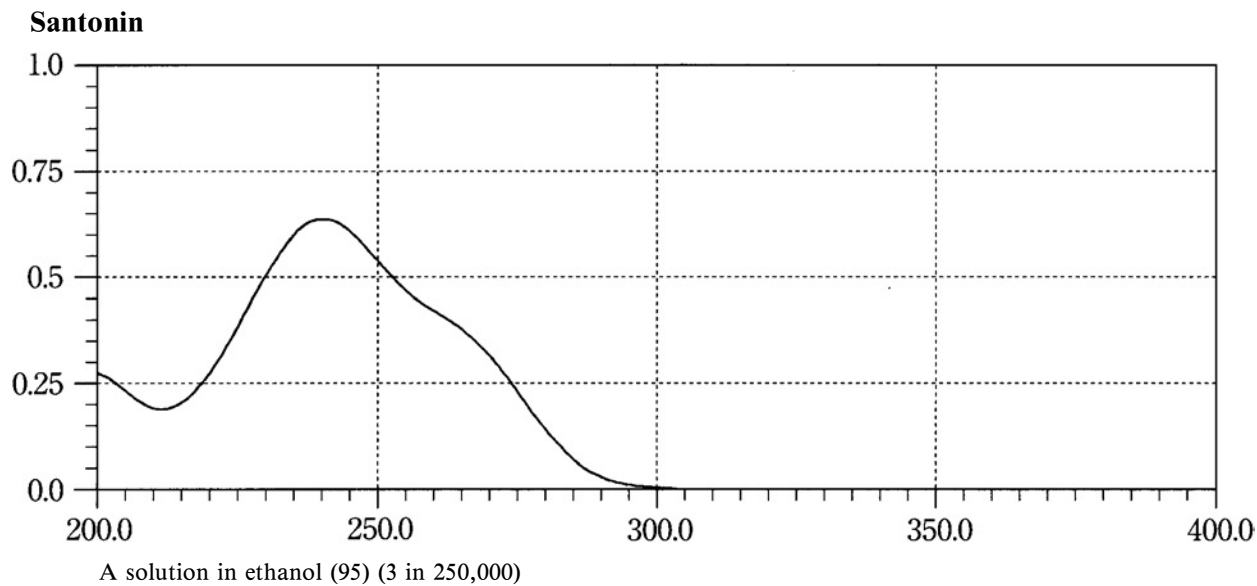
A solution in ethanol (99.5) (1 in 10,000)

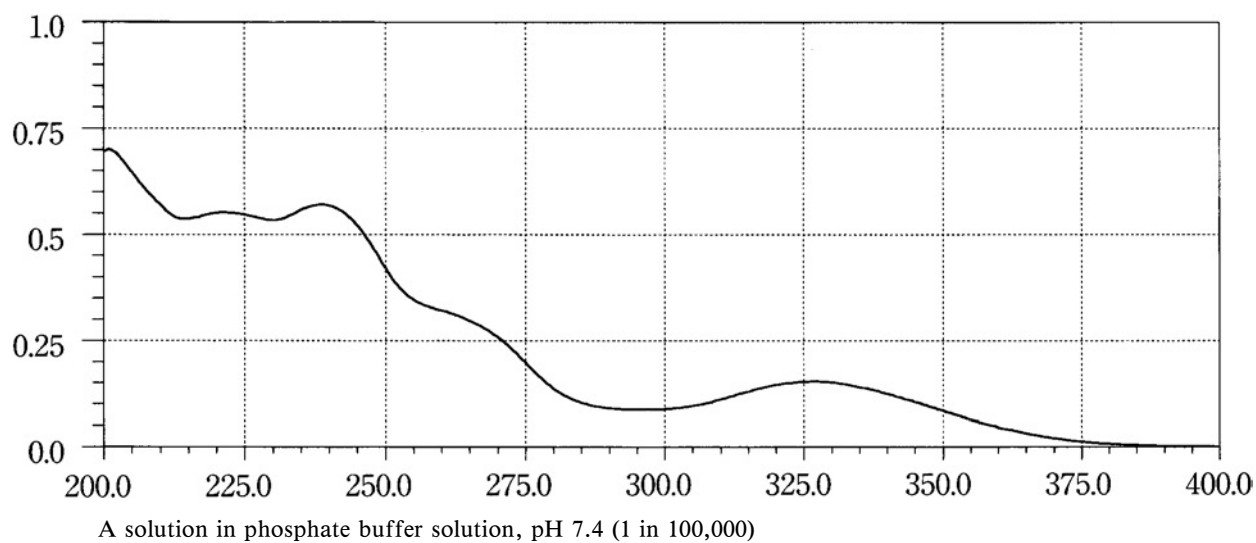
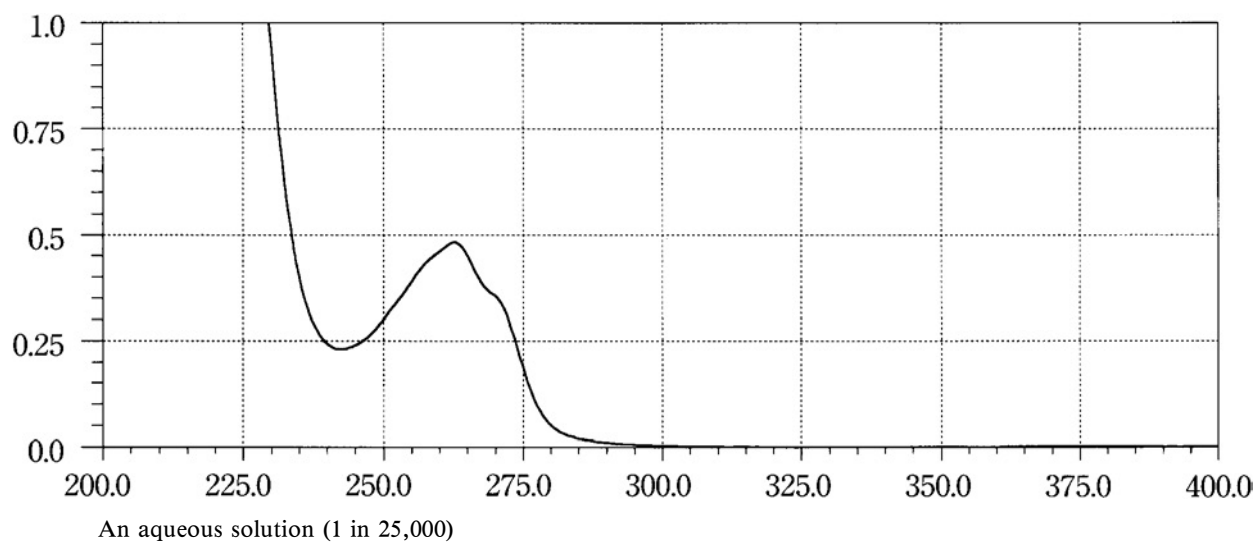
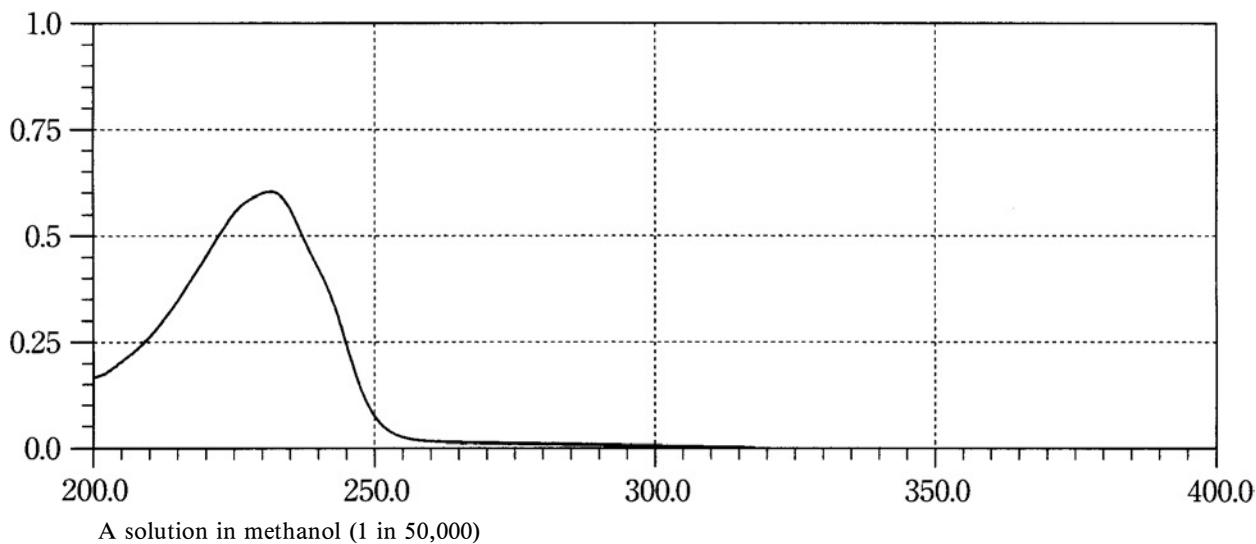
Salazosulfapyridine

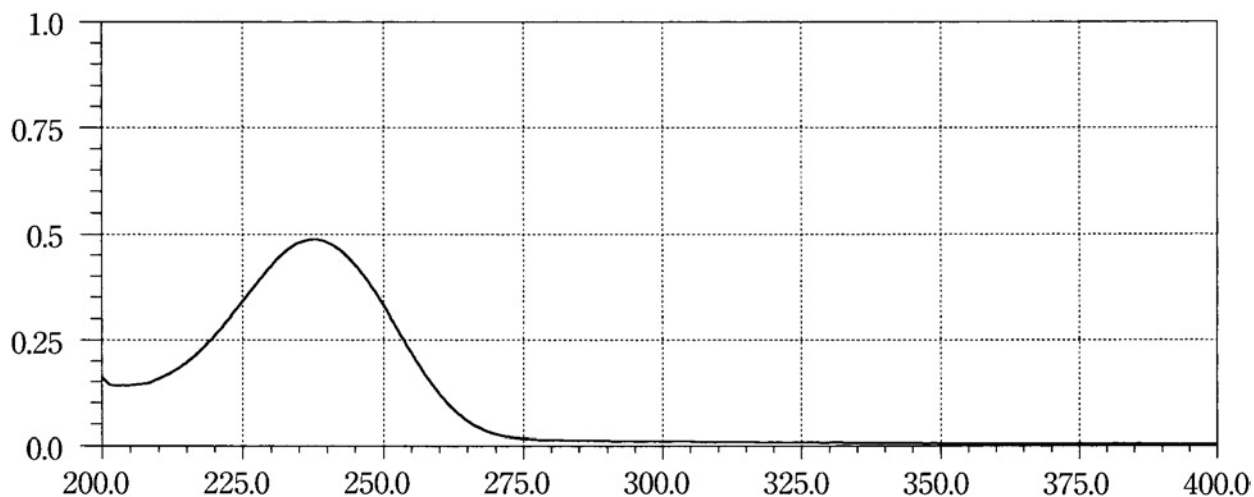
A solution in dilute sodium hydroxide TS (1 in 100,000)

Salbutamol Sulfate

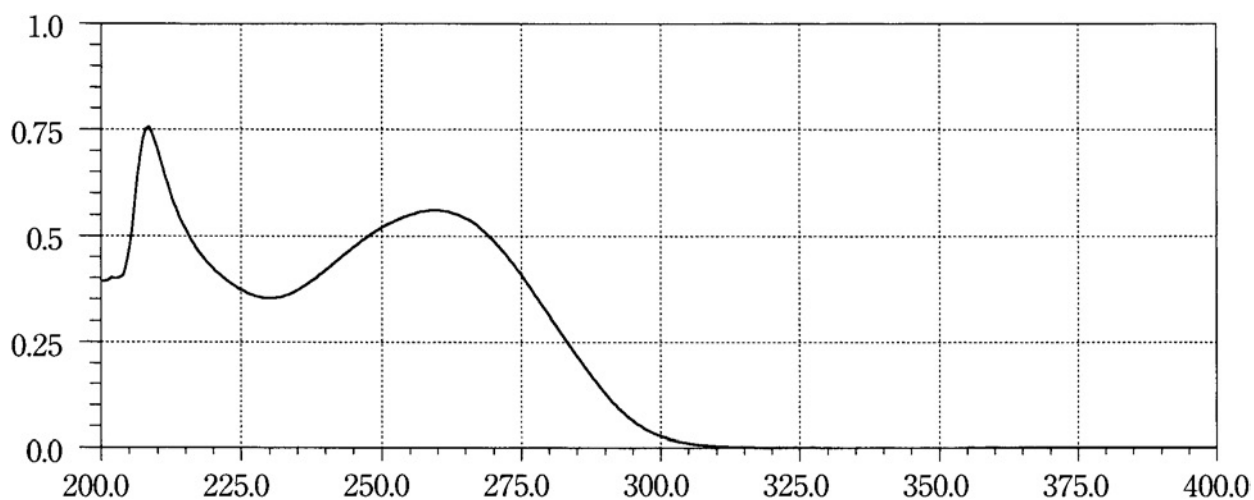
A solution in 0.1 mol/L hydrochloric acid TS (1 in 12,500)



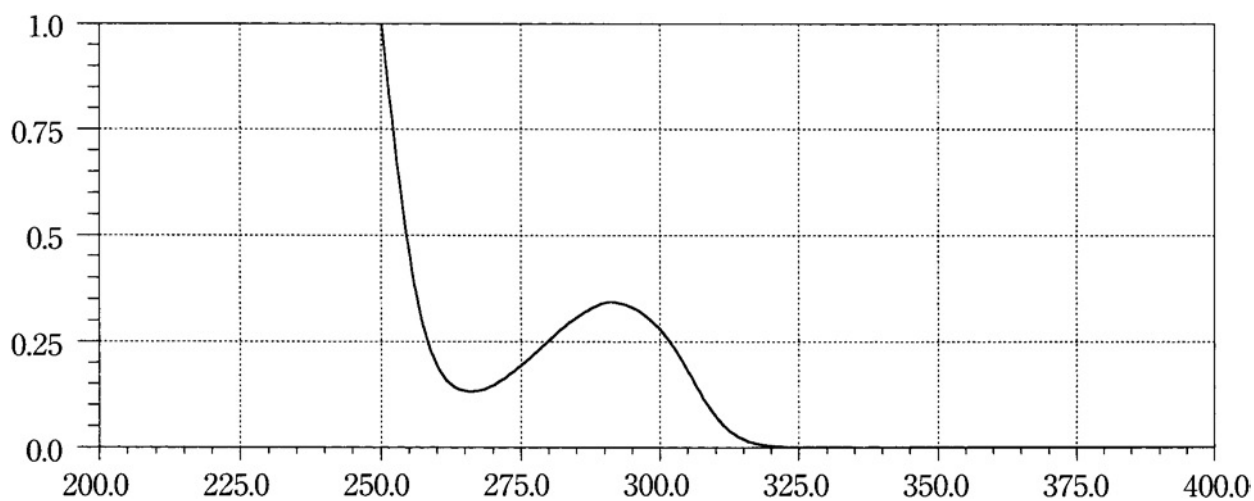
Sodium Cromoglicate**Sodium Picosulfate Hydrate****Spiramycin Acetate**

Spironolactone

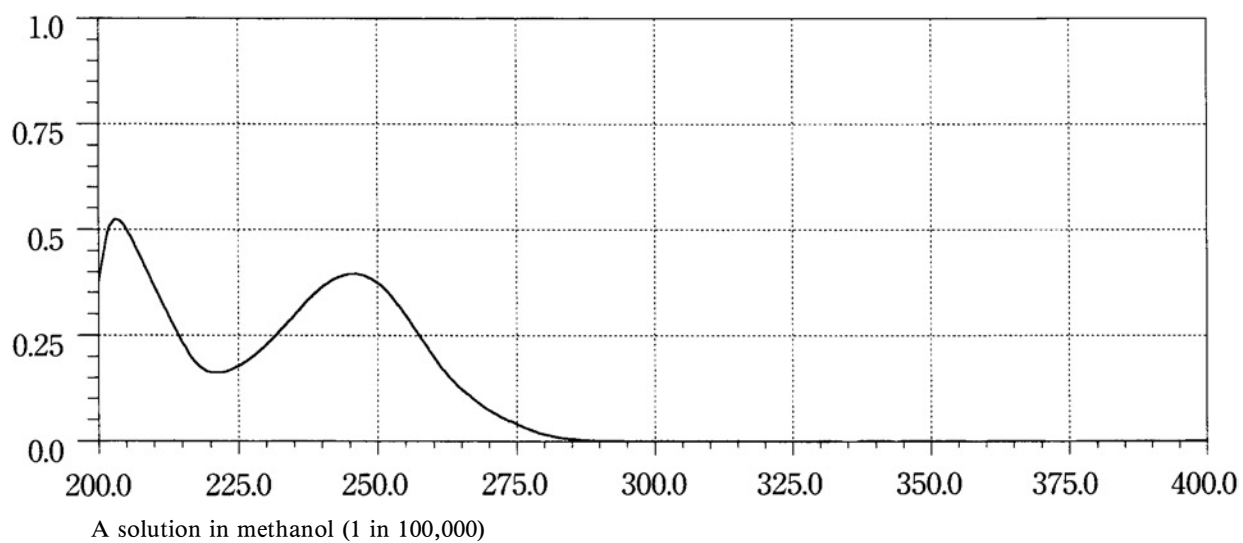
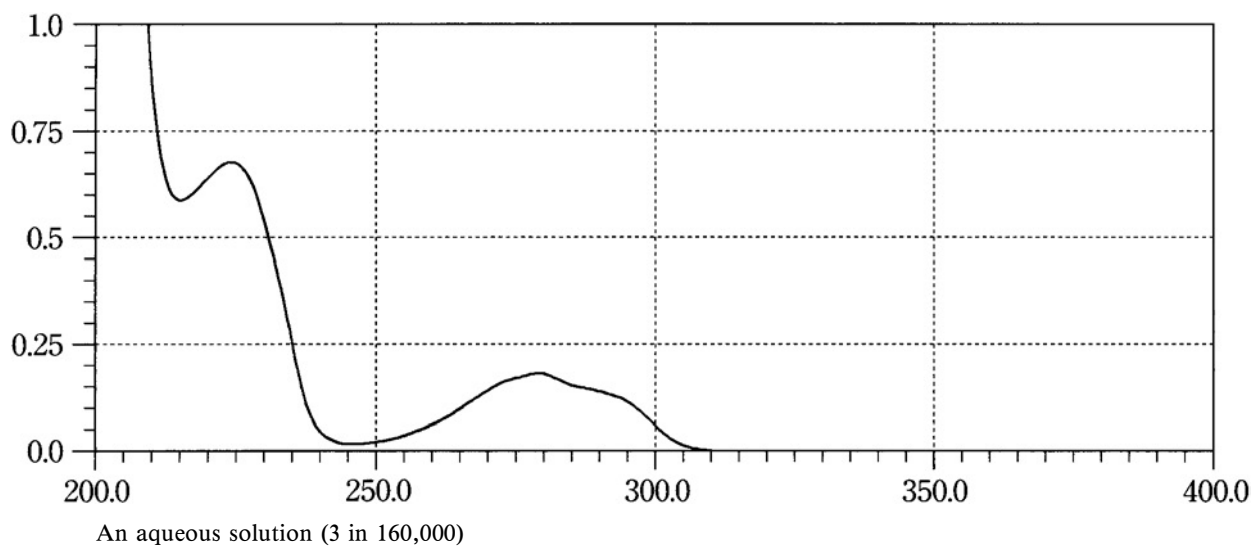
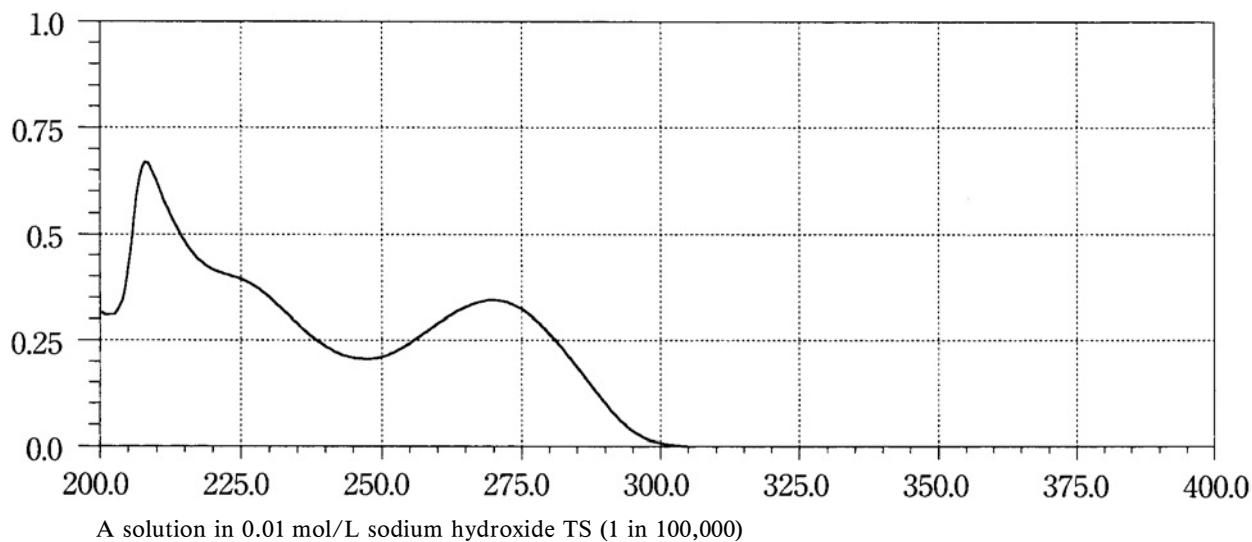
A solution in methanol (1 in 100,000)

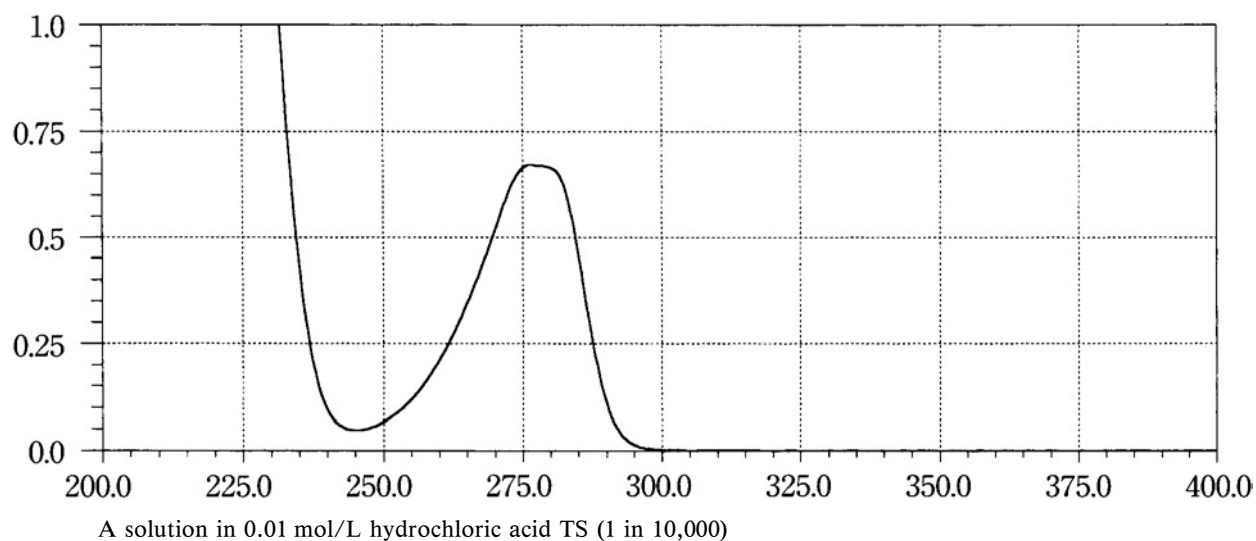
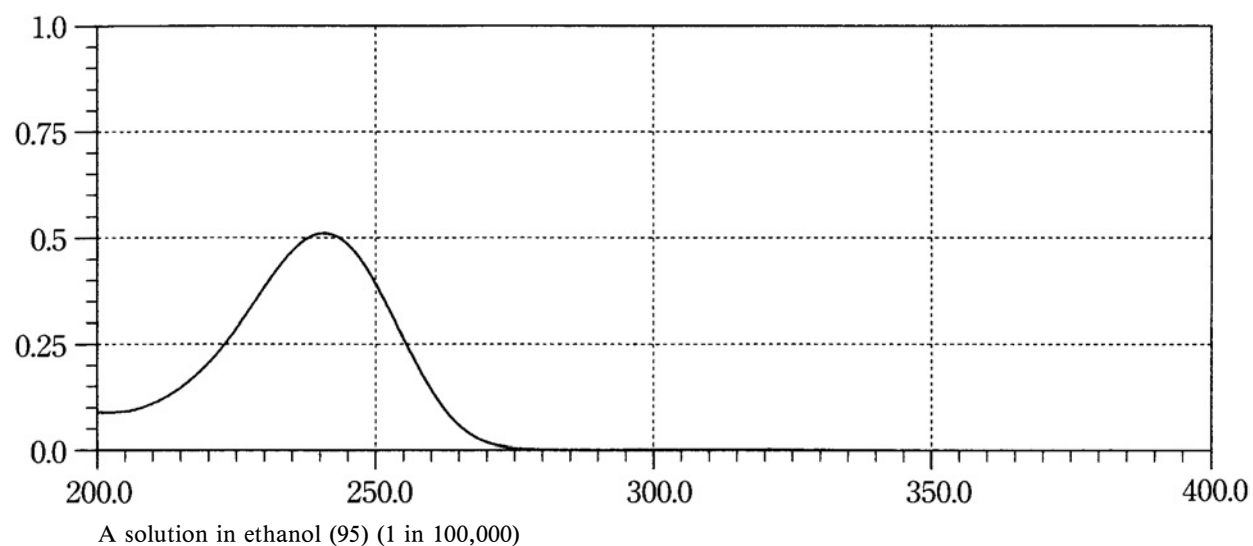
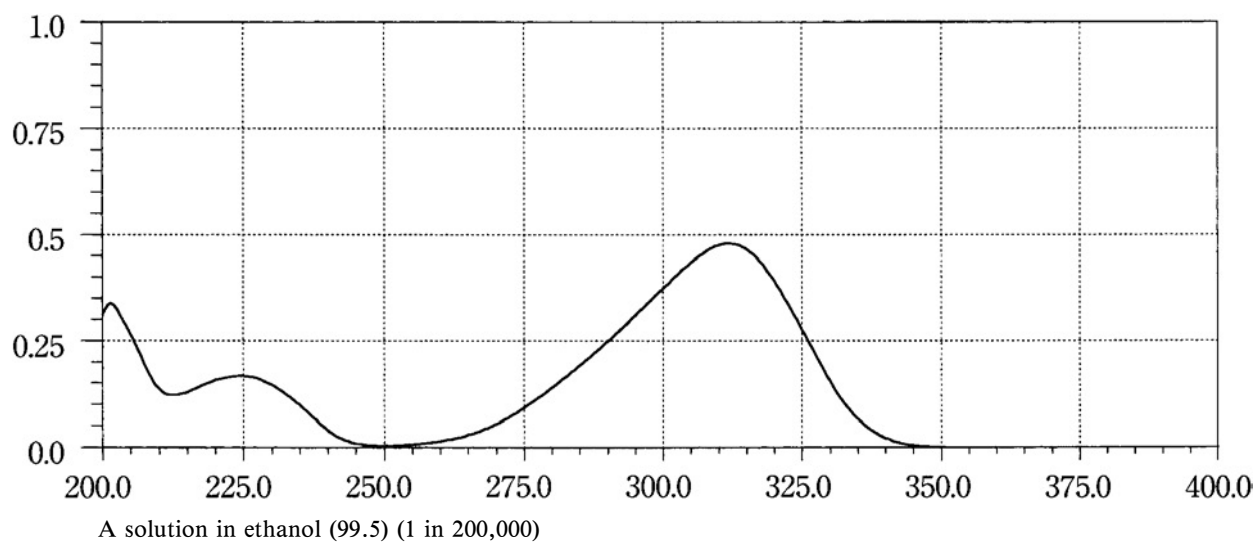
Sulfinpyrazone

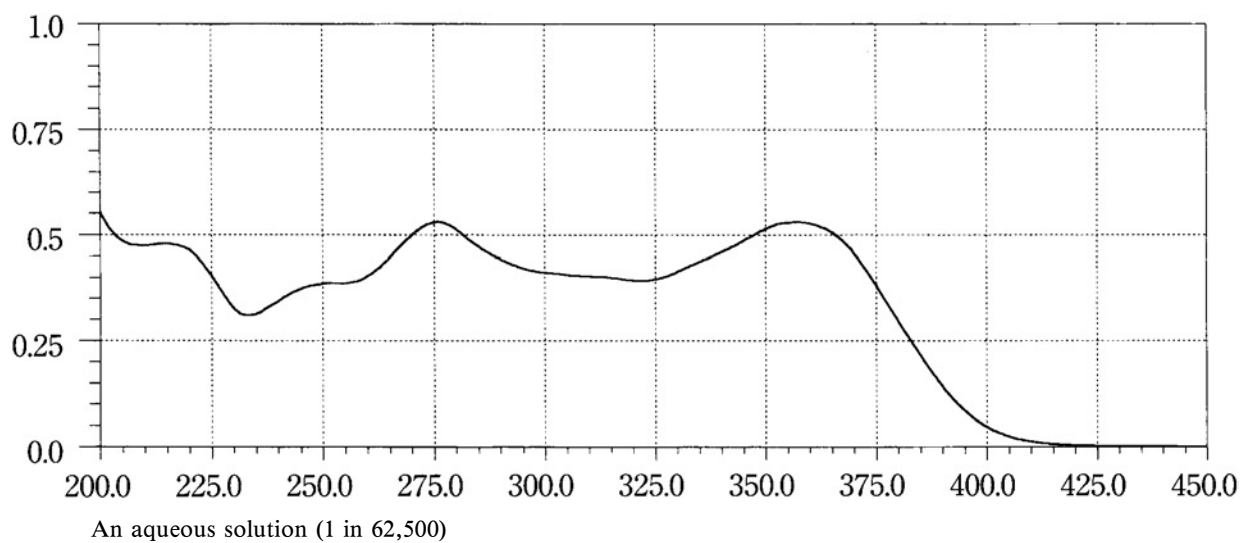
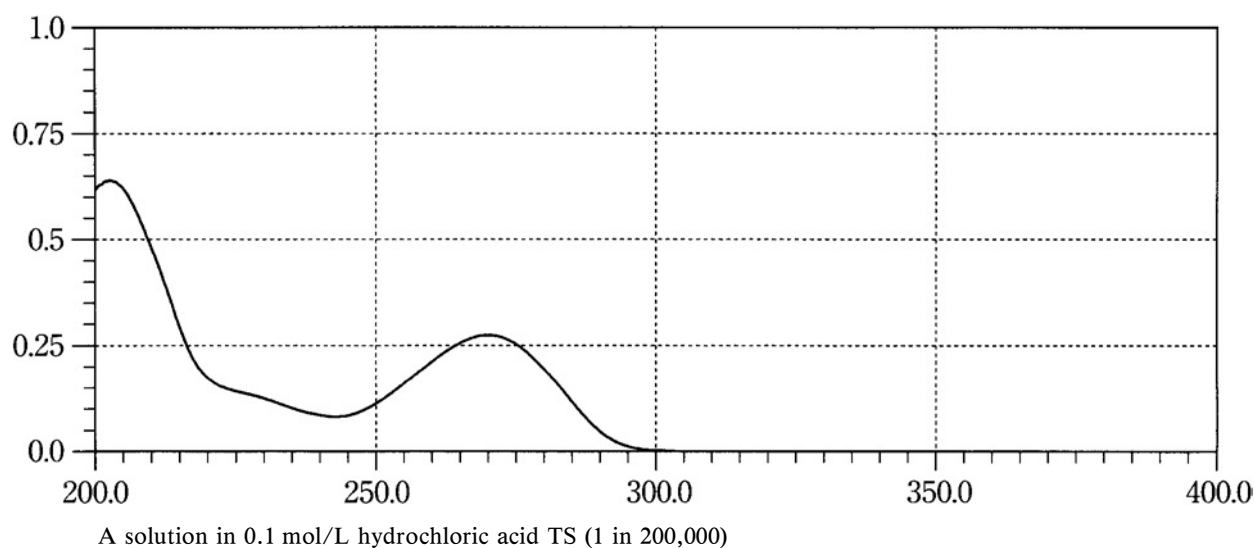
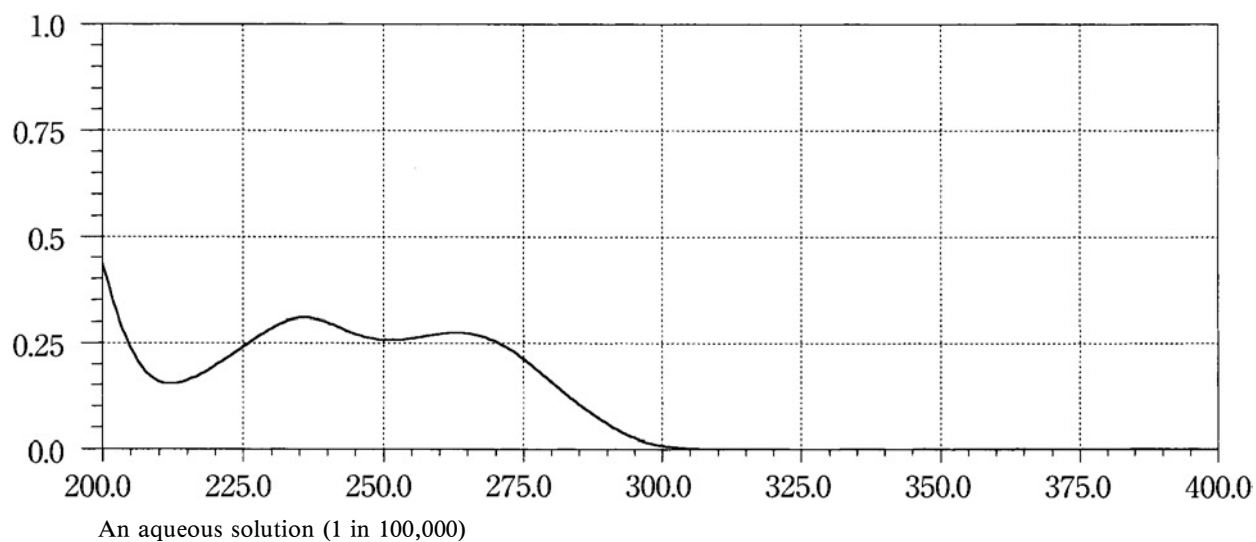
A solution in 0.01 mol/L sodium hydroxide TS (1 in 100,000)

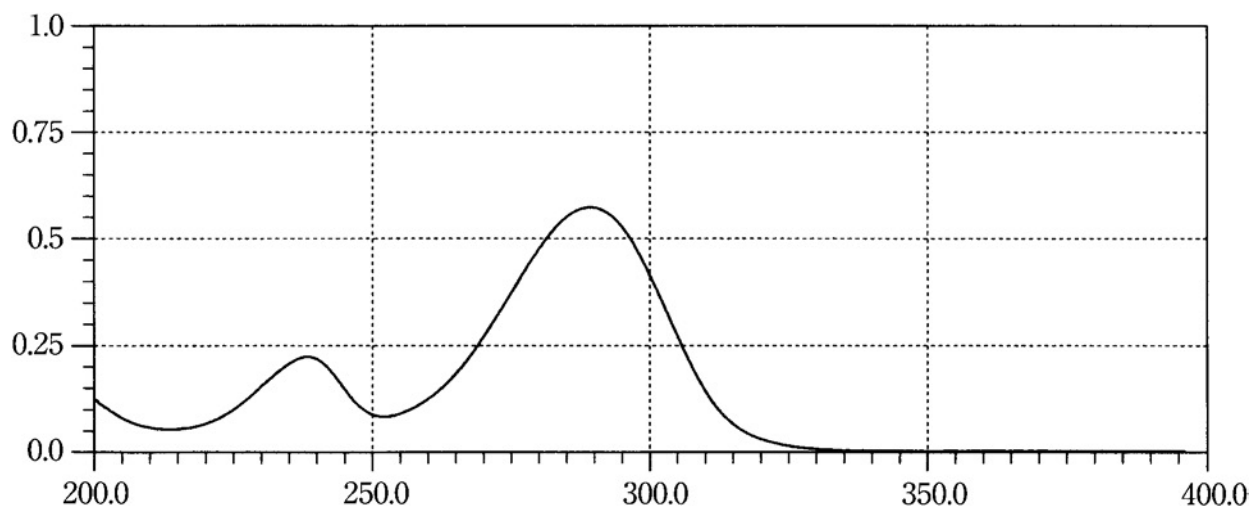
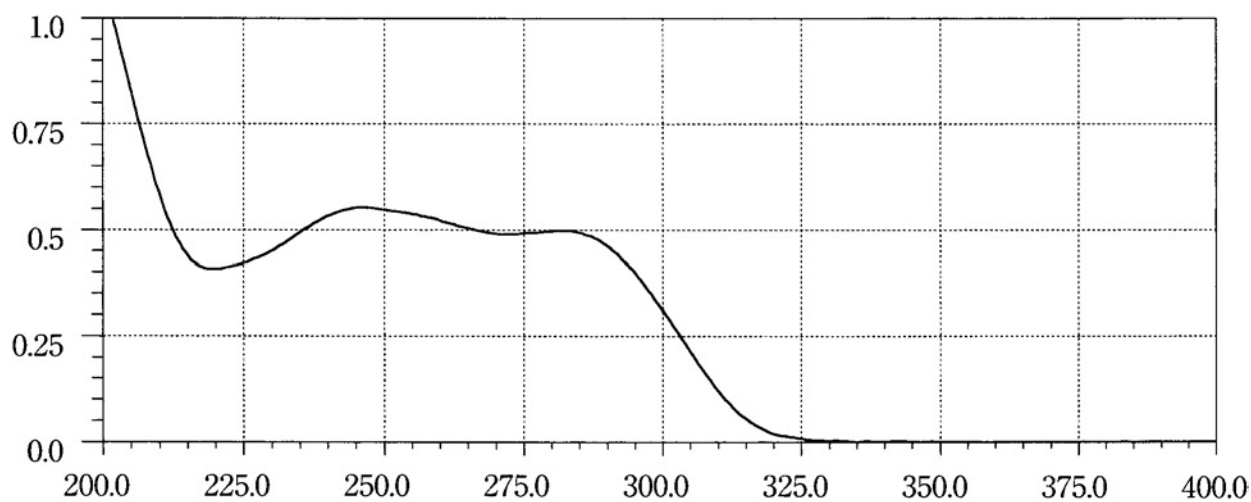
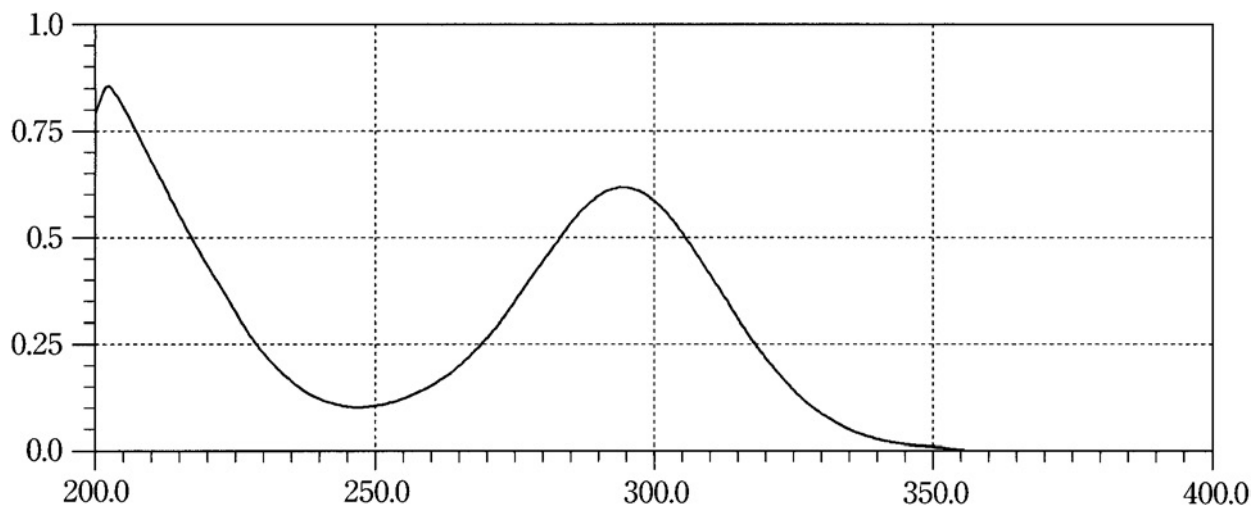
Sulpiride

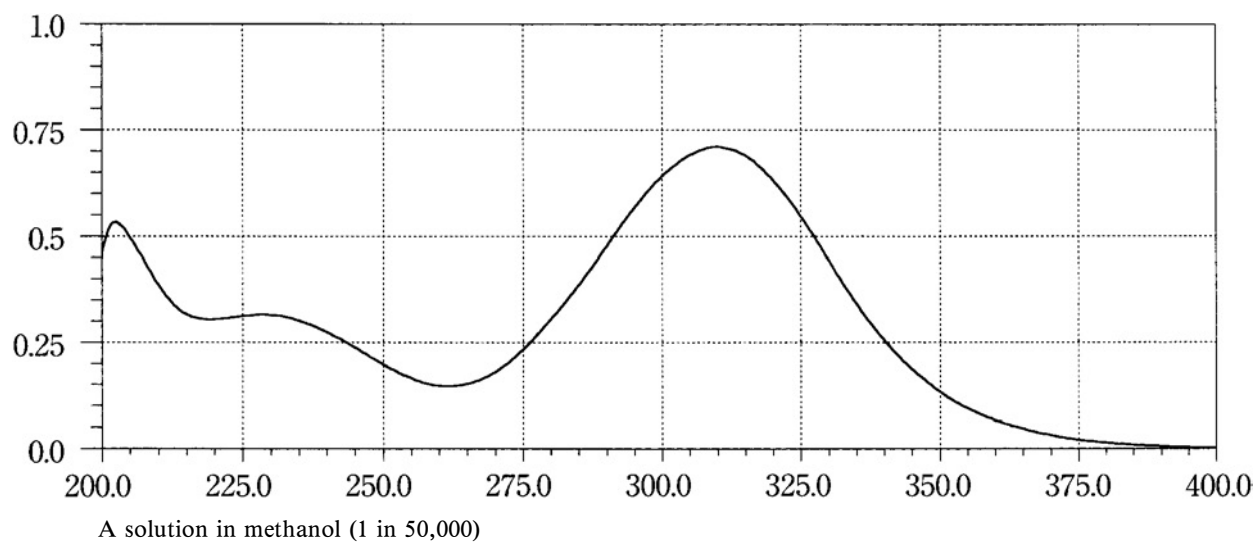
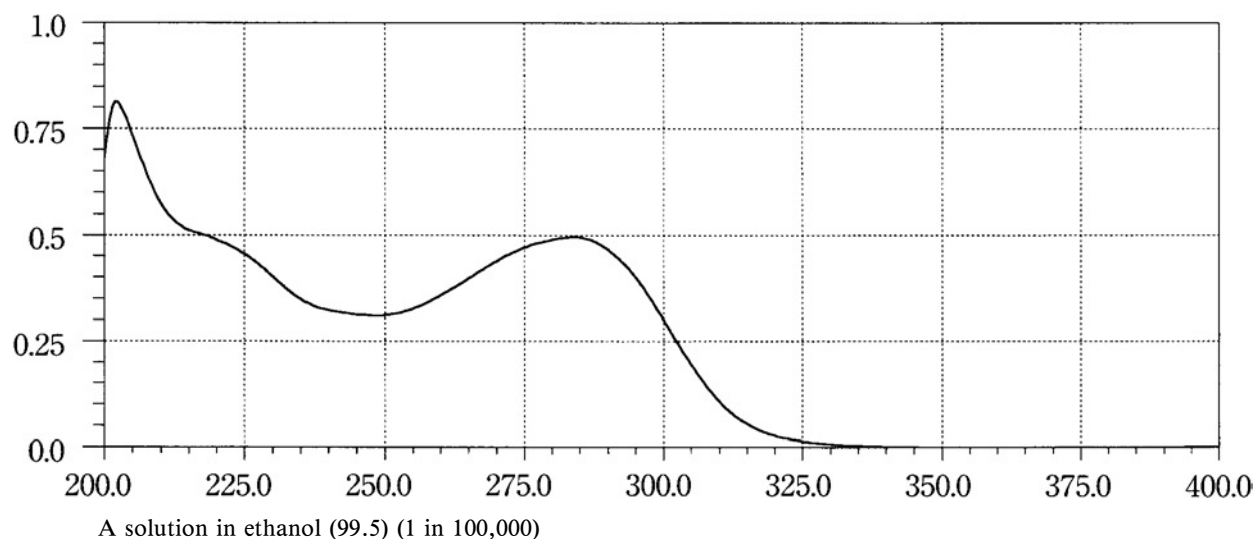
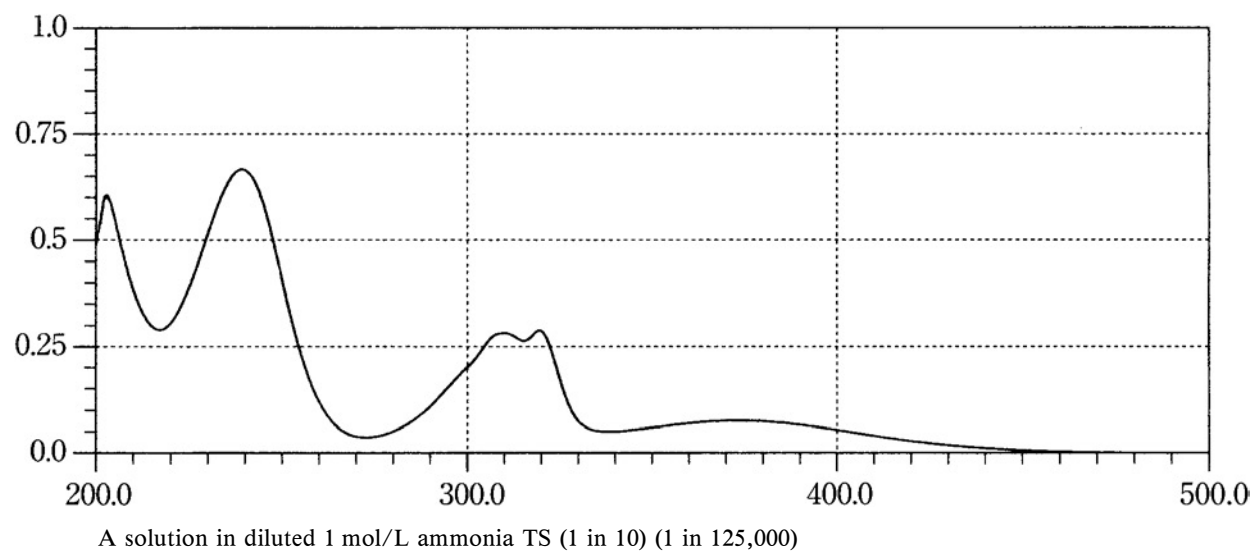
A solution prepared as follows: To 5 mL of a solution in 0.05 mol/L sulfuric acid TS (1 in 1000) add water to make 100 mL.

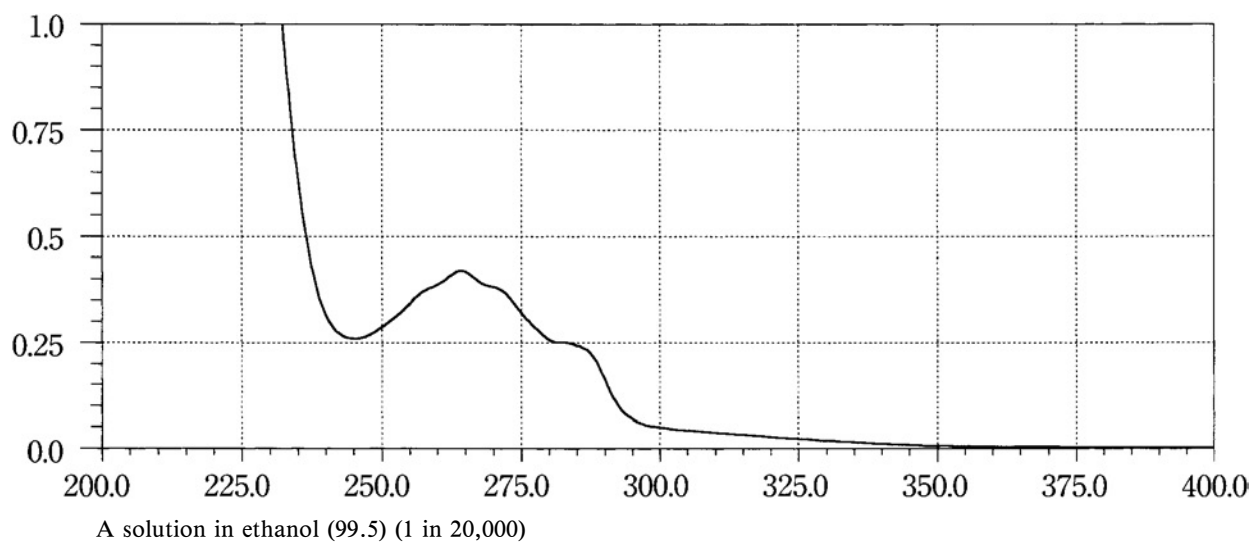
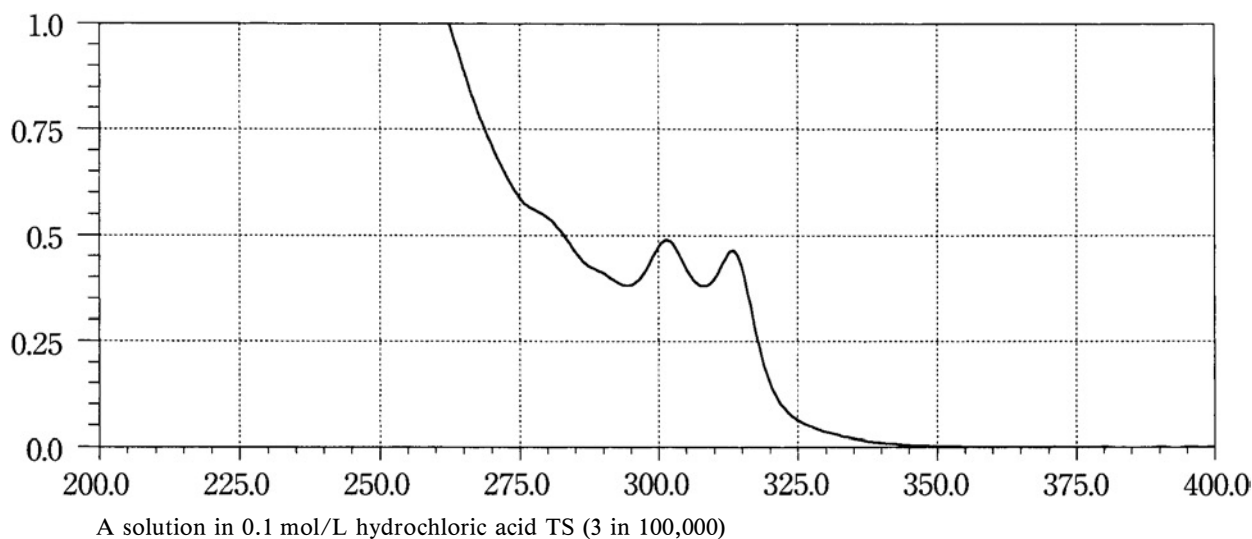
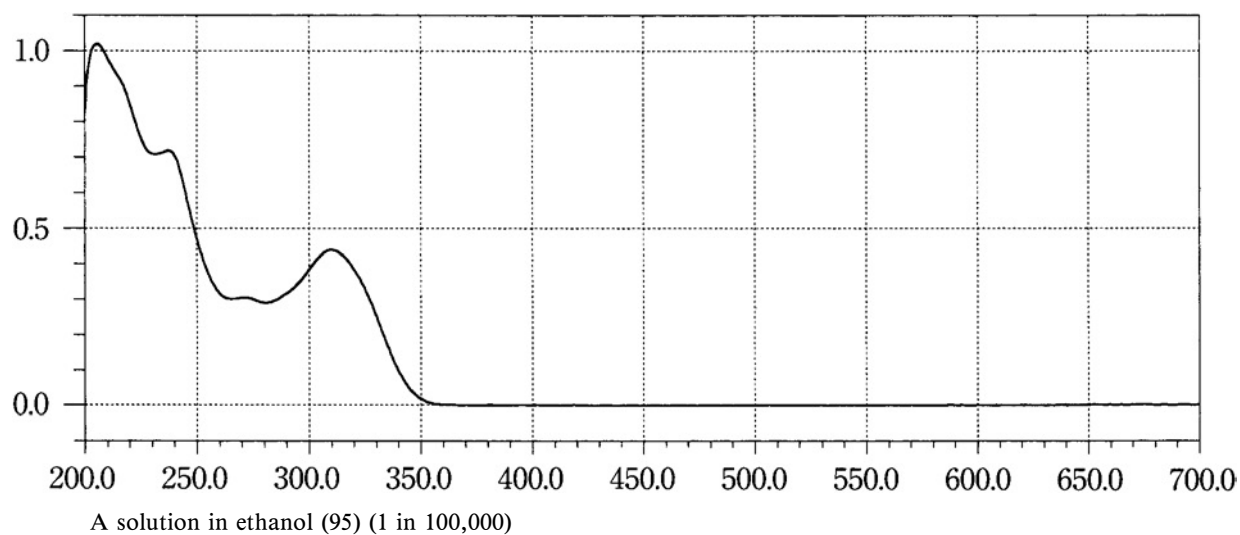
Sultiame**Tamsulosin Hydrochloride****Tegafur**

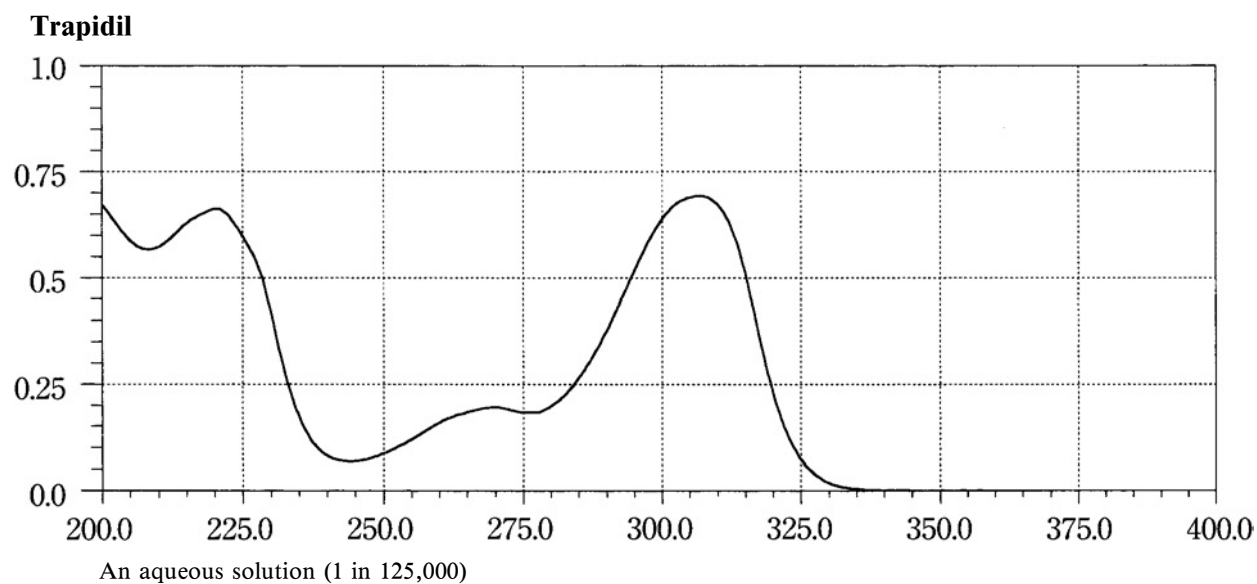
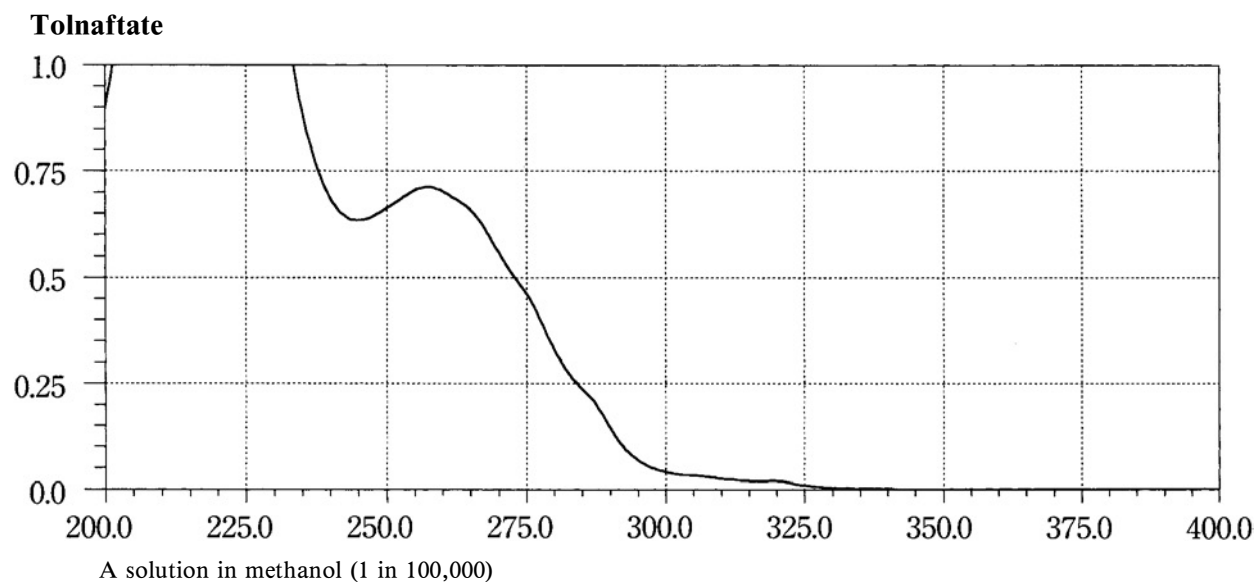
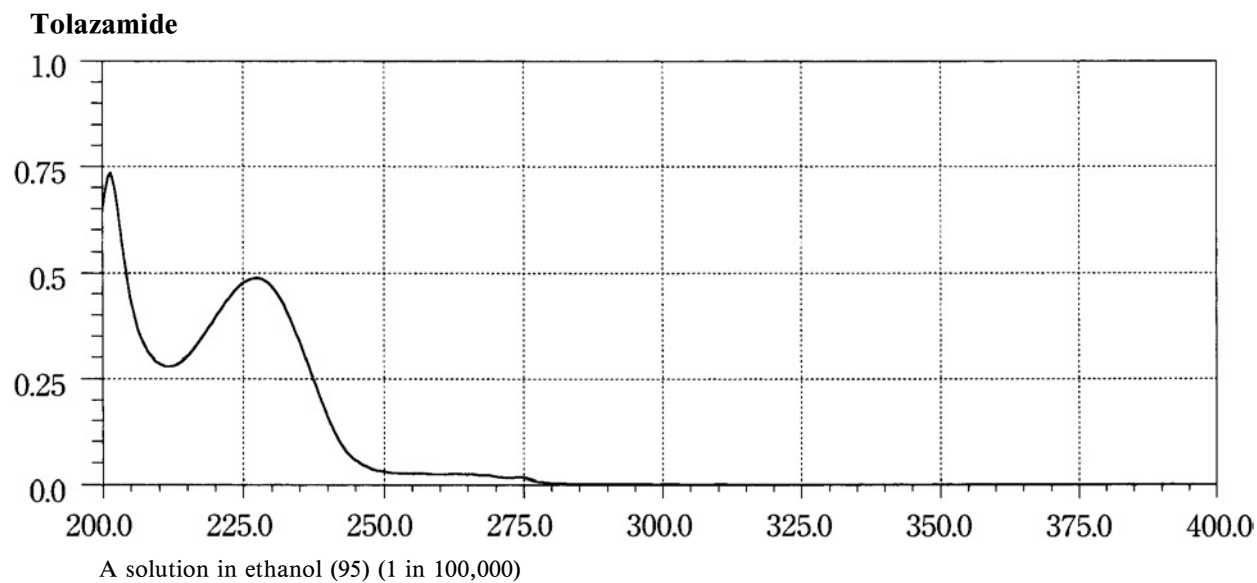
Terbutaline Sulfate**Testosterone Propionate****Tetracaine Hydrochloride**

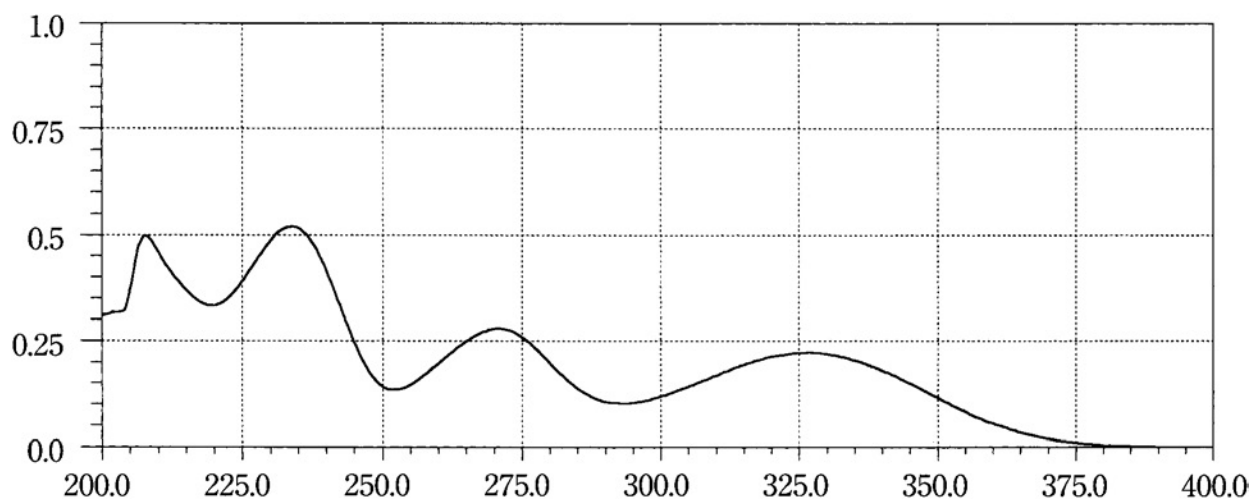
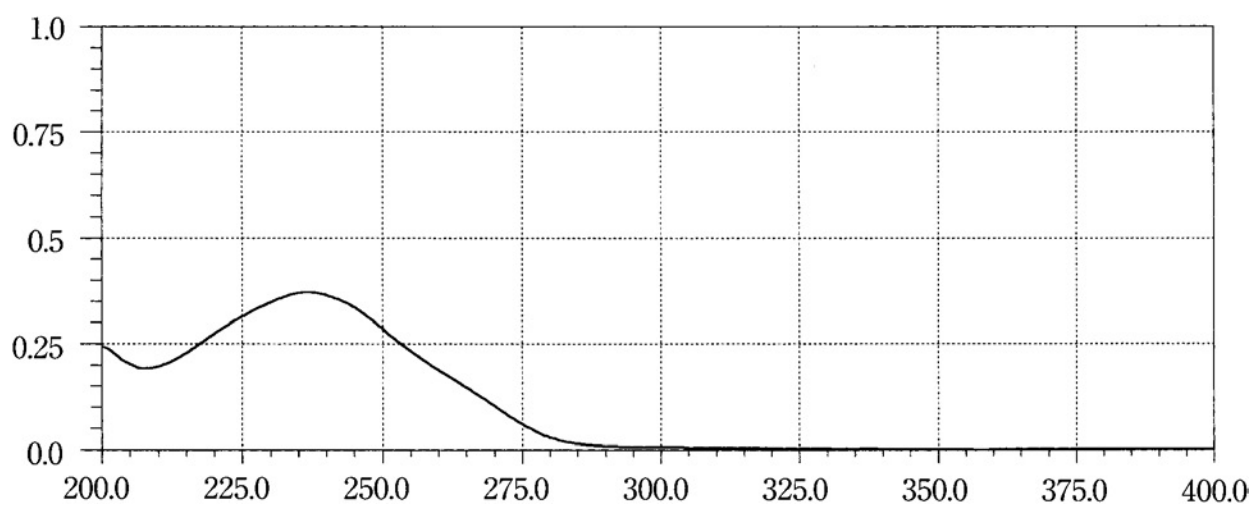
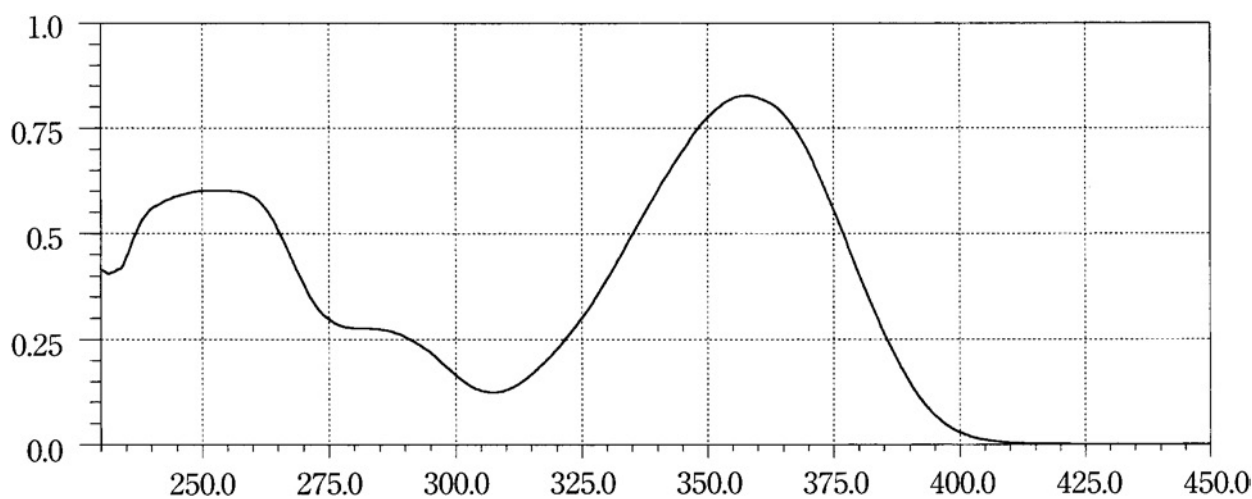
Tetracycline Hydrochloride**Theophylline****Thiamine Chloride Hydrochloride**

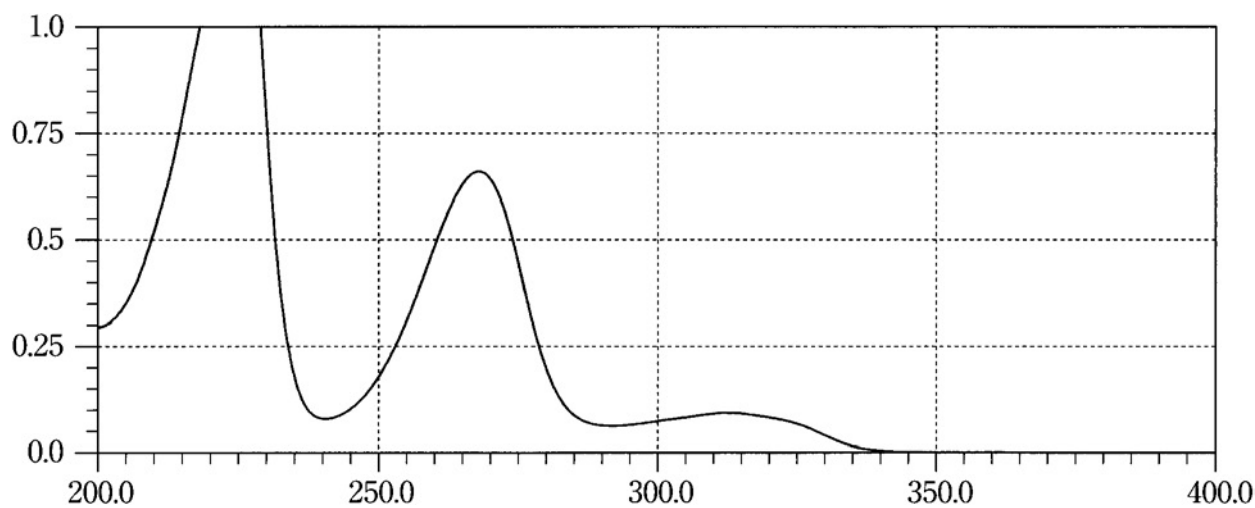
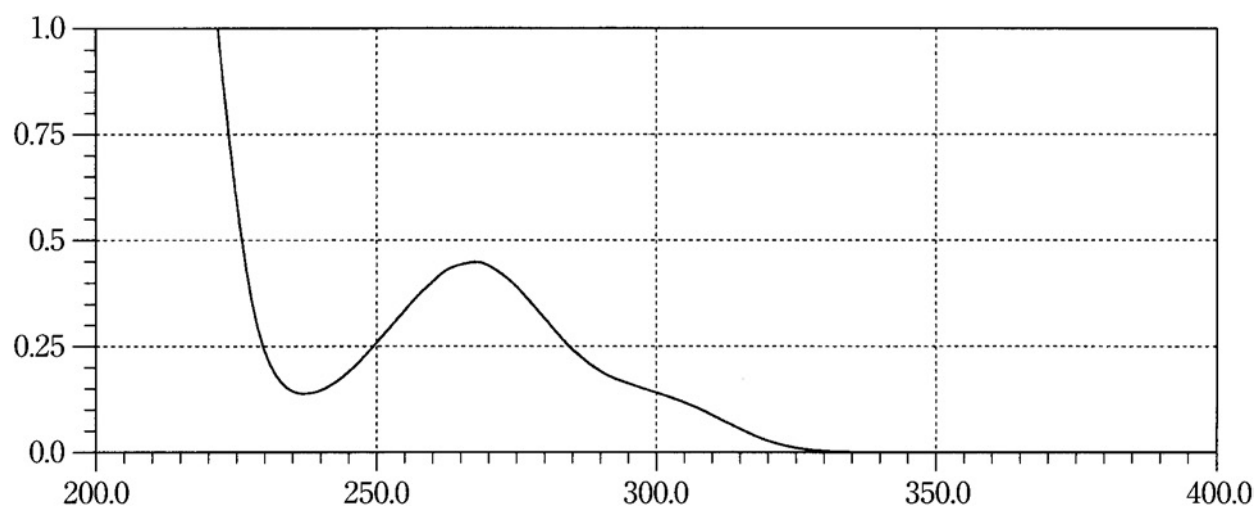
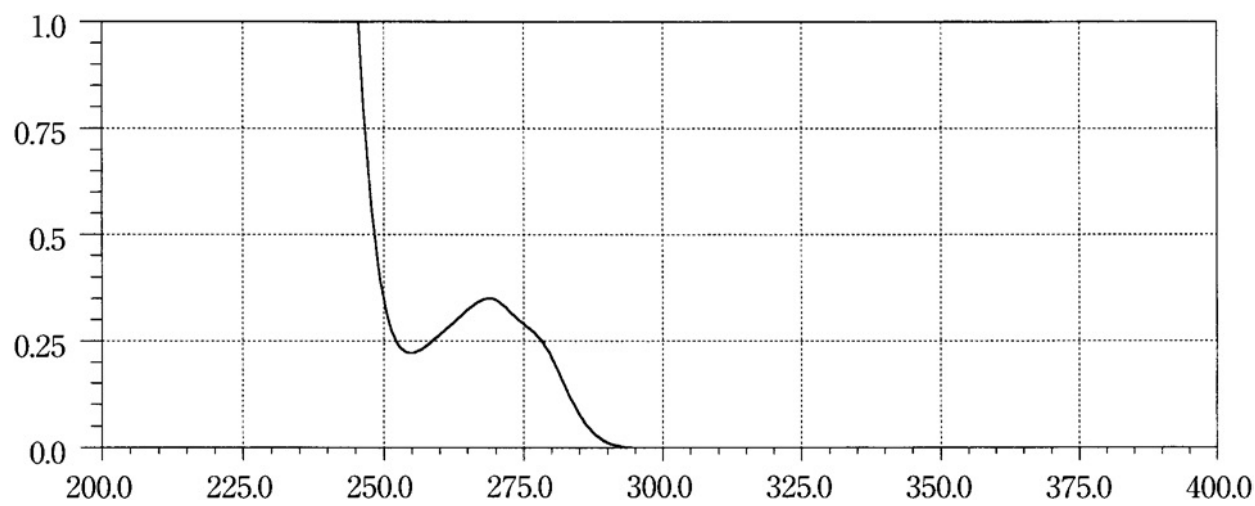
Thiamylal Sodium**Timepidium Bromide Hydrate****Timolol Maleate**

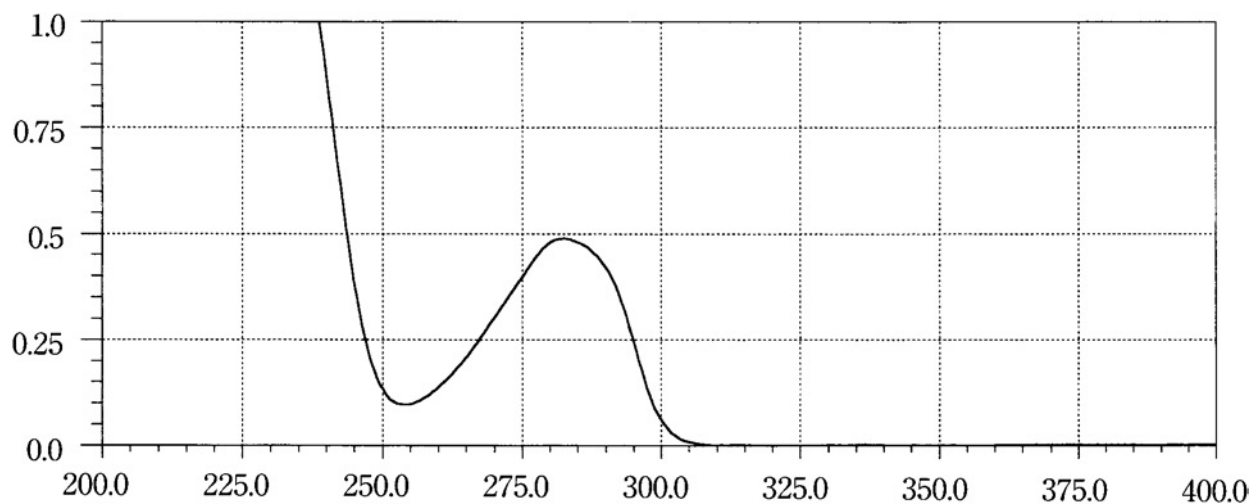
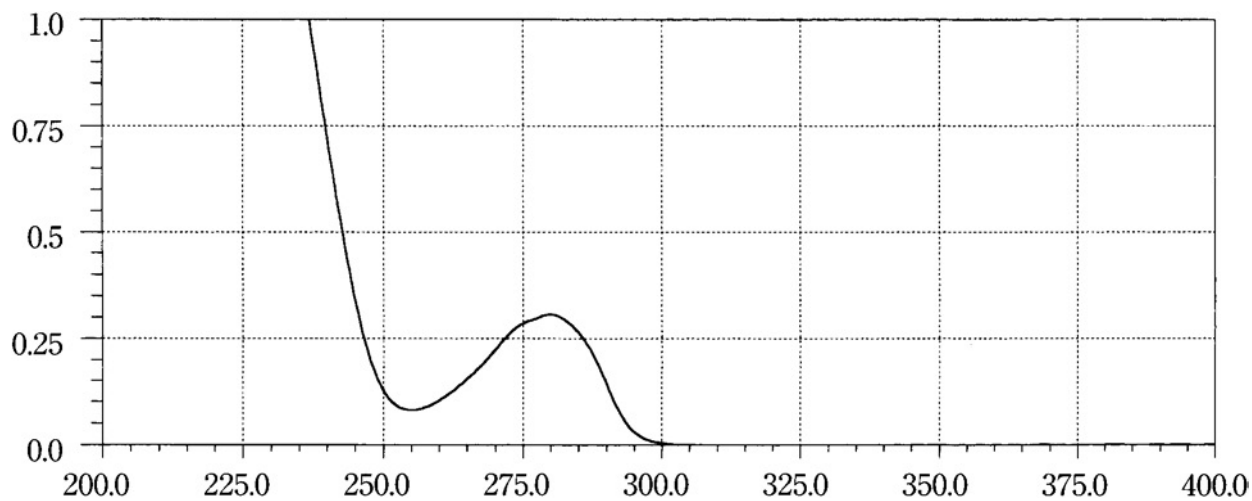
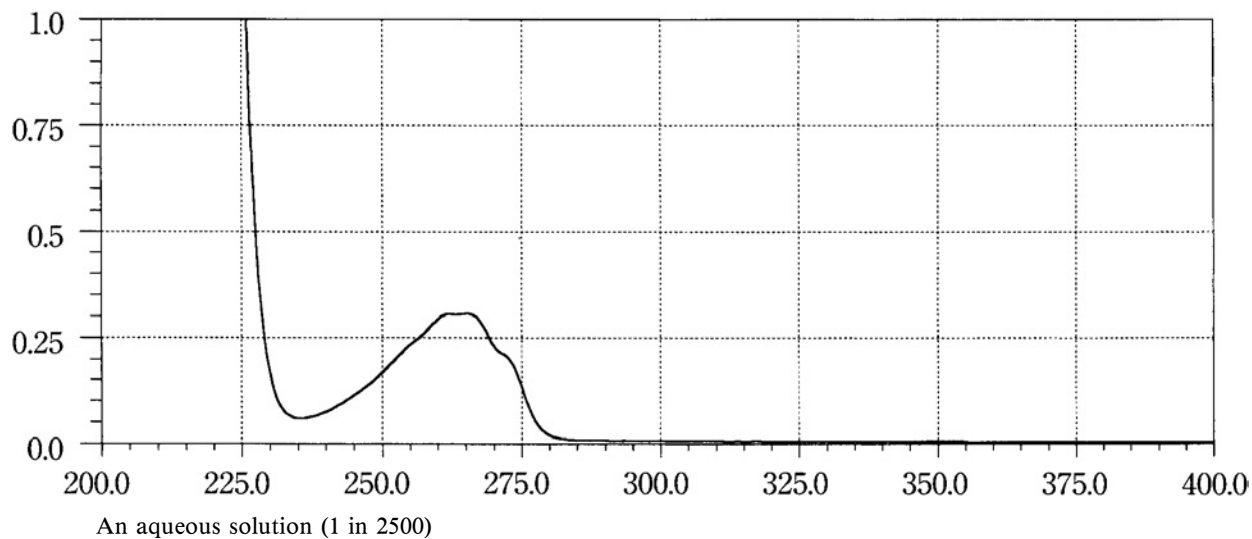
Tinidazole**Tipepidine Hibenzate****Tizanidine Hydrochloride**

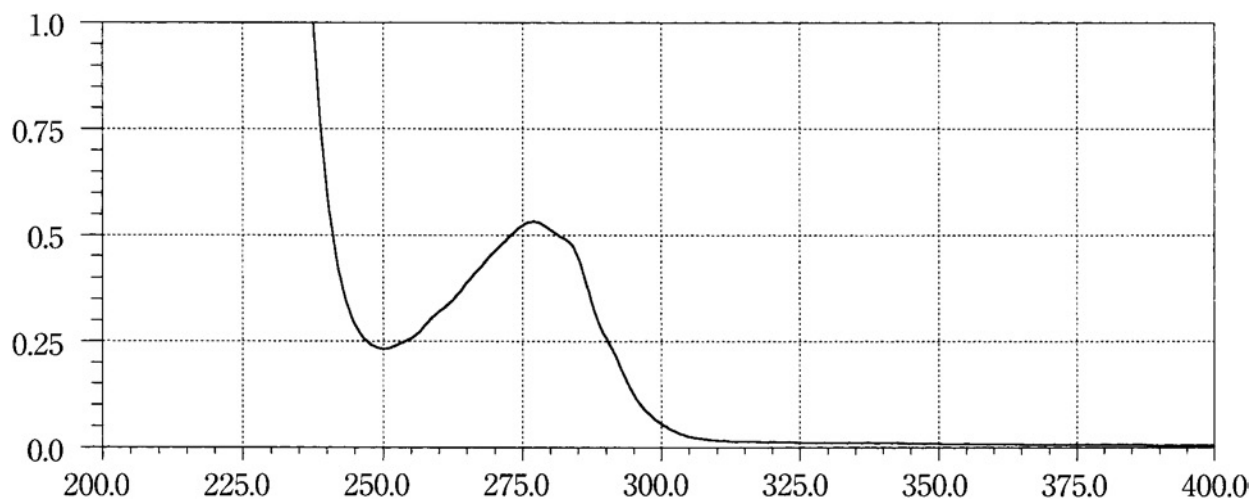
Tocopherol Nicotinate**Todalazine Hydrochloride Hydrate****Tofisopam**



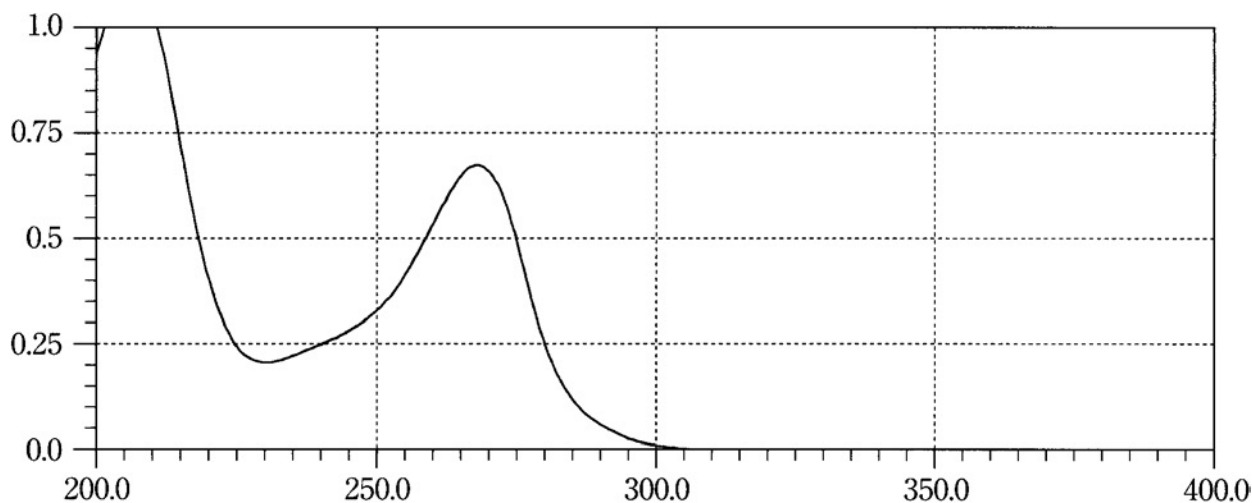
Trepibutone**Triamcinolone Acetonide****Triamterene**

Trichlormethiazide**Trimebutine Maleate****Trimetazidine Hydrochloride**

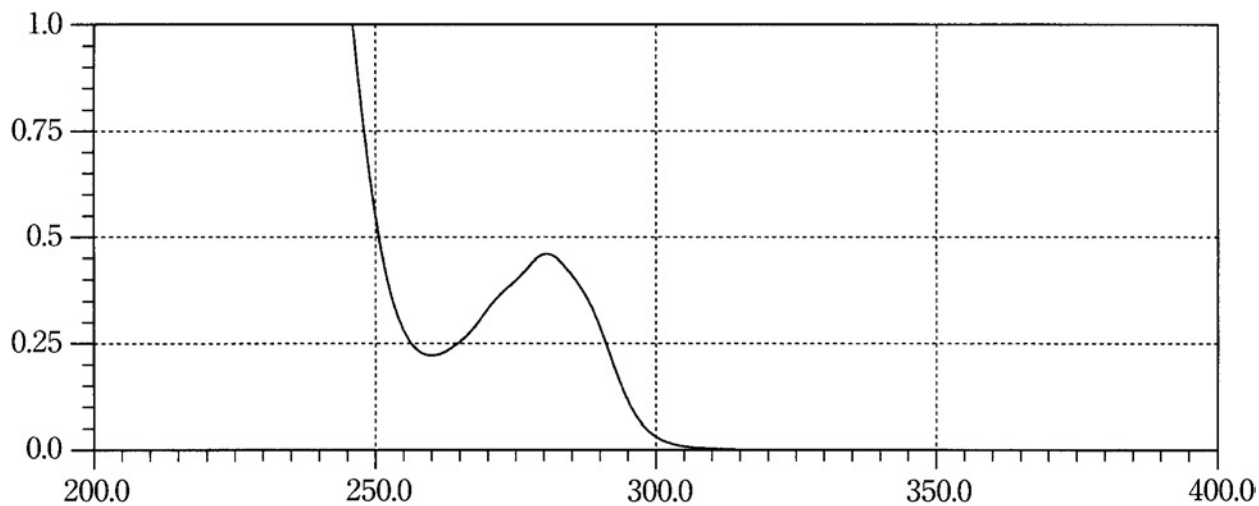
Trimetoquinol Hydrochloride Hydrate**Tubocurarine Chloride Hydrochloride Hydrate****Tulobuterol Hydrochloride**

Ulinastatin

An aqueous solution 2000 units/mL

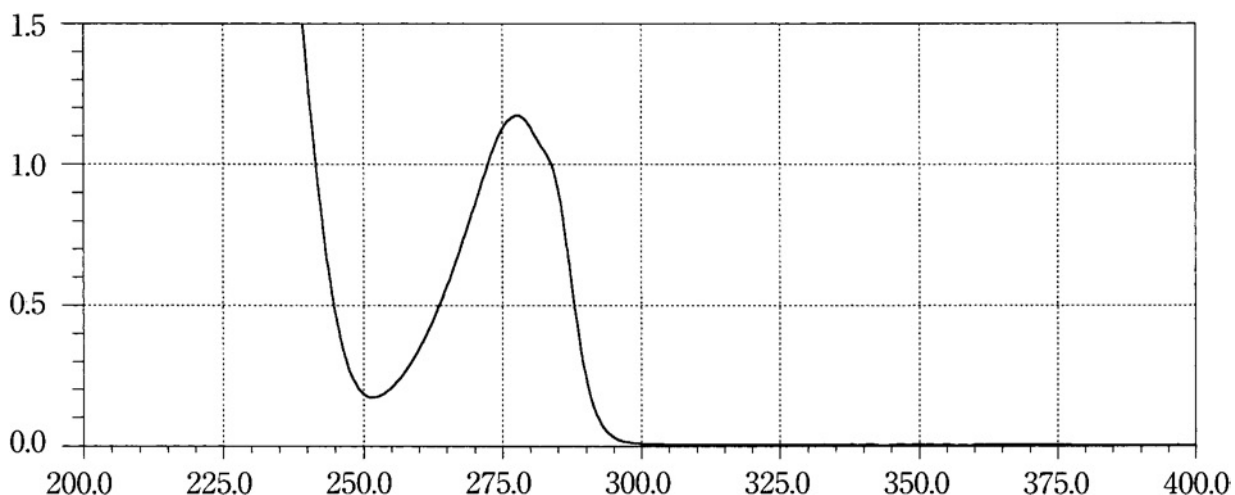
Urapidil

A solution in ethanol (95) (1 in 100,000)

Vancomycin Hydrochloride

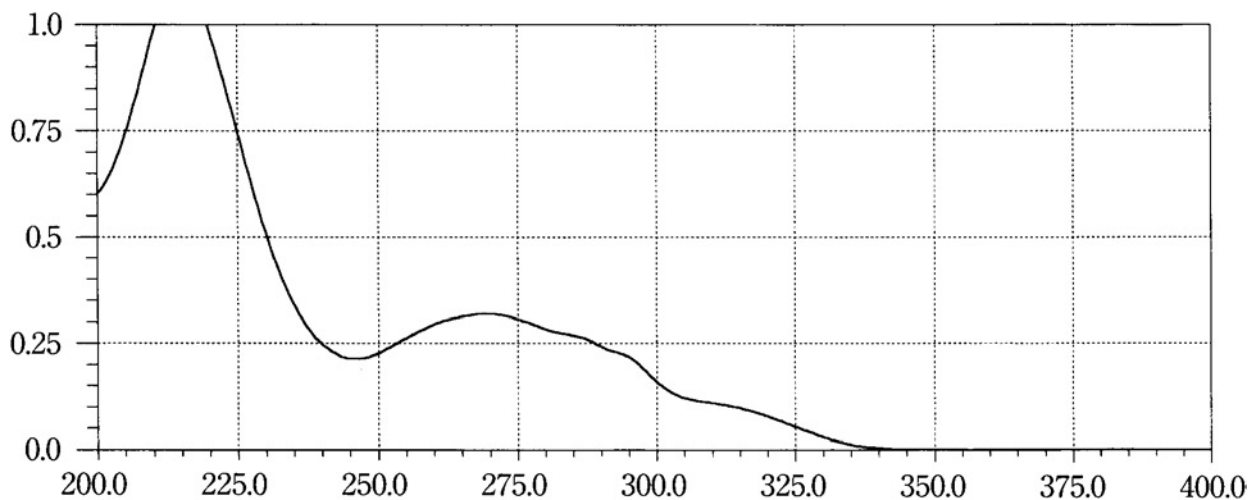
An aqueous solution (1 in 10,000)

Verapamil Hydrochloride



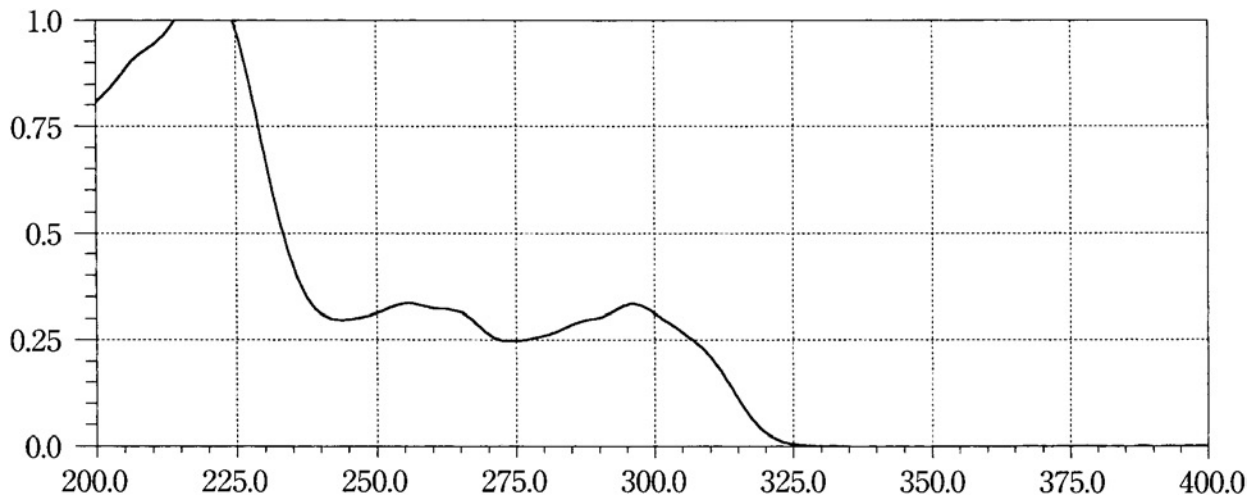
A solution in 0.01 mol/L hydrochloric acid TS (1 in 50,000)

Vinblastine Sulfate

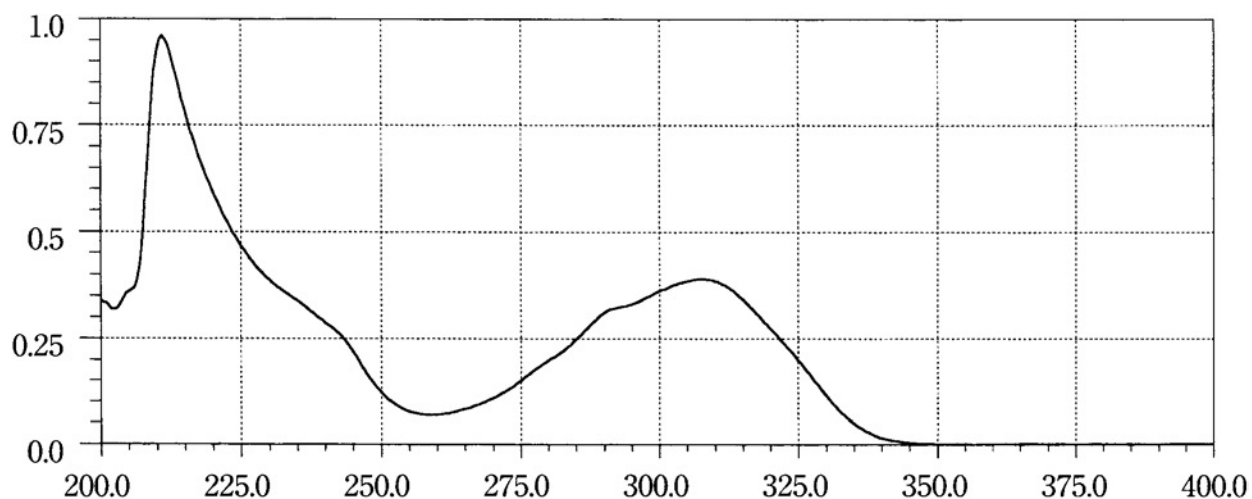


An aqueous solution (1 in 50,000)

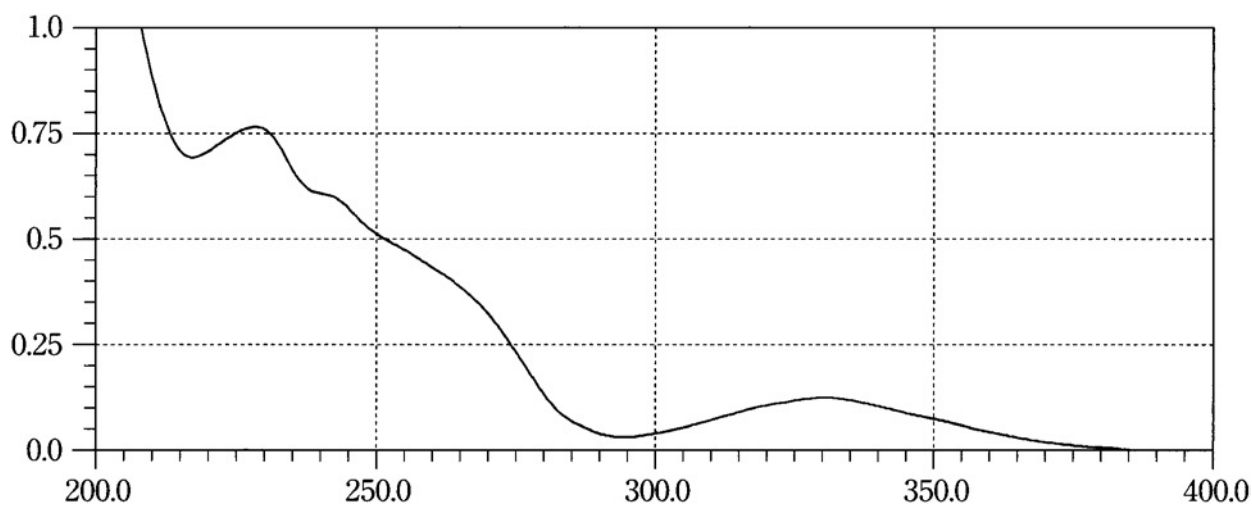
Vincristine Sulfate



An aqueous solution (1 in 50,000)

Warfarin Potassium

A solution in 0.02 mol/L potassium hydroxide TS (1 in 100,000)

Zaltoprofen

A solution in ethanol (99.5) (1 in 100,000)

GENERAL INFORMATION

1. Amino Acid Analysis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

Amino acid analysis refers to the methodology used to determine the amino acid composition or content of proteins, peptides, and other pharmaceutical preparations. Proteins and peptides are macromolecules consisting of covalently bonded amino acid residues organized as a linear polymer. The sequence of the amino acids in a protein or peptide determines the properties of the molecule. Proteins are considered large molecules that commonly exist as folded structures with a specific conformation, while peptides are smaller and may consist of only a few amino acids. Amino acid analysis can be used to quantify protein and peptides, to determine the identity of proteins or peptides based on their amino acid composition, to support protein and peptide structure analysis, to evaluate fragmentation strategies for peptide mapping, and to detect atypical amino acids that might be present in a protein or peptide. It is necessary to hydrolyze a protein/peptide to its individual amino acid constituents before amino acid analysis. Following protein/peptide hydrolysis, the amino acid analysis procedure can be the same as that practiced for free amino acids in other pharmaceutical preparations. The amino acid constituents of the test sample are typically derivatized for analysis.

Apparatus

Methods used for amino acid analysis are usually based on a chromatographic separation of the amino acids present in the test sample. Current techniques take advantage of the automated chromatographic instrumentation designed for analytical methodologies. An amino acid analysis instrument will typically be a low-pressure or high-pressure liquid chromatograph capable of generating mobile phase gradients that separate the amino acid analytes on a chromatographic column. The instrument must have postcolumn derivatization capability, unless the sample is analyzed using precolumn derivatization. The detector is usually an ultraviolet-visible or fluorescence detector depending on the derivatization method used. A recording device (e.g., integrator) is used for transforming the analog signal from the detector and for quantitation. It is preferred that instrumentation be dedicated particularly for amino acid analysis.

General Precautions

Background contamination is always a concern for the analyst in performing amino acid analysis. High purity reagents are necessary (e.g., low purity hydrochloric acid can contribute to glycine contamination). Analytical reagents are changed routinely every few weeks using only high-pressure liquid chromatography (HPLC) grade solvents. Potential microbial contamination and foreign material that might be present in the solvents are reduced by filtering solvents before

use, keeping solvent reservoirs covered, and not placing amino acid analysis instrumentation in direct sunlight.

Laboratory practices can determine the quality of the amino acid analysis. Place the instrumentation in a low traffic area of the laboratory. Keep the laboratory clean. Clean and calibrate pipets according to a maintenance schedule. Keep pipet tips in a covered box; the analysts may not handle pipet tips with their hands. The analysts may wear powder-free latex or equivalent gloves. Limit the number of times a test sample vial is opened and closed because dust can contribute to elevated levels of glycine, serine, and alanine.

A well-maintained instrument is necessary for acceptable amino acid analysis results. If the instrument is used on a routine basis, it is to be checked daily for leaks, detector and lamp stability, and the ability of the column to maintain resolution of the individual amino acids. Clean or replace all instrument filters and other maintenance items on a routine schedule.

Reference Standard Material

Acceptable amino acid standards are commercially available for amino acid analysis and typically consist of an aqueous mixture of amino acids. When determining amino acid composition, protein or peptide standards are analyzed with the test material as a control to demonstrate the integrity of the entire procedure. Highly purified bovine serum albumin has been used as a protein standard for this purpose.

Calibration of Instrumentation

Calibration of amino acid analysis instrumentation typically involves analyzing the amino acid standard, which consists of a mixture of amino acids at a number of concentrations, to determine the response factor and range of analysis for each amino acid. The concentration of each amino acid in the standard is known. In the calibration procedure, the analyst dilutes the amino acid standard to several different analyte levels within the expected linear range of the amino acid analysis technique. Then, replicates at each of the different analyte levels can be analyzed. Peak areas obtained for each amino acid are plotted versus the known concentration for each of the amino acids in the standard dilution. These results will allow the analyst to determine the range of amino acid concentrations where the peak area of a given amino acid is an approximately linear function of the amino acid concentration. It is important that the analyst prepare the samples for amino acid analysis so that they are within the analytical limits (e.g., linear working range) of the technique employed in order to obtain accurate and repeatable results.

Four to six amino acid standard levels are analyzed to determine a response factor for each amino acid. The response factor is calculated as the average peak area or peak height per nmol of amino acid present in the standard. A calibration file consisting of the response factor for each amino acid is prepared and used to calculate the concentration of each amino acid present in the test sample. This calculation involves dividing the peak area corresponding to a given ami-

no acid by the response factor for that amino acid to give the nmol of the amino acid. For routine analysis, a single-point calibration may be sufficient; however, the calibration file is updated frequently and tested by the analysis of analytical controls to ensure its integrity.

Repeatability

Consistent high quality amino acid analysis results from an analytical laboratory require attention to the repeatability of the assay. During analysis of the chromatographic separation of the amino acids or their derivatives, numerous peaks can be observed on the chromatogram that correspond to the amino acids. The large number of peaks makes it necessary to have an amino acid analysis system that can repeatedly identify the peaks based on retention time and integrate the peak areas for quantitation. A typical repeatability evaluation involves preparing a standard amino acid solution and analyzing many replicates (i.e., six analyses or more) of the same standard solution. The relative standard deviation (RSD) is determined for the retention time and integrated peak area of each amino acid. An evaluation of the repeatability is expanded to include multiple assays conducted over several days by different analysts. Multiple assays include the preparation of standard dilutions from starting materials to determine the variation due to sample handling. Often the amino acid composition of a standard protein (e.g., bovine serum albumin) is analyzed as part of the repeatability evaluation. By evaluating the replicate variation (i.e., RSD), the laboratory can establish analytical limits to ensure that the analyses from the laboratory are under control. It is desirable to establish the lowest practical variation limits to ensure the best results. Areas to focus on to lower the variability of the amino acid analysis include sample preparation, high background spectral interference due to quality of reagents and/or laboratory practices, instrument performance and maintenance, data analysis and interpretation, and analyst performance and habits. All parameters involved are fully investigated in the scope of the validation work.

Sample Preparation

Accurate results from amino acid analysis require purified protein and peptide samples. Buffer components (e.g., salts, urea, detergents) can interfere with the amino acid analysis and are removed from the sample before analysis. Methods that utilize postcolumn derivatization of the amino acids are generally not affected by buffer components to the extent seen with precolumn derivatization methods. It is desirable to limit the number of sample manipulations to reduce potential background contamination, to improve analyte recovery, and to reduce labor. Common techniques used to remove buffer components from protein samples include the following methods: (1) injecting the protein sample onto a reversed-phase HPLC system, eluting the protein with a volatile solvent containing a sufficient organic component, and drying the sample in a vacuum centrifuge; (2) dialysis against a volatile buffer or water; (3) centrifugal ultrafiltration for buffer replacement with a volatile buffer or water; (4) precipitating the protein from the buffer using an organic solvent (e.g., acetone); and (5) gel filtration.

Internal Standards

It is recommended that an internal standard be used to monitor physical and chemical losses and variations during amino acid analysis. An accurately known amount of internal

standard can be added to a protein solution prior to hydrolysis. The recovery of the internal standard gives the general recovery of the amino acids of the protein solution. Free amino acids, however, do not behave in the same way as protein-bound amino acids during hydrolysis because their rates of release or destruction are variable. Therefore, the use of an internal standard to correct for losses during hydrolysis may give unreliable results. It will be necessary to take this point under consideration when interpreting the results. Internal standards can also be added to the mixture of amino acids after hydrolysis to correct for differences in sample application and changes in reagent stability and flow rates. Ideally, an internal standard is an unnaturally occurring primary amino acid that is commercially available and inexpensive. It should also be stable during hydrolysis, its response factor should be linear with concentration, and it needs to elute with a unique retention time without overlapping other amino acids. Commonly used amino acid standards include norleucine, nitrotyrosine, and α -aminobutyric acid.

Protein Hydrolysis

Hydrolysis of protein and peptide samples is necessary for amino acid analysis of these molecules. The glassware used for hydrolysis must be very clean to avoid erroneous results. Glove powders and fingerprints on hydrolysis tubes may cause contamination. To clean glass hydrolysis tubes, boil tubes for 1 hour in 1 mol/L hydrochloric acid or soak tubes in concentrated nitric acid or in a mixture of concentrated hydrochloric acid and concentrated nitric acid (1:1). Clean hydrolysis tubes are rinsed with high-purity water followed by a rinse with HPLC grade methanol, dried overnight in an oven, and stored covered until use. Alternatively, pyrolysis of clean glassware at 500°C for 4 hours may also be used to eliminate contamination from hydrolysis tubes. Adequate disposable laboratory material can also be used.

Acid hydrolysis is the most common method for hydrolyzing a protein sample before amino acid analysis. The acid hydrolysis technique can contribute to the variation of the analysis due to complete or partial destruction of several amino acids. Tryptophan is destroyed; serine and threonine are partially destroyed; methionine might undergo oxidation; and cysteine is typically recovered as cystine (but cystine recovery is usually poor because of partial destruction or reduction to cysteine). Application of adequate vacuum (\leq less than 200 μ m of mercury or 26.7 Pa) or introduction of an inert gas (argon) in the headspace of the reaction vessel can reduce the level of oxidative destruction. In peptide bonds involving isoleucine and valine the amido bonds of Ile-Ile, Val-Val, Ile-Val, and Val-Ile are partially cleaved; and asparagine and glutamine are deamidated, resulting in aspartic acid and glutamic acid, respectively. The loss of tryptophan, asparagine, and glutamine during an acid hydrolysis limits quantitation to 17 amino acids. Some of the hydrolysis techniques described are used to address these concerns. Some of the hydrolysis techniques described (i.e., *Methods 4-11*) may cause modifications to other amino acids. Therefore, the benefits of using a given hydrolysis technique are weighed against the concerns with the technique and are tested adequately before employing a method other than acid hydrolysis.

A time-course study (i.e., amino acid analysis at acid hydrolysis times of 24, 48, and 72 hours) is often employed to analyze the starting concentration of amino acids that are

partially destroyed or slow to cleave. By plotting the observed concentration of labile amino acids (i.e., serine and threonine) versus hydrolysis time, the line can be extrapolated to the origin to determine the starting concentration of these amino acids. Time-course hydrolysis studies are also used with amino acids that are slow to cleave (e.g., isoleucine and valine). During the hydrolysis time course, the analyst will observe a plateau in these residues. The level of this plateau is taken as the residue concentration. If the hydrolysis time is too long, the residue concentration of the sample will begin to decrease, indicating destruction by the hydrolysis conditions.

An acceptable alternative to the time-course study is to subject an amino acid calibration standard to the same hydrolysis conditions as the test sample. The amino acid in free form may not completely represent the rate of destruction of labile amino acids within a peptide or protein during the hydrolysis. This is especially true for peptide bonds that are slow to cleave (e.g., Ile-Val bonds). However, this technique will allow the analyst to account for some residue destruction. Microwave acid hydrolysis has been used and is rapid but requires special equipment as well as special precautions. The optimal conditions for microwave hydrolysis must be investigated for each individual protein/peptide sample. The microwave hydrolysis technique typically requires only a few minutes, but even a deviation of one minute may give inadequate results (e.g., incomplete hydrolysis or destruction of labile amino acids). Complete proteolysis, using a mixture of proteases, has been used but can be complicated, requires the proper controls, and is typically more applicable to peptides than proteins.

Note: During initial analyses of an unknown protein, experiments with various hydrolysis time and temperature conditions are conducted to determine the optimal conditions.

Method 1

Acid hydrolysis using hydrochloric acid containing phenol is the most common procedure used for protein/peptide hydrolysis preceding amino acid analysis. The addition of phenol to the reaction prevents the halogenation of tyrosine.

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol.

Procedure—

Liquid Phase Hydrolysis Place the protein or peptide sample in a hydrolysis tube, and dry. [Note: The sample is dried so that water in the sample will not dilute the acid used for the hydrolysis.] Add 200 μL of *Hydrolysis Solution* per 500 μg of lyophilized protein. Freeze the sample tube in a dry ice-acetone bath, and flame seal in vacuum. Samples are typically hydrolyzed at 110°C for 24 hours in vacuum or inert atmosphere to prevent oxidation. Longer hydrolysis times (e.g., 48 and 72 hours) are investigated if there is a concern that the protein is not completely hydrolyzed.

Vapor Phase Hydrolysis This is one of the most common acid hydrolysis procedures, and it is preferred for microanalysis when only small amounts of the sample are available. Contamination of the sample from the acid reagent is also minimized by using vapor phase hydrolysis. Place vials containing the dried samples in a vessel that contains an appropriate amount of *Hydrolysis Solution*. The *Hydrolysis Solution* does not come in contact with the test sample. Apply an inert atmosphere or vacuum (\leq less than 200 μm of

mercury or 26.7 Pa) to the headspace of the vessel, and heat to about 110°C for a 24-hour hydrolysis time. Acid vapor hydrolyzes the dried sample. Any condensation of the acid in the sample vials is minimized. After hydrolysis, dry the test sample in vacuum to remove any residual acid.

Method 2

Tryptophan oxidation during hydrolysis is decreased by using mercaptoethanesulfonic acid (MESA) as the reducing acid.

Hydrolysis Solution 2.5 mol/L MESA solution.

Vapor Phase Hydrolysis About 1 to 100 μg of the protein/peptide under test is dried in a hydrolysis tube. The hydrolysis tube is placed in a larger tube with about 200 μL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the *Hydrolysis Solution*. The hydrolysis tube is heated to 170°C to 185°C for about 12.5 minutes. After hydrolysis, the hydrolysis tube is dried in vacuum for 15 minutes to remove the residual acid.

Method 3

Tryptophan oxidation during hydrolysis is prevented by using thioglycolic acid (TGA) as the reducing acid.

Hydrolysis Solution A solution containing 7 mol/L hydrochloric acid, 10% of trifluoroacetic acid, 20% of thioglycolic acid, and 1% of phenol.

Vapor Phase Hydrolysis About 10 to 50 μg of the protein/peptide under test is dried in a sample tube. The sample tube is placed in a larger tube with about 200 μL of the *Hydrolysis Solution*. The larger tube is sealed in vacuum (about 50 μm of mercury or 6.7 Pa) to vaporize the TGA. The sample tube is heated to 166°C for about 15 to 30 minutes. After hydrolysis, the sample tube is dried in vacuum for 5 minutes to remove the residual acid. Recovery of tryptophan by this method may be dependent on the amount of sample present.

Method 4

Cysteine-cystine and methionine oxidation is performed with performic acid before the protein hydrolysis.

Oxidation Solution The performic acid is prepared fresh by mixing formic acid and 30 percent hydrogen peroxide (9:1), and incubated at room temperature for 1 hour.

Procedure The protein/peptide sample is dissolved in 20 μL of formic acid, and heated at 50°C for 5 minutes; then 100 μL of the *Oxidation Solution* is added. The oxidation is allowed to proceed for 10 to 30 minutes. In this reaction, cysteine is converted to cysteic acid and methionine is converted to methionine sulfone. The excess reagent is removed from the sample in a vacuum centrifuge. This technique may cause modifications to tyrosine residues in the presence of halides. The oxidized protein can then be acid hydrolyzed using *Method 1* or *Method 2*.

Method 5

Cysteine-cystine oxidation is accomplished during the liquid phase hydrolysis with sodium azide.

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.2% of phenol, to which is added sodium azide to obtain a final concentration of 0.2% (w/v). The added phenol prevents halogenation of tyrosine.

Liquid Phase Hydrolysis The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the sodium azide present in the *Hydrolysis So-*

lution. This technique allows better tyrosine recovery than *Method 4*, but it is not quantitative for methionine. Methionine is converted to a mixture of the parent methionine and its two oxidative products, methionine sulfoxide and methionine sulfone.

Method 6

Cysteine-cystine oxidation is accomplished with dimethyl sulfoxide (DMSO).

Hydrolysis Solution 6 mol/L hydrochloric acid containing 0.1% to 1.0% of phenol, to which DMSO is added to obtain a final concentration of 2% (v/v).

Vapor Phase Hydrolysis The protein/peptide hydrolysis is conducted at about 110°C for 24 hours. During the hydrolysis, the cysteine-cystine present in the sample is converted to cysteic acid by the DMSO present in the *Hydrolysis Solution*. As an approach to limit variability and compensate for partial destruction, it is recommended to evaluate the cysteic acid recovery from oxidative hydrolyses of standard proteins containing 1 to 8 mol of cysteine. The response factors from protein/peptide hydrolysates are typically about 30% lower than those for nonhydrolyzed cysteic acid standards. Because histidine, methionine, tyrosine, and tryptophan are also modified, a complete compositional analysis is not obtained with this technique.

Method 7

Cysteine-cystine reduction and alkylation is accomplished by a vapor phase pyridylethylation reaction.

Reducing Solution Transfer 83.3 μL of pyridine, 16.7 μL of 4-vinylpyridine, 16.7 μL of tributylphosphine, and 83.3 μL of water to a suitable container, and mix.

Procedure Add the protein/peptide (between 1 and 100 μg) to a hydrolysis tube, and place in a larger tube. Transfer the *Reducing Solution* to the large tube, seal in vacuum (about 50 μm of mercury or 6.7 Pa), and incubate at about 100°C for 5 minutes. Then remove the inner hydrolysis tube, and dry it in a vacuum desiccator for 15 minutes to remove residual reagents. The pyridylethylated protein/peptide can then be acid hydrolyzed using previously described procedures. The pyridylethylation reaction is performed simultaneously with a protein standard sample containing 1 to 8 mol of cysteine to improve accuracy in the pyridylethyl-cysteine recovery. Longer incubation times for the pyridylethylation reaction can cause modifications to the α -amino terminal group and the ϵ -amino group of lysine in the protein.

Method 8

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase pyridylethylation reaction.

Stock Solutions Prepare and filter three solutions: 1 mol/L Tris hydrochloride (pH 8.5) containing 4 mmol/L disodium dihydrogen ethylenediamine tetraacetate (*Stock Solution A*), 8 mol/L guanidine hydrochloride (*Stock Solution B*), and 10% of 2-mercaptoethanol in water (*Stock Solution C*).

Reducing Solution Prepare a mixture of *Stock Solution B* and *Stock Solution A* (3:1) to obtain a buffered solution of 6 mol/L guanidine hydrochloride in 0.25 mol/L Tris hydrochloride.

Procedure Dissolve about 10 μg of the test sample in 50 μL of the *Reducing Solution*, and add about 2.5 μL of *Stock Solution C*. Store under nitrogen or argon for 2 hours at

room temperature in the dark. To achieve the pyridylethylation reaction, add about 2 μL of 4-vinylpyridine to the protein solution, and incubate for an additional 2 hours at room temperature in the dark. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis.

Method 9

Cysteine-cystine reduction and alkylation is accomplished by a liquid phase carboxymethylation reaction.

Stock Solutions Prepare as directed for *Method 8*.

Carboxymethylation Solution Prepare a solution containing 100 mg of iodoacetamide per mL of ethanol (95).

Buffer Solution Use the *Reducing Solution*, prepared as directed for *Method 8*.

Procedure Dissolve the test sample in 50 μL of the *Buffer Solution*, and add about 2.5 μL of *Stock Solution C*. Store under nitrogen or argon for 2 hours at room temperature in the dark. Add the *Carboxymethylation Solution* in a ratio 1.5 fold per total theoretical content of thiols, and incubate for an additional 30 minutes at room temperature in the dark. [Note: If the thiol content of the protein is unknown, then add 5 μL of 100 mmol/L iodoacetamide for every 20 nmol of protein present.] The reaction is stopped by adding excess of 2-mercaptoethanol. The protein/peptide is desalted by collecting the protein/peptide fraction from a reversed-phase HPLC separation. The collected sample can be dried in a vacuum centrifuge before acid hydrolysis. The S-carboxyamidomethyl-cysteine formed will be converted to S-carboxymethylcysteine during acid hydrolysis.

Method 10

Cysteine-cystine is reacted with dithiodiglycolic acid or dithiodipropionic acid to produce a mixed disulfide. [Note: The choice of dithiodiglycolic acid or dithiodipropionic acid depends on the required resolution of the amino acid analysis method.]

Reducing Solution A solution containing 10 mg of dithiodiglycolic acid (or dithiodipropionic acid) per mL of 0.2 mol/L sodium hydroxide.

Procedure Transfer about 20 μg of the test sample to a hydrolysis tube, and add 5 μL of the *Reducing Solution*. Add 10 μL of isopropyl alcohol, and then remove all of the sample liquid by vacuum centrifugation. The sample is then hydrolyzed using *Method 1*. This method has the advantage that other amino acid residues are not derivatized by side reactions, and the sample does not need to be desalted prior to hydrolysis.

Method 11

Asparagine and glutamine are converted to aspartic acid and glutamic acid, respectively, during acid hydrolysis. Asparagine and aspartic acid residues are added and represented by *Asx*, while glutamine and glutamic acid residues are added and represented by *Glx*. Proteins/peptides can be reacted with bis(1,1-trifluoroacetoxy)iodobenzene (BTI) to convert the asparagine and glutamine residues to diaminopropionic acid and diaminobutyric acid residues, respectively, upon acid hydrolysis. These conversions allow the analyst to determine the asparagine and glutamine content of a protein/peptide in the presence of aspartic acid and glutamic acid residues.

Reducing Solutions Prepare and filter three solutions: a

solution of 10 mmol/L trifluoroacetic acid (*Solution A*), a solution of 5 mol/L guanidine hydrochloride and 10 mmol/L trifluoroacetic acid (*Solution B*), and a freshly prepared solution of *N,N*-dimethylformamide containing 36 mg of BTI per mL (*Solution C*).

Procedure In a clean hydrolysis tube, transfer about 200 μg of the test sample, and add 2 mL of *Solution A* or *Solution B* and 2 mL of *Solution C*. Seal the hydrolysis tube in vacuum. Heat the sample at 60°C for 4 hours in the dark. The sample is then dialyzed with water to remove the excess reagents. Extract the dialyzed sample three times with equal volumes of *n*-butyl acetate, and then lyophilize. The protein can then be acid hydrolyzed using previously described procedures. The α,β -diaminopropionic and α,γ -diaminobutyric acid residues do not typically resolve from the lysine residues upon ion-exchange chromatography based on amino acid analysis. Therefore, when using ion-exchange as the mode of amino acid separation, the asparagine and glutamine contents are the quantitative difference in the aspartic acid and glutamic acid content assayed with underivatized and BTI-derivatized acid hydrolysis. [Note: The threonine, methionine, cysteine, tyrosine, and histidine assayed content can be altered by BTI derivatization; a hydrolysis without BTI will have to be performed if the analyst is interested in the composition of these other amino acid residues of the protein/peptide.]

Methodologies of Amino Acid Analysis General Principles

Many amino acid analysis techniques exist, and the choice of any one technique often depends on the sensitivity required from the assay. In general, about one-half of the amino acid analysis techniques employed rely on the separation of the free amino acids by ion-exchange chromatography followed by postcolumn derivatization (e.g., with ninhydrin or *o*-phthalaldehyde). Postcolumn detection techniques can be used with samples that contain small amounts of buffer components, such as salts and urea, and generally require between 5 and 10 μg of protein sample per analysis. The remaining amino acid techniques typically involve precolumn derivatization of the free amino acids (e.g., phenyl isothiocyanate; 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate or *o*-phthalaldehyde; (dimethylamino)azobenzenesulfonyl chloride; 9-fluorenylmethylchloroformate; and, 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole) followed by reversed-phase HPLC. Precolumn derivatization techniques are very sensitive and usually require between 0.5 and 1.0 μg of protein sample per analysis but may be influenced by buffer salts in the samples. Precolumn derivatization techniques may also result in multiple derivatives of a given amino acid, which complicates the result interpretation. Postcolumn derivatization techniques are generally influenced less by performance variation of the assay than precolumn derivatization techniques.

The following *Methods* may be used for quantitative amino acid analysis. Instruments and reagents for these procedures are available commercially. Furthermore, many modifications of these methodologies exist with different reagent preparations, reaction procedures, chromatographic systems, etc. Specific parameters may vary according to the exact equipment and procedure used. Many laboratories will utilize more than one amino acid analysis technique to exploit the advantages offered by each. In each of these *Methods*, the analog signal is visualized by means of a data acquisition sys-

tem, and the peak areas are integrated for quantification purposes.

Method 1—Postcolumn Ninhydrin Detection General Principle

Ion-exchange chromatography with postcolumn ninhydrin detection is one of the most common methods employed for quantitative amino acid analysis. As a rule, a Li-based cation-exchange system is employed for the analysis of the more complex physiological samples, and the faster Na-based cation-exchange system is used for the more simplistic amino acid mixtures obtained with protein hydrolysates (typically containing 17 amino acid components). Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. A temperature gradient is often employed to enhance separation.

When the amino acid reacts with ninhydrin, the reactant has characteristic purple or yellow color. Amino acids, except imino acid, give a purple color, and show the maximum absorption at 570 nm. The imino acids such as proline give a yellow color, and show the maximum absorption at 440 nm. The postcolumn reaction between ninhydrin and amino acid eluted from column is monitored at 440 and 570 nm, and the chromatogram obtained is used for the determination of amino acid composition.

Detection limit is considered to be 10 pmol for most of the amino acid derivatives, but 50 pmol for proline. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good composition data, samples larger than 1 μg before hydrolysis are best suited for this amino acid analysis of protein/peptide.

Method 2—Postcolumn OPA Fluorometric Detection General Principle

o-Phthalaldehyde (OPA) reacts with primary amines in the presence of thiol compound, to form highly fluorescent isoindole products. This reaction is utilized for the postcolumn derivatization in analysis of amino acids by ion-exchange chromatography. The rule of the separation is the same as *Method 1*. Instruments and reagents for this form of amino acid analysis are available commercially. Many modifications of this methodology exist.

Although OPA does not react with secondary amines (imino acids such as proline) to form fluorescent substances, the oxidation with sodium hypochlorite allows secondary amines to react with OPA. The procedure employs a strongly acidic cation-exchange column for separation of free amino acids followed by postcolumn oxidation with sodium hypochlorite and postcolumn derivatization using OPA and thiol compound such as *N*-acetyl-L-cysteine and 2-mercaptoethanol. The derivatization of primary amino acids are not noticeably affected by the continuous supply of sodium hypochlorite.

Separation of the amino acids on an ion-exchange column is accomplished through a combination of changes in pH and cation strength. After postcolumn derivatization of eluted amino acids with OPA, the reactant passes through the fluorometric detector. Fluorescence intensity of OPA-derivatized amino acids are monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limit is considered to be a few tens of picomole level for most of the amino acid derivatives. Response lineari-

ty is obtained in the range of a few picomole level to a few tens of nanomole level. To obtain good compositional data, the starting with greater than 500 ng of sample before hydrolysis is best suited for the amino acid analysis of protein/peptide.

Method 3—Precolumn PITC Derivatization General Principle

Phenylisothiocyanate (PITC) reacts with amino acids to form phenylthiocarbamyl (PTC) derivatives which can be detected with high sensitivity at 245 nm. Therefore, precolumn derivatization of amino acids with PITC followed by a reversed-phase HPLC separation with UV detection is used to analyze the amino acid composition.

After the reagent is removed under vacuum, the derivatized amino acids can be stored dry and frozen for several weeks with no significant degradation. If the solution for injection is kept cold, no noticeable loss in chromatographic response occurs after three days.

Separation of the PTC-amino acids on a reversed-phase HPLC with ODS column is accomplished through a combination of changes in concentrations of acetonitrile and buffer ionic strength. PTC-amino acids eluted from column are monitored at 254 nm.

Detection limit is considered to be 1 pmol for most of the amino acid derivatives. Response linearity is obtained in the range of 20 to 500 pmol with correlation coefficients exceeding 0.999. To obtain good compositional data, samples larger than 500 ng of protein/peptide before hydrolysis is best suited for this amino acid analysis of proteins/peptides.

Method 4—Precolumn AQC Derivatization General Principle

Precolumn derivatization of amino acids with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) followed by reversed-phase HPLC separation with fluorometric detection is used.

6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) reacts with amino acids to form stable, fluorescent unsymmetric urea derivatives (AQC-amino acids) which are readily amenable to analysis by reversed-phase HPLC. Therefore, precolumn derivatization of amino acids with AQC followed by reversed-phase HPLC separation is used to analyze the amino acid composition.

Separation of the AQC-amino acids on ODS column is accomplished through a combination of changes in concentrations of acetonitrile and salt. Selective fluorescence detection of the derivatives with excitation wavelength at 250 nm and emission wavelength at 395 nm allows for the direct injection of the reaction mixture with no significant interference from the only major fluorescent reagent by-product, 6-aminoquinoline. Excess reagent is rapidly hydrolyzed ($t_{1/2} < 15$ seconds) to yield 6-aminoquinoline, *N*-hydroxysuccinimide and carbon dioxide, and after 1 minute no further derivatization can take place.

Peak areas for AQC-amino acids are essentially unchanged for at least 1 week at room temperature, and the derivatives have more than sufficient stability to allow for overnight automated chromatographic analysis.

Detection limit is considered to be ranging from ca. 40 to 320 fmol for each amino acid, except for Cys. Detection limit for Cys is approximately 800 fmol. Response linearity is obtained in the range of 2.5 to 200 $\mu\text{mol/L}$ with correlation coefficients exceeding 0.999. Good compositional data could

be obtained from the analysis of derivatized protein hydrolysates containing as little as 30 ng of protein/peptide.

Method 5—Precolumn OPA Derivatization General Principle

Precolumn derivatization of amino acids with *o*-phthalaldehyde (OPA) followed by reversed-phase HPLC separation with fluorometric detection is used. This technique does not detect amino acids that exist as secondary amines (e.g., proline).

o-Phthalaldehyde (OPA) in conjunction with a thiol reagent reacts with primary amine groups to form highly fluorescent isoindole products. 2-Mercaptoethanol or 3-mercaptopropionic acid can be used as the thiol. OPA itself does not fluoresce and consequently produces no interfering peaks. In addition, its solubility and stability in aqueous solution, along with the rapid kinetics for the reaction, make it amenable to automated derivatization and analysis using an autosampler to mix the sample with the reagent. However, lack of reactivity with secondary amino acids has been predominant drawback. This method does not detect amino acids that exist as secondary amines (e.g., proline). To compensate for this drawback, this technique may be combined with another technique described in *Method 7* or *Method 8*.

Precolumn derivatization of amino acids with OPA is followed by a reversed-phase HPLC separation. Because of the instability of the OPA-amino acid derivative, HPLC separation and analysis are performed immediately following derivatization. The liquid chromatograph is equipped with a fluorometric detector for the detection of derivatized amino acids. Fluorescence intensity of OPA-derivatized amino acids is monitored with an excitation wavelength of 348 nm and an emission wavelength of 450 nm.

Detection limits as low as 50 fmol via fluorescence have been reported, although the practical limit of analysis remains at 1 pmol.

Method 6—Precolumn DABS-Cl Derivatization General Principle

Precolumn derivatization of amino acids with (dimethylamino)azobenzenesulfonyl chloride (DABS-Cl) followed by reversed-phase HPLC separation with visible light detection is used.

(Dimethylamino)azobenzenesulfonyl chloride (DABS-Cl) is a chromophoric reagent employed for the labeling of amino acids. Amino acids labeled with DABS-Cl (DABS-amino acids) are highly stable and show the maximum absorption at 436 nm.

DABS-amino acids, all 19 naturally occurring amino acids derivatives, can be separated on an ODS column of a reversed-phase HPLC by employing gradient systems consisting of acetonitrile and aqueous buffer mixture. Separated DABS-amino acids eluted from column are detected at 436 nm in the visible region.

This *Method* can analyze the imino acids such as proline together with the amino acids at the same degree of sensitivity, DABS-Cl derivatization method permits the simultaneous quantification of tryptophan residues by previous hydrolysis of the protein/peptide with sulfonic acids such as mercaptoethanesulfonic acid, *p*-toluenesulfonic acid or methanesulfonic acid described under *Method 2* in "Protein Hydrolysis". The other acid-labile residues, asparagine and glutamine, can also be analysed by previous conversion into diaminopropionic acid and diamino butyric acid, respectively,

by treatment of protein/peptide with BTI described under *Method 11* in "Protein Hydrolysis".

The non-proteinogenic amino acid, norleucine cannot be used as internal standard in this method, as this compound is eluted in a chromatographic region crowded with peaks of primary amino acids. Nitrotyrosine can be used as an internal standard, because it is eluted in a clean region.

Detection limit of DABS-amino acid is about 1 pmol. As little as 2 to 5 pmol of an individual DABS-amino acid can be quantitatively analysed with reliability, and only 10 to 30 ng of the dabsylated protein hydrolysate is required for each analysis.

Method 7—Precolumn FMOC-Cl Derivatization General Principle

Precolumn derivatization of amino acids with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by reversed-phase HPLC separation with fluorometric detection is used.

9-Fluorenylmethyl chloroformate (FMOC-Cl) reacts with both primary and secondary amino acids to form highly fluorescent products. The reaction of FMOC-Cl with amino acid proceeds under mild conditions in aqueous solution and is completed in 30 seconds. The derivatives are stable, only the histidine derivative showing any breakdown. Although FMOC-Cl is fluorescent itself, the reagent excess and fluorescent side-products can be eliminated without loss of FMOC-amino acids.

FMOC-amino acids are separated by a reversed-phase HPLC using ODS column. The separation is carried out by gradient elution varied linearly from a mixture of acetonitrile methanol and acetic acid buffer (10:40:50) to a mixture of acetonitrile and acetic acid buffer (50:50), and 20 amino acid derivatives are separated in 20 minutes. Each derivative eluted from column is monitored by a fluorometric detector set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm.

The detection limit is in the low fmol range. A linearity range of 0.1 to 50 $\mu\text{mol/L}$ is obtained for most of the amino acids.

Method 8—Precolumn NBD-F Derivatization General Principle

Precolumn derivatization of amino acids with 7-fluoro-4-nitrobenzo-2-oxa-1.3-diazole (NBD-F) followed by reversed-phase HPLC separation with fluorometric detection is used.

7-fluoro-4-nitrobenzo-2-oxa-1.3-diazole (NBD-F) reacts with both primary and secondary amino acids to form highly fluorescent products. Amino acids are derivatized with NBD-F by heating to 60°C for 5 minutes.

NBD-amino acid derivatives are separated on an ODS column of a reversed-phase HPLC by employing gradient elution system consisting of acetonitrile and aqueous buffer mixture, and 17 amino acid derivatives are separated in 35 minutes. ϵ -Aminocaproic acid can be used as an internal standard, because it is eluted in a clean chromatographic region. Each derivative eluted from column is monitored by a fluorometric detector set at an excitation wavelength of 480 nm and an emission wavelength of 530 nm.

The sensitivity of this method is almost the same as for precolumn OPA derivatization method (*Method 5*), excluding proline to which OPA is not reactive, and might be advantageous for NBD-F against OPA. The detection limit for each amino acid is about 10 fmol. Profile analysis was

achieved for about 1.5 μg of protein hydrolysates in the final precolumn labeling reaction mixture for HPLC.

Data Calculation and Analysis

When determining the amino acid content of a protein/peptide hydrolysate, it should be noted that the acid hydrolysis step destroys tryptophan and cysteine. Serine and threonine are partially destroyed by acid hydrolysis, while isoleucine and valine residues may be only partially cleaved. Methionine can undergo oxidation during acid hydrolysis, and some amino acids (e.g., glycine and serine) are common contaminants. Application of adequate vacuum (≤ 0.0267 kPa) or introduction of inert gas (argon) in the headspace of the reaction vessel during vapor phase hydrolysis can reduce the level of oxidative destruction. Therefore, the quantitative results obtained for cysteine, tryptophan, threonine, isoleucine, valine, methionine, glycine, and serine from a protein/peptide hydrolysate may be variable and may warrant further investigation and consideration.

Calculations

Amino Acid Mole Percent This is the number of specific amino acid residues per 100 residues in a protein. This result may be useful for evaluating amino acid analysis data when the molecular weight of the protein under investigation is unknown. This information can be used to corroborate the identity of a protein/peptide and has other applications. Carefully identify and integrate the peaks obtained as directed for each *Procedure*. Calculate the mole percent for each amino acid present in the test sample by the formula:

$$100r_U/r,$$

in which r_U is the peak response, in nmol, of the amino acid under test; and r is the sum of peak responses, in nmol, for all amino acids present in the test sample. Comparison of the mole percent of the amino acids under test to data from known proteins can help establish or corroborate the identity of the sample protein.

Unknown Protein Samples This data analysis technique can be used to estimate the protein concentration of an unknown protein sample using the amino acid analysis data. Calculate the mass, in μg , of each recovered amino acid by the formula:

$$mM_w/1000,$$

in which m is the recovered quantity, in nmol, of the amino acid under test; and M_w is the average molecular weight for that amino acid, corrected for the weight of the water molecule that was eliminated during peptide bond formation. The sum of the masses of the recovered amino acids will give an estimate of the total mass of the protein analyzed after appropriate correction for partially and completely destroyed amino acids. If the molecular weight of the unknown protein is available (i.e., by SDS-PAGE analysis or mass spectroscopy), the amino acid composition of the unknown protein can be predicted. Calculate the number of residues of each amino acid by the formula:

$$m/(1000M/M_{WT}),$$

in which m is the recovered quantity, in nmol, of the amino acid under test; M is the total mass, in μg , of the protein; and M_{WT} is the molecular weight of the unknown protein.

Known Protein Samples This data analysis technique can be used to investigate the amino acid composition and protein concentration of a protein sample of known molecular weight and amino acid composition using the amino acid analysis data. When the composition of the protein being

analyzed is known, one can exploit the fact that some amino acids are recovered well, while other amino acid recoveries may be compromised because of complete or partial destruction (e.g., tryptophan, cysteine, threonine, serine, methionine), incomplete bond cleavage (i.e., for isoleucine and valine) and free amino acid contamination (i.e., by glycine and serine).

Because those amino acids that are recovered best represent the protein, these amino acids are chosen to quantify the amount of protein. Well-recovered amino acids are, typically, aspartate-asparagine, glutamate-glutamine, alanine, leucine, phenylalanine, lysine, and arginine. This list can be modified based on experience with one's own analysis system. Divide the quantity, in nmol, of each of the well-recovered amino acids by the expected number of residues for that amino acid to obtain the protein content based on each well-recovered amino acid. Average the protein content results calculated. The protein content determined for each of the well-recovered amino acids should be evenly distributed about the mean. Discard protein content values for those amino acids that have an unacceptable deviation from the mean. Typically \geq greater than 5% variation from the mean is considered unacceptable. Recalculate the mean protein content from the remaining values to obtain the protein content of the sample. Divide the content of each amino acid by the calculated mean protein content to determine the amino acid composition of the sample by analysis.

Calculate the relative compositional error, in percentage, by the formula:

$$100m/m_s,$$

in which m is the experimentally determined quantity, in nmol per amino acid residue, of the amino acid under test; and m_s is the known residue value for that amino acid. The average relative compositional error is the average of the absolute values of the relative compositional errors of the individual amino acids, typically excluding tryptophan and cysteine from this calculation. The average relative compositional error can provide important information on the stability of analysis run over time. The agreement in the amino acid composition between the protein sample and the known composition can be used to corroborate the identity and purity of the protein in the sample.

2. Aristolochic Acid

Aristolochic acid, which occurs in plants of *Aristolochiaceae*, is suspected to cause renal damage. It is also reported to be oncogenic (see References).

Aristolochic acid toxicity will not be a problem if crude drugs of the origin and parts designated in the JP are used, but there may be differences in crude drug nomenclature between different countries, and it is known that crude drug preparations not meeting the specifications of the JP are circulating in some countries. Consequently, when crude drugs or their preparations are used, it is important that the materials should not include any plant containing aristolochic acid.

Since Supplement I to JP14, the test for aristolochic acid I was added to the Purity under *Asiasarum* Root, which consists of the rhizome and root. Because the aerial part of the plant may contain aristolochic acid and may have been improperly contaminated in *Asiasarum* Root.

It is considered that *Akebia* Stem, *Sinomenium* Stem and *Saussurea* Root do not contain aristolochic acid, unless plants of origin other than that designated in the JP are used. However, contamination of aristolochic acid might occur, as mentioned above. In this case, the test described in the Purity under *Asiasarum* Root is useful for checking the presence of aristolochic acid.

References:

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3. Basic Requirements for Viral Safety of Biotechnological/Biological Products listed in Japanese Pharmacopoeia

Introduction

The primary role of specification of biotechnological/biological products listed in Japanese Pharmacopoeia (JP) is not only for securing quality control or consistency of the quality but also for assuring their efficacy and safety. In the meantime, the requirements to assure quality and safety of drugs have come to be quite strict recently, and a rigid attitude addressing safety assurance is expected for biotechnological/biological products. The key points for quality and safety assurance of biotechnological/biological products are selection and appropriate evaluation of source material, appropriate evaluation of manufacturing process and maintenance of manufacturing consistency, and control of specific physical properties of the products. Now, how to assure quality and safety of such drugs within a scope of JP has come to be questioned. This General Information describes what sorts of approaches are available to overcome these issues.

It is desired that quality and safety assurance of JP listed products are achieved by state-of-the-art methods and concepts which reflect progress of science and accumulation of experiences. This General Information challenges to show the highest level of current scientific speculation. It is expected that this information will contribute to promotion of scientific understanding of quality and safety assurance of not only JP listed products but also the other biotechnological/biological products and to promotion of active discussion of each Official Monograph in JP.

1. Fundamental measures to ensure viral safety of JP listed biotechnological/biological products

The biotechnological/biological product JP includes the products derived from living tissue and body fluid (urine, blood, etc.) of mammals, etc. Protein drugs derived from cell lines of human or animal origin (e.g., recombinant DNA drug, cell culture drug) are also included. The fundamental measures required for comprehensive viral safety of JP listed biotechnological/biological products are as follows: 1) acquaintance of possible virus contamination (source of contamination); 2) careful examination of eligibility of raw materials and their sources, e.g. human/animal, and thorough analysis and screening of the sample chosen as a substrate for drug production (e.g., pooled body fluid, cell

bank, etc.) to determine any virus contamination and determination of type and nature of the virus, if contaminated; 3) evaluation to determine virus titer and virus-like particles hazardous to human, if exists; 4) selection of production related material (e.g., reagent, immune antibody column) free from infectious or pathogenic virus; 5) performance of virus free test at an appropriate stage of manufacturing including the final product, if necessary; 6) adoption of effective viral clearance method in the manufacturing process to remove/inactivate virus. Combined method sometimes achieves higher level of clearance; 7) development of a deliberate viral clearance scheme; 8) performance of the test to evaluate viral removal and inactivation. It is considered that the stepwise and supplemental adoption of the said measures will contribute to ensure viral safety and its improvement.

2. Safety assurance measures described in the Official Monograph and this General Information

As mentioned in above 1, this General Information describes, in package, points to be concerned with and concrete information on the measures taken for viral safety of JP listed products. Except where any specific caution is provided in Official Monograph of a product in question, Official Monograph provides in general that "Any raw material, substrate for drug production and production related material used for production of drug should be derived from healthy animals and should be shown to be free of latent virus which is infectious or pathogenic to human", "Cell line and culture method well evaluated in aspects of appropriateness and rationality on viral safety are used for production, and the presence of infectious or pathogenic latent virus to human in process related materials derived from living organisms should be denied". and "biotechnological/biological drug should be produced through a manufacturing process which is capable of removing infectious or pathogenic virus", etc., to raise awareness on viral safety and on necessity to conduct test and process evaluation for viral safety.

3. Items and contents described in this General Information

As for viral safety of protein drug derived from cell line of human or animal origin, there is a Notice in Japan entitled "Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin" (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare) to reflect the internationally harmonized ICH Guideline, and as for blood plasma protein fraction preparations, there is a document entitled "Guideline for ensuring viral safety of blood plasma protein fraction preparations". This General Information for ensuring viral safety of JP listed biotechnological/biological products has been written, referencing the contents of those guidelines, to cover general points and their details to be concerned for ensuring viral safety of not only JP listed biotechnological/biological products but also all products which would be listed in JP in future, i.e., biological products derived from living tissue and body fluid, such as urine, and protein drugs derived from cell line of human or animal origin (Table 1).

3.1 Purpose

The purpose of this document is to propose the comprehensive concepts of the measures to be taken for ensuring viral safety of biotechnological/biological products derived

Table 1. Items described in General Information for Viral Safety Assurance of JP listed Biotechnological/Biological Product

I. Introduction

1. Fundamental measures to ensure viral safety of JP listed biotechnological/biological products
2. Safety assurance measures described in the Official Monograph and this General Information
3. Items and contents described in this General Information

II. General Matters

1. Purpose
2. Background
3. Unknown risk on the measures taken for ensuring viral safety
4. Applicable range
5. Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)
6. Basis for ensuring viral safety
7. Limit of virus test
8. Roles of viral clearance studies

III. Raw material/substrate for drug production

1. Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto
2. Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

IV. Points of concern with respect to manufacturing and virus testing

1. Virus test conducted in advance of purification process
2. Virus test as an acceptance test of an intermediate material, etc.
3. Virus test on a final product

V. Process evaluation on viral clearance

1. Rationale, objective and general items to be concerned with respect to viral clearance process evaluation
2. Selection of virus
3. Design of viral clearance studies
4. Interpretation of viral clearance studies
 - 1) Evaluation of viral clearance factor
 - 2) Calculation of viral clearance index
 - 3) Interpretation of results and items to be concerned at evaluation

VI. Statistics

1. Statistical considerations for assessing virus assays
2. Reproducibility and confidence limit of viral clearance studies

VII. Re-evaluation of viral clearance

VIII. Measurement for viral clearance studies

1. Measurement of virus infective titer
2. Testing by nucleic-acid amplification test (NAT)

IX. Reporting and preservation

X. Others

from living tissue or body fluid of mammals, etc. and of protein drugs derived from cell lines of human or animal origin. That is to say, this document describes the measures and the points of concern on the items, such as ① consideration of the source of virus contamination; ② appropriate evaluation on eligibility at selecting the raw material and on qualification

of its source, e.g. human or animal; ③ virus test, and its analysis and evaluation at a stage of cell substrate for drug production; ④ appropriate evaluation to choose product related materials derived from living organisms (e.g. reagent, immune antibody column, etc.); ⑤ conduct of necessary virus test on the product at an appropriate stage of manufacturing; ⑥ development of viral clearance test scheme; ⑦ performance and evaluation of viral clearance test. This document is also purposed to comprehensively describe in details that supplemental and combining adoption of the said measures will contribute to secure viral safety and its improvement.

3.2 Background

One of the most important issues to be cautioned for safety of a biological product, which is directly derived from human or animal, or of a protein drug, which is derived from cell line of human or animal origin (recombinant DNA derived product, cell culture derived product, etc.), is risk of virus contamination. Virus contamination may cause serious situation at clinical use once it occurs. Virus contamination may be from a raw material or from a cell substrate for drug production, or may be from an adventitious factor introduced to the manufacturing process.

JP listed biological drugs or protein drugs derived from cell line have achieved drastic contribution to the medical society, and to date, there has not been any evidence of any safety problem on them caused by virus. But, social requirement of health hazard prevention is strong, and it is now very important to prevent accidental incidence, taking security measures carefully supported by scientific rationality. It is always great concern among the persons involved that under what sort of viewpoint and to what extent we have to pursue for ensuring viral safety of a biotechnological/biological product. Before discussing these issues, two fundamental points have to be reconfirmed. One is that; we have to consider scientific, medical, and social profiles a drug has. In other words, "Medicine is a social asset which is utilized in medical practice paying attention to the risk and benefit from the standpoints of science and society". It is the destiny and the mission of the medical/pharmaceutical society to realize prompt and stable supply of such a social asset, drug, among the medical work front to bring gospel to the patients.

The other is that; issue of viral safety is independent from safety of the components of a drug per se (narrow sense of safety). It is important to consider that this is the matter of general safety of drug (broad sense of safety). In case of a drug which has been used for a long time in the medical front, such as a JP listed product, its broad sense of safety is considered to have been established epidemiologically, and its usage past records have a great meaning. However, different from safety of drug per se (its components), taking into account any possibility of virus contamination, we have to say that only the results accumulated can not always assure viral safety of a drug used in future. Accordingly, the basis for securing broad sense of viral safety of JP listed biotechnological/biological products is to pay every attention to the measures to take for prevention, while evaluating the accumulated results.

Adopting strict regulations and conducting tests at maximum level to the extent theoretically considered may be the ways off assuring safety, but applying such way generally, without sufficient scientific review of the ways and evaluation of usage results, causes excessive requirement of regulation

and test not having scientific rationality. As the results, effective and prompt supply of an important drug, already having enough accumulation of experiences, to the medical work front will be hampered, and the drug, a social asset, may not to be utilized effectively. Medicine is a sword used in medical field having double-edge named effectiveness and safety. Effectiveness and safety factors have to be derived as the fruits of leading edge of science, and relatively evaluated on a balance sheet of usefulness. Usefulness evaluation should not be unbalanced in a way that too much emphasis is placed on safety concern without back-up of appropriate scientific rationality. A drug can play an important role as a social asset only when well balanced appropriate scientific usefulness evaluation in addition to social concern of the age are given. In other words, drug is a common asset utilized by society for medication as a fruit of science of the age, and the key point of its utilization lies on a balance of risk and benefit produced from scientific and social evaluation. So, those factors have to be taken into account when target and pursuance levels for ensuring viral safety of a JP listed biotechnological/biological product are reviewed.

And, in general, the risk and benefit of drugs should be considered with the relative comparison to alternative drugs or medical treatment. The usefulness of certain drug should be reviewed finally after the competitive assessment on the risk and benefit on the alternative drugs, relevant drugs and/or alternative medical treatment.

Under such background, the purpose of this article is to describe the scientific and rational measures to be taken for ensuring viral safety of JP listed biotechnological/biological products. Giving scientific and rational measures mean that; appropriate and effective measures, elaborated from the current scientific level, are given to the issues assumable under the current scientific knowledge. In other words, possible contaminant virus is assumed to have the natures of genus, morph, particle size, physical/chemical properties, etc. which are within the range of knowledge of existing virology, and is those assumed to exist in human and animal, tissue and body fluid, which are the source of biotechnological/biological product, reagent, material, additives, etc. Accordingly, viral clearance studies using a detection method which target those viruses have to be designed.

3.3 Unknown risk on the measures taken for ensuring viral safety

There are known and unknown risks.

It is easy to determine a test method and an evaluation standard on the known risk, which exists in the drug per se (pharmaceutical component) or inevitably exists due to a quality threshold, and quantification of such risk is possible. In other words, it is easy to evaluate the known risk on a balance sheet in relation to the benefit, and we can say that valuation even in this respect has been established to some extent.

On the other hand, as for the unknown risk which is inevitable for ensuring viral safety, the subject of the risk can not be defined and quantitative concept is hard to introduce, and, therefore, taking a counter measure and evaluating its effect are not so easy. Therefore, this is the subject to be challenged calling upon wisdom of the related parties among the society of drug.

Talking about the unknown risk, there are view points that say "It is risky because it is unknown." and "What are the unknowns, and how do we cope with them in ensuring safe-

ty?”.

The view of “It is risky because it is unknown.” is already nothing but a sort of evaluation result, and directly connects to a final decision if it can be used as a drug. Such evaluation/decision has to be made based upon a rational, scientific or social judgment.

For example, in the case that “In a manufacturing process of drug, virus, virus-like particle or retrovirus was detected, but its identification could not be confirmed, and, therefore, its risk can not be denied.”, the evaluation of “It is risky because it is unknown.” is scientifically rational and reasonable. On the other hand, however, if we reach a decision of “It is risky because it is unknown.” due to the reason that “In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a ‘concern’ that something unknown may exist.”, it can not be said that such evaluation is based upon a rational, scientific or social judgment. It goes without saying that the utmost care has to be taken for viral safety, but the substance of ‘concern’ has to be at least clearly explainable. Otherwise, the ‘concern’ may result in causing contradiction in the meaningful mission to utilize a social asset, drug, in medical practice.

From scientific view point, we should not be narrow minded by saying “it is risky” because “there is a ‘concern’ that something unknown may exist”, but challenge to clarify the subject of “What is unknown, and how to cope with it for ensuring safety” using wisdom. What is important at the time is to define “what is unknown” based upon current scientific knowledge. Only through this way, is it possible for us to elaborate the measures for ensuring safety.

Once we chase up the substance of unknown risk for viral safety without premises of “what is unknown”, “unknown” will be an endless question because it theoretically remains unresolved forever. If this kind of approach is taken, the issue and the measure can not be scientifically connected to each other, which will result in the excessive requirement of regulation and of test to be conducted. Yet, it is unlikely that the measure which has no relation with science will be effective to the subject of “What is unknown is unknown.”

For example, “what is unknown” at the “evaluation of a purification process which can completely clear up every virus that contaminated in a manufacturing process” should be the subject of “what sort of existing virus that contaminated is unknown”, not on the subject of “what sort of virus that exist in the world is unknown. In the former subject, the premise of the study is based on all the knowledge on viruses including DNA/RNA-virus, virus with/without envelope, particle size, physical/chemical properties, etc. The premise is that the virus contaminated should be within range of existing wisdom and knowledge of virus such as species, type, nature, etc., even though the virus that contaminated is unknown. Under such premise, when evaluation is made on a purification process to decide its capability of clearing a derived virus, which is within the range of existing wisdom and learning, specific viral clearance studies designed to combine a few model viruses with different natures, such as type of nucleic acid, with/without envelope, particle size, physical/chemical properties, etc., would be enough to simulate every sort of the virus already known, and will be a “good measure for ensuring safety”.

The issue of “the sort of viruses that exist in the world is unknown” may be a future study item, but it is not an appropriate subject for the viral clearance test. Further, even if

the subject of “unknown viruses, which have a particle size smaller than that of currently known viruses, may exist” or “unknown viruses, which have special physical/chemical properties that can not be matched to any of the currently known viruses, may exist” is set up as an armchair theory, any experimental work can not be pursued under the current scientific level, since such virus model is not available. Further, any viral clearance test performed by using the currently available methods and technologies will be meaningless “for ensuring safety”, since particle size or natures of such speculated virus are unknown. Likewise, any counter measures can not be taken on the subject of “unknown virus, which can not be detected by currently available screening method, may exist”, and conducting any virus detection test at any stage will be useless “for ensuring safety”.

The requirement of regulations or tests excessively over scientific rationality will raise human, economical and temporal burden to the pharmaceutical companies, and will adversely affect prompt, effective and economical supply of a drug to the medical front. As drug is a sort of social asset, which has to be scientifically evaluated, how to assure maximization of its safety by means of scientifically rational approach at minimum human, economical and time resources is important.

It is also important to reconfirm that achievement of those issues is on the premise that appropriate measures are taken on the supply source of drugs. For example, in a case of “In a manufacturing process of drug, virus, virus-like particle or retrovirus was not detected, but there is a ‘concern’ that something unknown may exist.”, appropriateness of the test, which resulted in the judgment that “virus, virus-like particle or retrovirus was not detected in a process of drug production”, should be a prerequisite premise when judged by science standard at the time. If there is any question on the premise, it is quite natural that the question of “there is a ‘concern’ that unknown something may exist.” will be effective.

3.4 Applicable range

This General Information is on JP listed biological products, derived from living tissue or body fluid, and protein drugs, derived from human or animal cell line, that in Japan. In the case of protein drugs derived from human or animal cell line, the products developed and approved before enforcement of the Notice Iyakushin No. 329 entitled “Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin” should have been treated under the Notice had there been one, and it is inevitable that some products approved after the Notice might not have been sufficiently treated. It is expected that such biodrug will be sufficiently examined to meet such General Information before being listed in JP. On the other hand, blood preparations listed in the biological products standard and covered by “Guideline for securing safety of blood plasma protein fraction preparations against virus”, are out of the scope of this General Information. Further, in case of a relatively lower molecular biogenous substance, such as amino acid, saccharide and glycerin, and of gelatin, which is even classified as infectious or pathogenic polymer, there are cases that viral contamination can not be considered due to its manufacturing or purification process, and that potent viral inactivation/removal procedure that can not be applied to protein, can be used, and, therefore, it is considered reasonable to omit such substances from the subject for application. However, some

part of this General Information may be used as reference. Further, a comprehensive assurance measure for viral safety is recommendable on a biotechnological/biological product not listed in JP using this document as a reference so long as it is similar to the biotechnological/biological product JP.

3.5 Possible viral contamination to a JP listed biotechnological/biological product (source of virus contamination)

Promoting awareness of virus contamination to a JP listed biotechnological/biological product (source of virus contamination) and citing countermeasure thereof are important for eradicating any possible virus contamination and raising probability of safety assurance. Many biotechnological/biological products are produced from a "substrate" which is derived from human or animal tissue, body fluid, etc. as an origin/raw material, and in purification or pharmaceutical processing of such products column materials or additives, which are living organism origin, are occasionally used. Accordingly, enough safety measures should be taken against diffusion of the contaminant virus. Further, as mentioned in Notice Iyakushin No. 329, any protein drug derived from cell lines of human or animal origin should be carefully examined with respect to the risk of virus contamination through the cell line, the cell substrate for drug production, and through the manufacturing process applied thereafter.

"Substrate for drug production" is defined as a starting material which is at a stage where it is deemed to be in a position to ensure quality/safety of an active substance. The "substrate for drug production" is sometimes tissue, body fluid, etc. of human or animal per se and pooled material such as urine, and sometimes a material after some treatment. In many cases, it is considered rational that starting point of full-scale test, evaluation and control should be at the stage of "substrate for drug production". The more strict levels of test, evaluation and control achieved at the stage of "substrate for drug production" can more rationalize evaluation and control of the raw material or individual level of upper stream. On the contrary, strict evaluation and control of the raw material or individual level at an upper stream stage can rationalize tests, evaluation or quality control at the stage of "substrate for drug production".

The measures taken for ensuring viral safety on a biotechnological/biological product currently listed in JP can be assumed from the provisions of manufacturing method, specification and test methods of each preparation. However, unitary principles or information with respect to the measures to be taken for ensuring viral safety, totally reviewing the entire process up to the final product rationally and comprehensively, including source/raw material/substrate, purification process, etc. have not been clarified. The most important thing for ensuring viral safety is to take thorough measures to eliminate the risk of virus contamination at any stage of source animal, raw material and substrate. Although not the cases of a biotechnological/biological product, known examples of a virus contamination from a raw material/substrate for drug production in old times are Hepatitis A Virus (HAV) or Hepatitis C Virus (HCV) contamination in blood protein fraction preparations. It is also well known that Human Immunodeficiency Virus (HIV) infection caused by blood plasma protein fraction preparations occurred in 1980s. The aim of this General Information is to show concrete guidelines for comprehensive viral safety assurance of the JP listed biotechnological/biological products. The pathogenic infectious viruses, currently known to con-

taminate to raw materials, etc. of drug and have to be cautioned, are HIV, HAV, Hepatitis B Virus (HBV), HCV, Human T-Lymphotropic Virus (HTLV-I/II), Human Parvovirus B19, Cytomegalovirus (CMV), etc. Biotechnological/biological products produced from raw material/cell substrate derived from tissue or body fluid of human or animal origin always have a risk of contamination of pathogenic or other latent virus. Therefore, safety measures should be thoroughly taken. There is also the case that a material, other than the biological component such as raw material/substrate, causes virus contamination. Using enzymatic or monoclonal antibody column or using albumin etc. as a stabilizer, is the example of the case, in which caution has to be taken on risk of virus contamination from the source animal or cell. Further, there is a possibility of contamination from environment or personnel in charge of production or at handling of the product. So, caution has to be taken on these respects as well.

In case of protein drugs derived from cell line of human or animal origin, there may be cases where latent or persistent infectious viruses (e.g., herpesvirus) or endogenous retroviruses exist in the cell. Further, adventitious viruses may be introduced through the routes such as: 1) derivation of a cell line from an infected animal; 2) use of a virus to drive a cell line; 3) use of a contaminated biological reagent (e.g., animal serum components); 4) contamination during cell handling. In the manufacturing process of drug, an adventitious virus may contaminate to the final product through the routes, such as 1) contamination through a reagent of living being origin, such as serum component, which is used for culturing, etc.; 2) use of a virus for introduction of a specific gene expression to code an objective protein; 3) contamination through a reagent used for purification such as monoclonal antibody affinity column; 4) contamination through an additive used for formulation production; 5) contamination at handling of cell and culture media, etc. It is reported that monitoring of cell culture parameter may be helpful for early detection of an adventitious viral contamination.

3.6 Basis for ensuring viral safety

Viral safety of a biotechnological/biological product produced from a raw material/substrate, which derived from tissue, body fluid, cell line, etc. of human or animal origin, can be achieved by supplemental and appropriate adoption of the following plural methods.

- (1) Acquaintance of possible virus contamination (source of contamination).
- (2) Careful examination of eligibility of the raw material and its source, i.e., human or animal, thorough analysis and screening of the sample chosen as the substrate for drug production to determine virus contamination and through examination of the type of virus and its nature, if contaminated.
- (3) Evaluation to determine hazardous properties of the virus or virus-like particle to human, if exists.
- (4) Choosing a product related material of living organism origin (e.g., reagent, immune anti-body column, etc.) which is free from infectious or pathogenic virus.
- (5) Conduct virus free test at an appropriate stage of manufacturing including the final product, if necessary.
- (6) Adoption of an effective method to remove/inactivate the virus in the manufacturing process for viral clearance. Combined processes sometimes achieve higher level of viral clearance.

- (7) Develop a deliberate viral clearance scheme.
- (8) Conduct test and evaluation to confirm removal/inactivation of the virus.

Manufacturer is responsible for explaining rationality of the way of approach adopted among the comprehensive strategy for viral safety on each product and its manufacturing process. At the time, the approach described in this General Information shall be applicable as far as possible.

3.7 Limit of virus test

Virus test has to be conducted to define existence of virus, but it should be noted that virus test alone can not reach a conclusion of inexistence of virus nor sufficient to secure safety of the product. Examples of a virus not being detected are as follows: 1) Due to statistical reason, there is an inherent quantitative limit, such as detection sensitivity at lower concentration depends upon the sample size. 2) Generally, every virus test has a detection limit, and any negative result of a virus test can not completely deny existence of a virus. 3) A virus test applied is not always appropriate in terms of specificity or sensitivity for detection of a virus which exists in the tissue or body fluid of human or animal origin.

Virus testing method is improved as science and technology progress, and it is important to apply scientifically the most advanced technology at the time of testing so that it can be possible to raise the assurance level of virus detection. It should be noted, however, that the limit as mentioned above can not always be completely overcome. Further, risk of virus contamination in a manufacturing process can not be completely denied, and, therefore, it is necessary to elaborate the countermeasure taken these effects into account.

Reliable assurance of viral free final product can not be obtained only by negative test results on the raw material/substrate for drug production or on the product in general, it is also necessary to demonstrate inactivation/removal capability of the purification process.

3.8 Roles of viral clearance studies

Under the premises as mentioned in the preceding clause that there is a limit of a virus test, that there is a possibility of existence of latent virus in a raw material/substrate for drug production and that there is a risk of entry of a non-endogenous virus in a manufacturing process, one of the important measures for viral safety is how to remove or inactivate the virus, which exists in a raw material, etc. and can not be detected, or the virus, which is contingently contaminated in a manufacturing process. The purpose of viral clearance study is to experimentally evaluate the viral removal/inactivation capability of a step that mounted in a manufacturing process. So, it is necessary to conduct an experimental scale spike test using an appropriate virus that is selected by taking account the properties, such as particle size, shape, with or without envelope, type of nucleic acid (DNA type, RNA type), heat and chemical treatment tolerance, etc., with an aim to determine removal/inactivation capability of the virus that can not be detected in a raw material or contingently contaminated.

As mentioned above, the role of viral clearance study is to speculate removal/inactivation capability of a process through a model test, and it contributes to give scientific basis to assure that a biotechnological/biological product of human or animal origin has reached an acceptable level in aspect of viral safety.

At a viral clearance study, it is necessary to adopt an appropriate approach method which is definitive and rational

and can assure viral safety of a final product, taking into consideration the source and the properties of the raw material/substrate as well as the manufacturing process.

4. Raw material/substrate for drug production

4.1 Issues relating to animal species and its region as a source of raw material/substrate for drug production and countermeasures to be taken thereto

For manufacturing JP listed biotechnological/biological products, which require measures for viral safety, a raw material/substrate derived mainly from human, bovine, swine or equine is used, and it is obvious that such human and animal has to be healthy nature. A wild animal should be avoided, and it is recommended to use animals derived from a colony controlled by an appropriate SPF (Specific Pathogen-Free) condition and bred under a well deigned hygienic control, including appropriate control for prevention of microbial contamination and contamination monitoring system. If a meat standard for food is available, an animal meeting this standard has to be used. The type of virus to be concerned about depend on animal species, but it may be possible to narrow down the virus for investigation by means of examining the hygiene control, applicability of a meat standard for food, etc. On the other hand, even with the animals of the same species, a different approach may be necessary depending upon the region where the specimen for a raw material/substrate is taken. For example, in case of obtaining raw material/substrate from blood or other specific region, it is necessary to be aware of the risk level, virus multiplication risk, etc. which may specifically exists depending upon its region. Such approach may be different from those applied to body waste such as urine, milk, etc. as a source of raw material/substrate. Further, caution has to be taken on transmissible spongiform encephalopathy (TSE) when pituitary gland, etc. is used as a raw material. This report does not include detailed explanation on TSE, but recommendations are to use raw material derived from 1) animals originated in the countries (area) where incidence of TSE has not been reported; 2) animals not infected by TSE; or 3) species of animal which has not been reported on TSE. It is recommended to discuss the matters concerned with TSE with the regulatory authority if there is any unclear point.

Followings are the raw material/substrate used for manufacturing biotechnological/biological products in Japan.

(1) Biological products derived from human

Blood plasma, placenta, urine, etc. derived from human are used as the sources of raw material of biotechnological/biological product. As for these raw materials, there are 2 cases: 1) Appropriateness can be confirmed by interview or by examination of the individual who supplies each raw material, and 2) Such sufficient interview or examination of the individual can not be made due to type of raw material. In case that sufficient examination of individual level is not possible, it is necessary to perform test to deny virus contamination at an appropriate manufacturing stage, for example, the stage to decide it as a substrate for drug production.

(2) Biological products derived from animal besides human

Insulin, gonadotropin, etc. are manufactured from blood plasma or from various organs of bovine, swine and equine.

(3) Protein drug derived from cell line of human or animal origin

In the case of protein drugs derived from cell line of human

or animal origin, a cell line of human or animal is the raw material per se, and the substrate for drug production is a cell bank prepared from cloned cell line (master cell bank or working cell bank). Examination at cell bank level is considered enough for viral safety qualification, but it goes without saying that the more appropriate and rational qualification evaluation test of cell bank can be realized when more information is available on the virus of the source animal or on prehistory of driving the cell line, the base of cell bank.

4.2 Qualification evaluation test on human or animal as a source of raw material/substrate for drug production

(1) Biological products derived from human

Body fluid etc. obtained from healthy human must be used for biological products production. Further, in case that interview or examination of the individual, who supplies the raw material, can be possible and is necessary, interview under an appropriate protocol and a serologic test well evaluated in aspects of specificity, sensitivity and accuracy have to be performed, so that only the raw material, which is denied latent HBV, HCV and HIV, will be used. In addition to the above, it is necessary to test for gene of HBV, HCV and HIV by a nucleic amplification test (NAT) well evaluated in aspects of specificity, sensitivity and accuracy.

In case of the raw material (e.g., urine), which can not be tested over the general medical examination of the individual who supplies the material, or of the raw material which is irrational to conduct individual test, the pooled raw material, as the substrate for drug production, has to be conducted at least to deny existence of HBV, HCV and HIV, using a method well evaluated in aspects of specificity, sensitivity and accuracy, such as the antigen test or NAT.

(2) Biological products derived from animal besides human

The animal used for manufacturing biological product has to be under appropriate health control, and has to be confirmed of its health by various tests. Further, it is necessary that the population, to which the animal belongs, has been under an appropriate breeding condition, and that no abnormal individual has been observed in the population. Further, it is necessary to demonstrate information or scientific basis which can deny known causes infection or disease to human, or to deny such animal inherent latent virus by serologic test or by nucleic amplification test (NAT). The infectious virus that is known to be common between human and animal, and known to cause infection in each animal are tentatively listed in Table 2. It is necessary that the table is completed under careful examination, and denial of all of them, by means of tests on individual animal, tissue, body fluid, etc. as a raw material, or on pooled raw material (as a direct substrate for drug production), is not always necessary. Table 2 can be used as reference information, in addition to the other information, such as; source of animal, health condition, health and breeding control, conformity to the meat standard for food, etc., to elaborate to which virus what kind of test has to be performed, and for which virus it is not always necessary to test for, etc. It is important to clarify and record the basis of choosing the virus and the test conducted thereof.

Table 2. Infectious viruses known to be common between human and animal and known to cause infection to each animal

	bovine	swine	sheep	goat	equine
Cowpox virus	⊙				
Paravaccinia virus	⊙	⊙	⊙	⊙	
Murray valley encephalitis virus	⊙	⊙			
Louping ill virus	⊙	⊙	⊙	⊙	
Wesselsbron virus			⊙		
Foot-and-mouth disease virus	⊙	⊙			
Japanese encephalitis virus		⊙			
Vesicular stomatitis virus		⊙			
Bovine papular stomatitis virus	⊙				
Orf virus			⊙		
Borna disease virus			⊙		⊙
Rabies virus	⊙	⊙	⊙	⊙	⊙
Influenza virus		⊙			
Hepatitis E virus		⊙			
Encephalomyocarditis virus	⊙	⊙			
Rotavirus	⊙				
Eastern equine encephalitis virus					⊙
Western equine encephalitis virus					⊙
Venezuelan equine encephalitis virus					⊙
Morbillivirus					⊙
Hendra virus					⊙
Nipah virus		⊙			
Transmissible gastroenteritis virus		⊙			
Porcine respiratory coronavirus		⊙			
Porcine epidemic diarrhea virus		⊙			
Hemagglutinating encephalomyelitis virus		⊙			
Porcine respiratory and reproductive syndrome virus		⊙			
Hog cholera virus		⊙			

Parainfluenza virus Type 3		◎			
Telfan/Teschen disease virus		◎			
Reovirus		◎			
Endogenous retrovirus		◎			
Porcine adenovirus		◎			
Porcine circovirus		◎			
Porcine parvovirus		◎			
Porcine poxvirus		◎			
Porcine cytomegalovirus		◎			
Pseudorabies virus		◎			
Russian spring-summer encephalitis virus			◎	◎	
Rift Valley fever virus			◎	◎	
Crimean-Congo hemorrhagic fever virus (Nairovirus)	◎		◎	◎	
Torovirus	◎				

(3) Protein drug derived from cell line of human or animal origin

It is important to conduct thorough investigation on latent endogenous and non-endogenous virus contamination in a master cell bank (MCB), which is the cell substrate for drug production, in accordance with the Notice Iyakushin No. 329 entitled "Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin". Further, it is necessary to conduct an appropriate adventitious virus test (e.g., *in vitro* and *in vivo* test) and a latent endogenous virus test on the cell at the limit of *in vitro* cell age (CAL) for drug production. Each WCB as a starting cell substrate for drug production should be tested for adventitious virus either by direct testing or by analysis of cells at the CAL, initiated from the WCB. When appropriate non-endogenous virus tests have been performed on the MCB and cells cultured up to or beyond the CAL have been derived from the WCB and used for testing for the presence of adventitious viruses, similar tests need not be performed on the initial WCB.

5. Points of concern with respect to manufacturing and virus testing

To ensure viral safety of a biological product derived from tissue, body fluid etc. of human or animal origin, it is necessary to exclude any possibility of virus contamination from a raw material, such as tissue and body fluid, or a substrate, paying attention to the source of virus contamination as mentioned in above 3.5, and to adopt appropriate manufacturing conditions and technologies in addition to enhancement of manufacturing environment, so that virus contamination in the course of process and handling and from operators, facilities and environment can be minimized.

In addition to the above, effective virus test and viral inactivation/removal technology, which are reflected by rapid progress of science, have to be introduced. Adoption of two

or more steps with different principles is recommended for virus inactivation/removal process. Further, it is important to minimize any possible virus derivation by using a reagent, which quality is equivalent to that of a drug. Examples of virus inactivation/removal processes are ① heating (It is reported that almost viruses are inactivated by heating at 55 – 60°C for 30 minutes with exceptions of hepatitis virus, etc. and that dry heating at 60°C for 10 – 24 hours is effective in case of the products of blood or urine origin.), ② treatment with organic solvent/surfactant (S/D treatment), ③ membrane filtration (15 – 50 nm), ④ acid treatment, ⑤ irradiation (γ -irradiation, etc.), ⑥ treatment with column chromatography (e.g. affinity chromatography, ion-exchange chromatography), ⑦ fractionation (e.g. organic solvent or ammonium sulfate fractionation), ⑧ extraction.

5.1 Virus test conducted in advance of purification process

(1) Biological products derived from human

In many cases, samples for virus test before purification process are body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. As mentioned in 4.2 (1), it is necessary to deny latent HBV, HCV and HIV by the test evaluated enough in aspects of specificity, sensitivity and accuracy. Even in a case that a non-purified bulk before purification process is produced from a substrate, it is not always necessary to conduct virus test again at the stage before purification, so long as the presence of any latent virus can be denied at the stage of substrate by an appropriate virus test, with cases where the non-purified bulk is made from the substrate by adding any reagent etc. of living organisms origin are an exception.

(2) Biological products derived from animal besides human

Similar to (1) above, samples for virus test before purification process are, in many cases, body fluid or tissue of individual collected as a raw material, or its pooled material or extraction as a substrate. In these cases, it is necessary to have a data, which can deny latent virus of probable cause of human infection or disease as mentioned in the above 4.2 (2), or to have a result of serologic test or nucleic amplification test (NAT) evaluated enough in aspects of specificity, sensitivity and accuracy. The concept, which is applied to a case that non-purified bulk before purification process is produced from substrate, is the same as those provided in the above 4.2 (1).

(3) Protein drug derived from cell line of human or animal origin

Generally, substrate in this case is cell bank, and the sample for testing before purification process is a harvested cell after cell culturing or unprocessed bulk which consists of single or pooled complex culture broth. The unprocessed bulk may be sometimes culture broth without cell. Denial of latent virus, which is determined by virus test at a MCB or WCB level, does not always deny latent virus in unprocessed bulk after culturing. Further, it is noted that the viral test at the CAL is meaningful as a validation but can not guarantee definite assurance of latent virus denial, since the test is generally performed only once. In case of using a serum or a component of blood origin in a culture medium, definite denial of latent virus at the level of unprocessed bulk can not be assured so long as the viral test has not been conducted on each lot at the CAL, since lot renewal can be a variable factor on viral contamination.

A representative sample of the unprocessed bulk, removed from the production reactor prior to further processing,

represents one of the most suitable levels at which the possibility of adventitious virus contamination can be determined with a high probability of detection. Appropriate testing for viruses should be performed at the unprocessed bulk level unless virus testing is made more sensitive by initial partial processing (e.g., unprocessed bulk may be toxic in test cell cultures, whereas partially processed bulk may not be toxic). In certain instances it may be more appropriate to test a mixture consisting of both intact and disrupted cells and their cell culture supernatants removed from the production reactor prior to further processing.

In case of unprocessed bulk, it is required to conduct virus test on at least 3 lots obtained from pilot scale or commercial scale production. It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material sources and results of viral clearance studies. Screening *in vitro* tests, using one or several cell lines, are generally employed to test unprocessed bulk. If appropriate, a NAT test or other suitable methods may be used.

Generally, harvest material in which adventitious virus has been detected should not be used to manufacture the product. If any adventitious viruses are detected at this level, the process should be carefully checked to determine the cause of the contamination, and appropriate actions taken.

5.2 Virus test as an acceptance test of an intermediate material, etc.

When a biological product is manufactured from tissue, body fluid etc. of human or animal origin, there are cases that an intermediate material, partially processed as a raw material or substrate by outside manufacturer, is purchased and used for manufacturing. In such case, if any test to meet this General Information has been conducted by such outside manufacturer, it is necessary for the manufacturer of the biological product, who purchased the intermediate material, to examine what sort of virus test has to be conducted as acceptance tests, and to keep record on the basis of rationality including the details of the test conducted.

On the other hand, if no test to meet this General Information has been conducted by such outside manufacturer of the raw material, all necessary virus free test has to be conducted to meet this General Information on the intermediate material regarding it as the direct substrate for drug production.

5.3 Virus test on a final product

Virus tests to be conducted on a final product (or on a product to reach the final product) has to be defined under comprehensive consideration of the type of raw material or substrate, the result of virus test conducted on raw material/substrate, the result of evaluation on viral removal/inactivation process, any possibility of virus contamination in the manufacturing process, etc. Comprehensive viral safety assurance can only be achieved by appropriate selection of the raw material/substrate, an appropriate virus test conducted on the raw material/substrate/intermediate material, the virus test conducted at an appropriate stage of manufacturing, an appropriate viral clearance test, etc. However, there are cases of having specific backgrounds, such as 1) use of the raw material derived from unspecified individual human, 2)

possible existence of virus at window period, 3) specific detection limit of virus test, etc. and in these cases, virus contamination to the final product may occur if there is any deficiency on the manufacturing process (e.g., damage of membrane filter) or any mix-up of the raw materials, etc. To avoid such accidental virus contamination, it may be recommended to conduct nucleic amplification test (NAT) on the final product focusing on the most risky virus among those that may possibly to exist in the raw material.

6. Process evaluation on viral clearance

6.1 Rationale, objective and general items to be concerned with respect to viral clearance process evaluation

Evaluation of a viral inactivation/removal process is important for ensuring safety of a biological product derived from tissue or body fluid of human or animal origin. Conducting evaluation on viral clearance is to assure, even to some extent, elimination of the virus, which may exist in a raw material, etc. or may be derived to the process due to unexpected situation. Viral clearance studies should be made by a carefully designed appropriate method, and has to be rationally evaluated.

The objective of viral clearance studies is to assess process step(s) that can be considered to be effective in inactivating/removing viruses and to estimate quantitatively the overall level of virus reduction obtained by the process. This should be achieved by the deliberate addition (“spiking”) of significant amounts of a virus at different manufacturing/purification steps and demonstrating its removal or inactivation during the subsequent steps. It is not necessary to evaluate or characterize every step of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps. It should be borne in mind that other steps in the process may have an indirect effect on the viral inactivation/removal achieved. Manufacturers should explain and justify the approach used in studies for evaluating viral clearance.

The reduction of virus infectivity may be achieved by removal of virus particles or by inactivation of viral infectivity. For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it is due to inactivation or removal. For inactivation steps, the study should be planned in such a way that samples are taken at different times and an inactivation curve constructed.

6.2 Selection of virus

To obtain broad range of information of viral inactivation/removal, it is desirable that a model virus used for viral clearance studies should be chosen from the viruses with broad range of characteristics in aspects of DNA/RNA, with or without envelope, particle size, significant resistance to physical/chemical treatment, etc. and it is necessary to combine about 3 model viruses to cover these characteristics.

At choice of a model virus, there are also the ways to choose a virus closely related to or having the same characteristics of the virus known to exist in the raw material. In such case, it is in principle recommendable to choose a virus which demonstrates a higher resistance to inactivation/removal treatment if two or more candidate viruses are available for choice. Further, a virus which can grow at a high titer is desirable for choice, although this may not always be possible. In addition to the above, choosing a virus, which will provide effective and reliable assay result at each step, is necessary, since sample condition to be tested at each step of

Table 3. Example of viruses which have been used for viral clearance studies

Virus	Family	Genus	Natural host	Genome	Env	Size (nm)	Shape	Resistance
Vesicular Stomatitis Virus	Rhabdo	Vesiculovirus	Equine Bovine	RNA	yes	70 × 150	Bullet	Low
Parainfluenza Virus	Paramyxo	Type 1,3 Respirovirus Type 2,4 Rubulavirus	Various	RNA	yes	100 – 200 +	Pleo-Spher	Low
MuLV	Retro	Type C oncovirus	Mouse	RNA	yes	80 – 110	Spherical	Low
Sindbis Virus	Toga	Alphavirus	Human	RNA	yes	60 – 70	Spherical	Low
BVDV	Flavi	Pestivirus	Bovine	RNA	yes	50 – 70	Pleo-Spher	Low
Pseudorabies Virus	Herpes	Varicellovirus	Swine	DNA	yes	120 – 200	Spherical	Med
Poliovirus Sabin Type 1	Picornia	Enterovirus	Human	RNA	no	25 – 30	Icosahedral	Med
Encephalomyocarditis Virus	Picornia	Cardiovirus	Mouse	RNA	no	25 – 30	Icosahedral	Med
Reovirus 3	Reo	Orthoreovirus	Various kind	RNA	no	60 – 80	Spherical	Med
SV 40	Papova	Polyomavirus	Monkey	DNA	no	40 – 50	Icosahedral	Very high
Parvovirus: canine, porcine	Parvo	Parvovirus	Canine Porcine	DNA	no	18 – 24	Icosahedral	Very high

a production process may influence the detection sensitivity. Consideration should also be given to health hazard which may pose to the personnel performing the clearance studies.

For the other items taken for consideration at choice of virus, the Notice, Iyakushin No. 329 can be used as a reference. Examples of the virus which have been used for viral clearance studies are shown in Table 3 which was derived from Iyakushin No. 329. However, the Notice, Iyakushin No. 329, is on viral safety of a product derived cell line of human or animal origin, and a more appropriate model virus has to be chosen taking into account the origin/raw material of biological products.

6.3 Design of viral clearance studies

The purpose of viral clearance studies is to quantitatively evaluate removal or inactivation capability of a process, in which a virus is intentionally spiked to a specific step of a manufacturing process.

Following are the precautions to be taken at planning viral clearance studies.

(1) Care should be taken in preparing the high-titer virus to avoid aggregation which may enhance physical removal and decrease inactivation thus distorting the correlation with actual production.

(2) Virus detection method gives great influence to viral clearance factor. Accordingly, it is advisable to gain detection sensitivity of the methods available in advance, and use a method with a detection sensitivity as high as possible. Quantitative infectivity assays should have adequate sensitivity and reproducibility in each manufacturing process, and should be performed with sufficient replicates to ensure adequate statistical validity of the result. Quantitative assays not associated with infectivity may be used if justified. Appropriate virus controls should be included in all infectivity assays to

ensure the sensitivity of the method. Also, the statistics of sampling virus when at low concentrations (for example, number of virus is 1-1000/L) should be considered.

(3) Viral clearance studies are performed in a miniature size system that simulates the actual production process of the biotechnological/biological product used by the manufacturer. It is inappropriate to introduce any virus into a production facility because of GMP constraints. Therefore, viral clearance studies should be conducted in a separate laboratory equipped for virological work and performed by staff with virological expertise in conjunction with production personnel involved in designing and preparing a scaled-down version of the purification process. The viral clearance studies should be performed under the basic concept of GLP.

(4) Each factor on a viral clearance study of a process, which is performed in miniature size, should reflect that of actual manufacturing as far as possible, and its rationality should be clarified. In case of chromatograph process, length of column bed, linear velocity, ratio of bed volume per velocity (in other words, contact time), buffer, type of column packing, pH, temperature, protein concentration, salt concentration and concentration of the objective product are all correspondent to those of the actual production. Further, similarity of elution profile should be achieved. For the other process, similar concept should be applied. If there is any factor which can not reflect the actual production, its effect to the result should be examined.

(5) It is desirable that two or more inactivation/removal processes of different principles are selected and examined.

(6) As for the process which is expected to inactivate/remove virus, each step should be evaluated in aspect of clearance capability, and carefully determined if it is the stage of inactivation, removal or their combination for designing the

test. Generally, in viral clearance test, a virus is spiked in each step which is the object of the test, and after passing through the process in question, the reduction level of infectivity is evaluated. But, in some case, it is accepted that a high potential virus is spiked at a step of the process, and virus concentration of each succeeding step is carefully monitored. When removal of virus is made by separation or fractionation, it is desirable to investigate how the virus is separated or fractionated (mass balance).

(7) For assessment of viral inactivation, unprocessed crude material or intermediate material should be spiked with infectious virus and the reduction factor calculated. It should be recognized that virus inactivation is not a simple, first order reaction and is usually more complex, with a fast "phase 1" and a slow "phase 2". The study should, therefore, be planned in such a way that samples are taken at different times and an inactivation curve constructed. It is recommended that studies for inactivation include at least one time point less than the minimum exposure time and greater than zero, in addition to the minimum exposure time. The reproducible clearance should be demonstrated in at least two independent studies. When there is a possibility that the virus is a human pathogen, it is very important that the effective inactivation process is designed and additional data are obtained. The initial virus load should be determined from the virus which can be detected in the spiked starting material. If this is not possible, the initial virus load may be calculated from the titer of the spiking virus preparation. Where inactivation is too rapid to plot an inactivation curve using process conditions, appropriate controls should be performed to demonstrate that infectivity is indeed lost by inactivation.

(8) If antibody against virus exists in an unprocessed material, caution should be taken at clearance studies, since it may affect the behavior of virus at viral removal or inactivation process.

(9) Virus spiked in unprocessed material should be sufficient enough to evaluate viral removal or inactivation capability of the process. However, the virus "spike" to be added to the unprocessed material should be as small as possible in comparison with the sample volume of the unprocessed material so as not to cause characteristic change of the material by addition of the virus nor to cause behavioral change of the protein in the material.

(10) It is desirable that the virus in the sample is subject for quantitative determination without applying ultracentrifuge, dialysis, storage, etc. as far as possible. However, there may be a case that any handling before quantitative test, such as remove procedure of inhibitor or toxic substance, storage for a period to realize test at a time, etc., is inevitable. If any manipulation, such as dilution, concentration, filtration, dialysis, storage, etc., is applied for preparation of the sample for testing, a parallel control test, which passes through a similar manipulation, should be conducted to assess infectivity variance at the manipulation.

(11) Buffers and product (desired protein or other component contained therein) should be evaluated independently for toxicity or interference in assays used to determine the virus titer, as these components may adversely affect the indicator cells. If the solutions are toxic to the indicator cells, dilution, adjustment of the pH, or dialysis of the buffer containing spiked virus might be necessary. If the product itself has anti-viral activity, the clearance study may need to be performed without the product in a "mock" run, although

omitting the product or substituting a similar protein that does not have anti-viral activity could affect the behaviour of the virus in some production steps.

(12) Many purification schemes use the same or similar buffers or columns, repetitively. The effects of this approach should be taken into account when analyzing the data. The effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used.

(13) Overall reduction factors may be underestimated where production conditions or buffers are too cytotoxic or virucidal and should be discussed on a case-by-case basis. Overall reduction factors may also be overestimated due to inherent limitations or inadequate design of viral clearance studies.

(14) It has to be noted that clearance capability of viral removal/inactivation process may vary depending upon the type of virus. The viral removal/inactivation process, which displays viral clearance by specific principle or mechanism, may be quite effective to the virus, which meets such mechanism of action, but not effective to the other type of viruses. For example, S/D treatment is generally effective to the virus with lipid membrane, but not effective to the virus not having such membrane. Further, some virus is resistant to the general heating process (55 – 60°C, 30 minutes). When clearance is expected for such virus, introduction of a further severe condition or process, which has different principle or mechanism, is necessary. Virus removal membrane filtration, which is different from S/D or heat treatment in aspect of principle, is effective to a broad range of virus that can not pass through the membrane. Affinity chromatography process, which specifically absorbs the objective protein, can thoroughly wash out the materials other than the objective protein including virus etc. and is generally effective for viral removal. Separation/fractionation of a virus from an objective protein is sometimes very difficult, but there are not so rare that ion exchange chromatography, ethanol fractionation, etc. is effective for clearance of a virus which can not be sufficiently inactivated or removed by the other process.

(15) Effective clearance may be achieved by any of the following: multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps. Separation methods may be dependent on the extremely specific physico-chemical properties of a virus which influence its interaction with gel matrices and precipitation properties. However, despite these potential variables, effective removal can be obtained by a combination of complementary separation steps or combinations of inactivation and separation steps. Well designed separation steps, such as chromatographic procedures, filtration steps and extractions, can be also effective virus removal steps provided that they are performed under appropriately controlled conditions.

(16) An effective virus removal step should give reproducible reduction of virus load shown by at least two independent studies.

(17) Over time and after repeated use, the ability of chromatography columns and other devices used in the purification scheme to clear virus may vary. Some estimate of the stability of the viral clearance after several uses may provide support for repeated use of such columns.

(18) The Notice, Iyakushin No. 329, would be used as a reference when viral clearance studies on biological products are designed.

6.4 Interpretation of viral clearance studies

6.4.1 Evaluation on viral clearance factor

Viral clearance factor is a logarithm of reduction ratio of viral amount (infectious titer) between each step applied for viral clearance of a manufacturing process. Total viral clearance factor throughout the process is sum of the viral clearance factor of each step appropriately evaluated.

Whether each and total viral clearance factor obtained are acceptable or should not be evaluated in aspects of every virus that can be realistically anticipated to derive into the raw material or the manufacturing process, and its rationality should be recorded.

In case that existence of any viral particle is recognized in a substrate for drug production, e.g., a substrate of rodent origin for biodrug production, it is important not only to demonstrate removal or inactivation of such virus, but also to demonstrate that the purification process has enough capability over the required level to assure safety of the final product at an appropriate level. The virus amount removed or inactivated in a manufacturing process should be compared with the virus amount assumed to exist in the substrate etc. used for manufacturing drug, and for this purpose, it is necessary to obtain the virus amount in the raw materials/substrate, etc. Such figure can be obtained by measuring infectious titer or by the other method such as transmission electron microscope (TEM). For evaluation of overall process, a virus amount, far larger than that assumed to exist in the amount of the raw materials/substrate which is equivalent to single administration of the final product, has to be removed. It is quite rare that existence of virus can be assumed in a substrate for drug production, with the exception of the substrate of rodent origin, and such suspicious raw material/substrate should not be used for manufacturing drug with a special exceptional case that the drug in question is not available from the other process and is clinically indispensable, and that the information including infectious properties of the virus particle assumed to exist has been clarified.

6.4.2 Calculation of viral clearance index

Viral clearance factor, "R", for viral removal/inactivation process can be calculated by the following formula.

$$R = \log[(V_1 \times T_1)/(V_2 \times T_2)]$$

In which

- R: Logarithm of reduction ratio
- V₁: Sample volume of the unprocessed material
- T₁: Virus amount (titer) of the unprocessed material
- V₂: Sample volume of the processed material
- T₂: Virus amount (titer) of the processed material

At the calculation of viral clearance factor, it is recommendable to use the virus titer detected in the sample preparation of the unprocessed material after addition of virus, not the viral titer added to the sample preparation wherever possible. If this is not possible, loaded virus amount is calculated from virus titer of the solution used for spike.

6.4.3 Interpretation of results and items to be concerned at evaluation

At the interpretation and the evaluation of the data on effectiveness of viral inactivation/removal process, there are various factors to be comprehensively taken into account, such as ① appropriateness of the virus used for the test, ② design of the viral clearance studies, ③ virus reduction ratio shown in logarithm, ④ time dependence of inactivation, ⑤ factors/items which give influence to the inactivation/

removal process, ⑥ sensitivity limit of virus assay method, ⑦ possible effect of the inactivation/removal process which is specific to certain class of viruses.

Additional items to be concerned at appropriate interpretation and evaluation of the viral clearance data are as follows:

(1) Behavior of virus used to the test

At interpretation of the vial clearance results, it is necessary to recognize that clearance mechanism may differ depending upon the virus used for the test. Virus used for a test is generally produced in tissue culture, but behavior of the virus prepared in the tissue culture may be different from that of the native virus. Examples are possible differences of purity and degree of aggregation between the native and the cultured viruses. Further, change of surface properties of a virus, e.g., addition of a sucrose chain which is ascribed to specific nature of a separation process, may give effect to the separation. These matters should be also considered at interpretation of the results.

(2) Design of test

Viral clearance test should have been designed taking into account variation factors of the manufacturing process and scaling down, but there still remain some variance from actual production scale. It is necessary to consider such variance at the interpretation of the data and limitation of the test.

(3) Acceptability of viral reduction data

Total viral clearance factor is expressed as a sum of logarithm of reduction ratio obtained at each step. The summation of the reduction factor of multiple steps, particularly of steps with little reduction (e.g., below 1 log₁₀), may overestimate viral removal/inactivation capability of the overall process. Therefore, virus titer of the order of 1 log₁₀ or less has to be ignored unless justified. Further, viral clearance factor achieved by repeated use of the same or similar method should be ignored for calculation unless justified.

(4) Time dependence of inactivation

Inactivation of virus infectivity frequently shows biphasic curve, which consists of a rapid initial phase and subsequent slow phase. It is possible that a virus not inactivated in a step may be more resistant to the subsequent step. For example, if an inactivated virus forms coagulation, it may be resistant to any chemical treatment and heating.

(5) Evaluation of viral reduction ratio shown logarithm

Viral clearance factor shown in logarithm of reduction ratio of virus titer can demonstrate drastic reduction of residual infectious virus, but there is a limit that infectious titer can never be reduced to zero. For example, reduction in infectivity of a preparation containing 8 log₁₀ infectious unit per mL by a factor of 8 log₁₀ leaves zero log₁₀ per mL or one infectious unit per mL, taking into account the detection limit of assay.

(6) Variable factor of manufacturing process

Minor variance of a variation factor of a manufacturing process, e.g., contact time of a spiked sample to a buffer or a column, will sometimes give influence to viral removal or inactivation effect. In such case, it may be necessary to investigate to what extent such variance of the factor has given influence to the process concerned in aspect of viral inactivation.

(7) Existence of anti-viral antiserum

Anti-viral antiserum that exists in the sample preparation used for a test may affect sensitivity of distribution or inactivation of a virus, which may result in not only defusing the

virus titer but complicating interpretation of the test result. So, existence of anti-viral antiserum is one of the important variable factors.

(8) Introduction of a new process for removal/inactivation

Viral clearance is an important factor for securing safety of drug. In case that an achievement level of infective clearance of a process is considered insufficient, a process which is characterized by inactivation/removal mechanism to meet the purpose or an inactivation/removal process which can mutually complement to the existence process has to be introduced.

(9) Limit of viral clearance studies

Viral clearance studies are useful for contributing to the assurance that an acceptable level of safety in the final product is achieved but do not by themselves establish safety. However, a number of factors in the design and execution of viral clearance studies may lead to an incorrect estimate of the ability of the process to remove virus infectivity, as described above.

7. Statistics

The viral clearance studies should include the use of statistical analysis of the data to evaluate the results. The study results should be statistically valid to support the conclusions reached.

7.1 Statistical considerations for assessing virus assays

Virus titrations suffer the problems of variation common to all biological assay systems. Assessment of the accuracy of the virus titrations and reduction factors derived from them and the validity of the assays should be performed to define the reliability of a study. The objective of statistical evaluation is to establish that the study has been carried out to an acceptable level of virological competence.

Assay

1. Assay methods may be either quantal or quantitative. Both quantal and quantitative assays are amenable to statistical evaluation.

2. Variation can arise within an assay as a result of dilution errors, statistical effects and differences within the assay system which are either unknown or difficult to control. These effects are likely to be greater when different assay runs are compared (between-assay variation) than when results within a single assay run are compared (within-assay variation).

3. The 95% confidence limits for results of within-assay variation normally should be on the order of $\pm 0.5 \log_{10}$ of the mean. Within-assay variation can be assessed by standard textbook methods. Between-assay variation can be monitored by the inclusion of a reference preparation, the estimate of whose potency should be within approximately $0.5 \log_{10}$ of the mean estimate established in the laboratory for the assay to be acceptable. Assays with lower precision may be acceptable with appropriate justification.

7.2 Reproducibility and confidence limit of viral clearance studies

An effective virus inactivation/removal step should give reproducible reduction of virus load shown by at least two independent studies. The 95% confidence limits for the reduction factor observed should be calculated wherever possible in studies of viral clearance. If the 95% confidence limits for the viral assays of the starting material are $\pm s$, and for the viral assays of the material after the step are $\pm a$, the 95% confidence limits for the reduction factor are $\pm \sqrt{s^2 + a^2}$.

8. Re-evaluation of viral clearance

Whenever significant changes in the production or purification process are made, the effect of that change, both direct and indirect, on viral clearance should be considered and the system re-evaluated as needed. Changes in process steps may also change the extent of viral clearance.

9. Measurement for viral clearance studies

9.1 Measurement of virus infective titer

Assay methods may be either quantal or quantitative. Quantal methods include infectivity assays in animals or in cultured cell infections dose (CCID) assays, in which the animal or cell culture is scored as either infected or not. Infectivity titers are then measured by the proportion of animals or culture infected. In quantitative methods, the infectivity measured varies continuously with the virus input. Quantitative methods include plaque assays where each plaque counted corresponds to a single infectious unit. Both quantal and quantitative assays are amenable to statistical evaluation.

9.2 Testing by nucleic-acid-amplification test (NAT)

NAT can detect individual or pooled raw material/cell substrate or virus genome at a high sensitivity even in a stage that serum test on each virus is negative. Further, it can detect HBV or HCV gene, which can not be measured in culture system. Window period can be drastically shortened at the test on HBV, HCV and HIV, and the method is expected to contribute as an effective measure for ensuring viral safety. However, depending upon a choice of primer, there may be a case that not all the subtype of objective virus can be detected by this method, and, therefore, it is recommendable to evaluate, in advance, if subtype of a broad range can be detected.

NAT will be an effective evaluation method for virus removal capability at viral clearance studies. However, in case of viral inactivation process, viral inactivation obtained by this method may be underrated, since there is a case that inactivated virus still shows positive on nucleic acid. Further, at introduction of NAT, cautions should be taken on rationality of detection sensitivity, choice of a standard which is used as run-control, quality assurance and maintenance of a reagent used for primer, interpretation of positive and negative results, etc.

10. Reporting and preservation

All the items relating to virus test and viral clearance studies should be reported and preserved.

11. Others

The Notice, Iyakushin No. 329, should be used as a reference at virus test and viral clearance studies.

Conclusion

As mentioned at the Introduction, assurance of quality/safety etc. of JP listed drugs should be achieved by state-of-the-art methods and concepts reflecting the progress of science and accumulation of experiences.

The basis for ensuring viral safety of JP listed biotechnological/biological products is detailed in this General Information. What is discussed here is that an almost equal level of measures are required for both development of new drugs and for existing products as well, which means that similar level of concerns should be paid on both existing and new products in aspect of viral safety. This document is intended to introduce a basic concept that quality and safety assurance of JP listed product should be based upon the most advanced

methods and concepts. This document has been written to cover all conceivable cases, which can be applied to all biotechnological/biological products. Therefore, there may be cases that it is not so rational to pursue virus tests and viral clearance studies in accordance with this document on each product, which has been used for a long time without any safety issue. So, it will be necessary to elaborate the most rational ways under a case-by-case principle taking into due consideration source, origin, type, manufacturing process, characteristics, usages at clinical stage, accumulation of the past usage record, etc. relating to such biotechnological/biological products.

4. Capillary Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

General Principles

Capillary electrophoresis is a physical method of analysis based on the migration, inside a capillary, of charged analytes dissolved in an electrolyte solution, under the influence of a direct-current electric field.

The migration velocity of an analyte under an electric field of intensity E , is determined by the electrophoretic mobility of the analyte and the electro-osmotic mobility of the buffer inside the capillary. The electrophoretic mobility of a solute (μ_{ep}) depends on the characteristics of the solute (electric charge, molecular size and shape) and those of the buffer in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and additives). The electrophoretic velocity (v_{ep}) of a solute, assuming a spherical shape, is given by the equation:

$$v_{ep} = \mu_{ep} E = \left(\frac{q}{6\pi\eta r} \right) \left(\frac{V}{L} \right)$$

- q : effective charge of the solute,
- η : viscosity of the electrolyte solution,
- r : Stoke's radius of the solute,
- V : applied voltage,
- L : total length of the capillary.

When an electric field is applied through the capillary filled with buffer, a flow of solvent is generated inside the capillary, called electro-osmotic flow. The velocity of the electro-osmotic flow depends on the electro-osmotic mobility (μ_{eo}) which in turn depends on the charge density on the capillary internal wall and the buffer characteristics. The electro-osmotic velocity (v_{eo}) is given by the equation:

$$v_{eo} = \mu_{eo} E = \left(\frac{\varepsilon\zeta}{\eta} \right) \left(\frac{V}{L} \right)$$

- ε : dielectric constant of the buffer,
- ζ : zeta potential of the capillary surface.

The velocity of the solute (v) is given by:

$$v = v_{ep} + v_{eo}$$

The electrophoretic mobility of the analyte and the electro-osmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute. In normal capillary electrophoresis, anions will migrate in the opposite

direction to the electro-osmotic flow and their velocities will be smaller than the electro-osmotic velocity. Cations will migrate in the same direction as the electro-osmotic flow and their velocities will be greater than the electro-osmotic velocity. Under conditions in which there is a fast electro-osmotic velocity with respect to the electrophoretic velocity of the solutes, both cations and anions can be separated in the same run.

The time (t) taken by the solute to migrate the distance (l) from the injection end of the capillary to the detection point (capillary effective length) is given by the expression:

$$t = \frac{l}{v_{ep} + v_{eo}} = \frac{l \times L}{(\mu_{ep} + \mu_{eo}) V}$$

In general, uncoated fused-silica capillaries above pH 3 have negative charge due to ionized silanol groups in the inner wall. Consequently, the electro-osmotic flow is from anode to cathode. The electro-osmotic flow must remain constant from run to run if good reproducibility is to be obtained in the migration velocity of the solutes. For some applications, it may be necessary to reduce or suppress the electro-osmotic flow by modifying the inner wall of the capillary or by changing the concentration, composition and/or pH of the buffer solution.

After the introduction of the sample into the capillary, each analyte ion of the sample migrates within the background electrolyte as an independent zone, according to its electrophoretic mobility. Zone dispersion, that is the spreading of each solute band, results from different phenomena. Under ideal conditions the sole contribution to the solute-zone broadening is molecular diffusion of the solute along the capillary (longitudinal diffusion). In this ideal case the efficiency of the zone, expressed as the number of theoretical plates (N), is given by:

$$N = \frac{(\mu_{ep} + \mu_{eo}) \times V \times L}{2 \times D \times L}$$

- D : molecular diffusion coefficient of the solute in the buffer.

In practice, other phenomena such as heat dissipation, sample adsorption onto the capillary wall, mismatched conductivity between sample and buffer, length of the injection plug, detector cell size and unlevelled buffer reservoirs can also significantly contribute to band dispersion.

Separation between two bands (expressed as the resolution, R_s) can be obtained by modifying the electrophoretic mobility of the analytes, the electro-osmotic mobility induced in the capillary and by increasing the efficiency for the band of each analyte, according to the equation:

$$R_s = \frac{\sqrt{N}(\mu_{epb} - \mu_{epa})}{4(\bar{\mu}_{ep} + \mu_{eo})}$$

- μ_{epa} and μ_{epb} : electrophoretic mobilities of the two analytes separated,

$\bar{\mu}_{ep}$: mean electrophoretic mobility of the two analytes

$$\bar{\mu}_{ep} = \frac{1}{2} (\mu_{epb} + \mu_{epa}).$$

Apparatus

An apparatus for capillary electrophoresis is composed of:—a high-voltage, controllable direct-current power supply, —two buffer reservoirs, held at the same level, containing the prescribed anodic and cathodic solutions,

- two electrode assemblies (the cathode and the anode), immersed in the buffer reservoirs and connected to the power supply,
- a separation capillary (usually made of fused-silica) which, when used with some specific types of detectors, has an optical viewing window aligned with the detector. The ends of the capillary are placed in the buffer reservoirs. The capillary is filled with the solution prescribed in the monograph,
- a suitable injection system,
- a detector able to monitor the amount of substances of interest passing through a segment of the separation capillary at a given time. It is usually based on absorption spectrophotometry (UV and visible) or fluorometry, but conductimetric, amperometric or mass spectrometric detection can be useful for specific applications. Indirect detection is an alternative method used to detect non-UV-absorbing and non-fluorescent compounds,
- a thermostatic system able to maintain a constant temperature inside the capillary is recommended to obtain a good separation reproducibility,
- a recorder and a suitable integrator or a computer.

The definition of the injection process and its automation are critical for precise quantitative analysis. Modes of injection include gravity, pressure or vacuum injection and electrokinetic injection. The amount of each sample component introduced electrokinetically depends on its electrophoretic mobility, leading to possible discrimination using this injection mode.

Use the capillary, the buffer solutions, the preconditioning method, the sample solution and the migration conditions prescribed in the monograph of the considered substance. The employed electrolytic solution is filtered to remove particles and degassed to avoid bubble formation that could interfere with the detection system or interrupt the electrical contact in the capillary during the separation run. A rigorous rinsing procedure should be developed for each analytical method to achieve reproducible migration times of the solutes.

1. Capillary Zone Electrophoresis

Principle

In capillary zone electrophoresis, analytes are separated in a capillary containing only buffer without any anticonvective medium. With this technique, separation takes place because the different components of the sample migrate as discrete bands with different velocities. The velocity of each band depends on the electrophoretic mobility of the solute and the electro-osmotic flow in the capillary (see General Principles). Coated capillaries can be used to increase the separation capacity of those substances adsorbing on fused-silica surfaces.

Using this mode of capillary electrophoresis, the analysis of both small ($M_r < 2000$) and large molecules ($2000 < M_r < 100,000$) can be accomplished. Due to the high efficiency achieved in free solution capillary electrophoresis, separation of molecules having only minute differences in their charge-to-mass ratio can be effected. This separation mode also allows the separation of chiral compounds by addition of chiral selectors to the separation buffer.

Optimization

Optimization of the separation is a complex process where several separation parameters can play a major role. The

main factors to be considered in the development of separations are instrumental and electrolytic solution parameters.

Instrumental parameters

Voltage: A Joule heating plot is useful in optimizing the applied voltage and column temperature. Separation time is inversely proportional to applied voltage. However, an increase in the voltage used can cause excessive heat production, giving rise to temperature and, as a result thereof, viscosity gradients in the buffer inside the capillary. This effect causes band broadening and decreases resolution.

Polarity: Electrode polarity can be normal (anode at the inlet and cathode at the outlet) and the electro-osmotic flow will move toward the cathode. If the electrode polarity is reversed, the electro-osmotic flow is away from the outlet and only charged analytes with electro-osmotic mobilities greater than the electro-osmotic flow will pass to the outlet.

Temperature: The main effect of temperature is observed on buffer viscosity and electrical conductivity, and therefore on migration velocity. In some cases, an increase in capillary temperature can cause a conformational change in proteins, modifying their migration time and the efficiency of the separation.

Capillary: The dimensions of the capillary (length and internal diameter) contribute to analysis time, efficiency of separations and load capacity. Increasing both effective length and total length can decrease the electric fields (working at constant voltage) which increases migration time. For a given buffer and electric field, heat dissipation, and hence sample band-broadening, depend on the internal diameter of the capillary. The latter also affects the detection limit, depending on the sample volume injected and the detection system employed.

Since the adsorption of the sample components on the capillary wall limits efficiency, methods to avoid these interactions should be considered in the development of a separation method. In the specific case of proteins, several strategies have been devised to avoid adsorption on the capillary wall. Some of these strategies (use of extreme pH and adsorption of positively charged buffer additives) only require modification of the buffer composition to prevent protein adsorption. In other strategies, the internal wall of the capillary is coated with a polymer, covalently bonded to the silica, that prevents interaction between the proteins and the negatively charged silica surface. For this purpose, ready-to-use capillaries with coatings consisting of neutral-hydrophilic, cationic and anionic polymers are available.

Electrolytic solution parameters

Buffer type and concentration: Suitable buffers for capillary electrophoresis have an appropriate buffer capacity in the pH range of choice and low mobility to minimize current generation.

Matching buffer-ion mobility to solute mobility, whenever possible, is important for minimizing band distortion. The type of sample solvent used is also important to achieve on-column sample focusing, which increases separation efficiency and improves detection.

An increase in buffer concentration (for a given pH) decreases electro-osmotic flow and solute velocity.

Buffer pH: The pH of the buffer can affect separation by modifying the charge of the analyte or additives, and by changing the electro-osmotic flow. In protein and peptide separation, changing the pH of the buffer from above to below the isoelectric point (pI) changes the net charge of the

solute from negative to positive. An increase in the buffer pH generally increases the electro-osmotic flow.

Organic solvents: Organic modifiers (methanol, acetonitrile, etc.) may be added to the aqueous buffer to increase the solubility of the solute or other additives and/or to affect the degree of ionization of the sample components. The addition of these organic modifiers to the buffer generally causes a decrease in the electro-osmotic flow.

Additives for chiral separations: For the separation of optical isomers, a chiral selector is added to the separation buffer. The most commonly used chiral selectors are cyclodextrins, but crown ethers, polysaccharides and proteins may also be used. Since chiral recognition is governed by the different interactions between the chiral selector and each of the enantiomers, the resolution achieved for the chiral compounds depends largely on the type of chiral selector used. In this regard, for the development of a given separation it may be useful to test cyclodextrins having a different cavity size (α -, β -, or γ -cyclodextrin) or modified cyclodextrins with neutral (methyl, ethyl, hydroxyalkyl, etc.) or ionizable (aminomethyl, carboxymethyl, sulfobutyl ether, etc.) groups. When using modified cyclodextrins, batch-to-batch variations in the degree of substitution of the cyclodextrins must be taken into account since it will influence the selectivity. Other factors controlling the resolution in chiral separations are concentration of chiral selector, composition and pH of the buffer and temperature. The use of organic additives, such as methanol or urea can also modify the resolution achieved.

2. Capillary Gel Electrophoresis

Principle

In capillary gel electrophoresis, separation takes place inside a capillary filled with a gel that acts as a molecular sieve. Molecules with similar charge-to-mass ratios are separated according to molecular size since smaller molecules move more freely through the network of the gel and therefore migrate faster than larger molecules. Different biological macromolecules (for example, proteins and DNA fragments), which often have similar charge-to-mass ratios, can thus be separated according to their molecular mass by capillary gel electrophoresis.

Characteristics of Gels

Two types of gels are used in capillary electrophoresis: permanently coated gels and dynamically coated gels. Permanently coated gels, such as cross-linked polyacrylamide, are prepared inside the capillary by polymerization of the monomers. They are usually bonded to the fused-silica wall and cannot be removed without destroying the capillary. If the gels are used for protein analysis under reducing conditions, the separation buffer usually contains sodium dodecyl sulfate and the samples are denatured by heating a mixture of sodium dodecyl sulfate and 2-mercaptoethanol or dithiothreitol before injection. When non-reducing conditions are used (for example, analysis of an intact antibody), 2-mercaptoethanol and dithiothreitol are not used. Separation in cross-linked gels can be optimized by modifying the separation buffer (as indicated in the free solution capillary electrophoresis section) and controlling the gel porosity during the gel preparation. For cross-linked polyacrylamide gels, the porosity can be modified by changing the concentration of acrylamide and/or the proportion of cross-linker. As a rule, a decrease in the porosity of the gel leads to a decrease

in the mobility of the solutes. Due to the rigidity of these gels, only electrokinetic injection can be used.

Dynamically coated gels are hydrophilic polymers, such as linear polyacrylamide, cellulose derivatives, dextran, etc., which can be dissolved in aqueous separation buffers giving rise to a separation medium that also acts as a molecular sieve. These separation media are easier to prepare than cross-linked polymers. They can be prepared in a vial and filled by pressure in a wall-coated capillary (with no electro-osmotic flow). Replacing the gel before every injection generally improves the separation reproducibility. The porosity of the gels can be increased by using polymers of higher molecular mass (at a given polymer concentration) or by decreasing the polymer concentration (for a given polymer molecular mass). A reduction in the gel porosity leads to a decrease in the mobility of the solute for the same buffer. Since the dissolution of these polymers in the buffer gives low viscosity solutions, both hydrodynamic and electrokinetic injection techniques can be used.

3. Capillary Isoelectric Focusing

Principle

In isoelectric focusing, the molecules migrate under the influence of the electric field, so long as they are charged, in a pH gradient generated by ampholytes having pI values in a wide range (poly-aminocarboxylic acids), dissolved in the separation buffer.

The three basic steps of isoelectric focusing are loading, focusing and mobilization.

Loading step: Two methods may be employed:

—loading in one step: the sample is mixed with ampholytes and introduced into the capillary either by pressure or vacuum;

—sequential loading: a leading buffer, then the ampholytes, then the sample mixed with ampholytes, again ampholytes alone and finally the terminating buffer are introduced into the capillary. The volume of the sample must be small enough not to modify the pH gradient.

Focusing step: When the voltage is applied, ampholytes migrate toward the cathode or the anode, according to their net charge, thus creating a pH gradient from anode (lower pH) to cathode (higher pH). During this step the components to be separated migrate until they reach a pH corresponding to their isoelectric point (pI) and the current drops to very low values.

Mobilization step: If mobilization is required for detection, use one of the following methods. Three methods are available:

—in the first method, mobilization is accomplished during the focusing step under the effect of the electro-osmotic flow; the electro-osmotic flow must be small enough to allow the focusing of the components;

—in the second method, mobilization is accomplished by applying positive pressure after the focusing step;

—in the third method, mobilization is achieved after the focusing step by adding salts to the cathode reservoir or the anode reservoir (depending on the direction chosen for mobilization) in order to alter the pH in the capillary when the voltage is applied. As the pH is changed, the proteins and ampholytes are mobilized in the direction of the reservoir which contains the added salts and pass the detector.

The separation achieved, expressed as ΔpI , depends on the

pH gradient (dpH/dx), the number of ampholytes having different pI values, the molecular diffusion coefficient (D), the intensity of the electric field (E) and the variation of the electrophoretic mobility of the analyte with the pH ($-d\mu/dpH$):

$$\Delta PI = 3 \sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

Optimization

The main parameters to be considered in the development of separations are:

Voltage: Capillary isoelectric focusing utilises very high electric fields, 300 V/cm to 1000 V/cm in the focusing step.

Capillary: The electro-osmotic flow must be reduced or suppressed depending on the mobilization strategy (see above). Coated capillaries tend to reduce the electro-osmotic flow.

Solutions: The anode buffer reservoir is filled with a solution with a pH lower than the pI of the most acidic ampholyte and the cathode reservoir is filled with a solution with a pH higher than the pI of the most basic ampholyte. Phosphoric acid for the anode and sodium hydroxide for the cathode are frequently used.

Addition of a polymer, such as methylcellulose, in the ampholyte solution tends to suppress convective forces (if any) and electro-osmotic flow by increasing the viscosity. Commercial ampholytes are available covering many pH ranges and may be mixed if necessary to obtain an expanded pH range. Broad pH ranges are used to estimate the isoelectric point whereas narrower ranges are employed to improve accuracy. Calibration can be done by correlating migration time with isoelectric point for a series of protein markers.

During the focusing step precipitation of proteins at their isoelectric point can be prevented, if necessary, using buffer additives such as glycerol, surfactants, urea or zwitterionic buffers. However, depending on the concentration, urea denatures proteins.

4. Micellar Electrokinetic Chromatography (MEKC)

Principle

In micellar electrokinetic chromatography, separation takes place in an electrolyte solution which contains a surfactant at a concentration above the critical micellar concentration (cmc). The solute molecules are distributed between the aqueous buffer and the pseudo-stationary phase composed of micelles, according to the partition coefficient of the solute. The technique can therefore be considered as a hybrid of electrophoresis and chromatography. It is a technique that can be used for the separation of both neutral and charged solutes, maintaining the efficiency, speed and instrumental suitability of capillary electrophoresis. One of the most widely used surfactants in MEKC is the anionic surfactant sodium dodecyl sulfate, although other surfactants, for example cationic surfactants such as cetyltrimethylammonium salts, are also used.

The separation mechanism is as follows. At neutral and alkaline pH, a strong electro-osmotic flow is generated and moves the separation buffer ions in the direction of the cathode. If sodium dodecyl sulfate is employed as the surfactant, the electrophoretic migration of the anionic micelle is in the opposite direction, towards the anode. As a result, the overall micelle migration velocity is slowed down compared to the bulk flow of the electrolytic solution. In the case of neutral solutes, since the analyte can partition between the

micelle and the aqueous buffer, and has no electrophoretic mobility, the analyte migration velocity will depend only on the partition coefficient between the micelle and the aqueous buffer. In the electropherogram, the peaks corresponding to each uncharged solute are always between that of the electro-osmotic flow marker and that of the micelle (the time elapsed between these two peaks is called the separation window). For electrically charged solutes, the migration velocity depends on both the partition coefficient of the solute between the micelle and the aqueous buffer, and on the electrophoretic mobility of the solute in the absence of micelle.

Since the mechanism in MEKC of neutral and weakly ionized solutes is essentially chromatographic, migration of the solute and resolution can be rationalized in terms of the retention factor of the solute (k'), also referred to as mass distribution ratio (D_m), which is the ratio of the number of moles of solute in the micelle to those in the mobile phase. For a neutral compound, k' is given by:

$$k' = \frac{t_R - t_0}{t_0 \left(1 - \frac{t_R}{t_{mc}}\right)} = K \frac{V_S}{V_M}$$

t_R : migration time of the solute,

t_0 : analysis time of an unretained solute (determined by injecting an electro-osmotic flow marker which does not enter the micelle, for instance methanol),

t_{mc} : micelle migration time (measured by injecting a micelle marker, such as Sudan III, which migrates while continuously associated in the micelle),

K : partition coefficient of the solute,

V_S : volume of the micellar phase,

V_M : volume of the mobile phase.

Likewise, the resolution between two closely-migrating solutes (R_S) is given by:

$$R_S = \frac{\sqrt{N}}{4} \times \frac{\alpha - 1}{\alpha} \times \frac{k'_b}{k'_b + 1} \times \frac{1 - \left(\frac{t_0}{t_{mc}}\right)}{1 + \left(\frac{t_0}{t_{mc}}\right)k'_a}$$

N : number of theoretical plates for one of the solutes,

α : selectivity,

k'_a and k'_b : retention factors for both solutes, respectively ($k'_b > k'_a$).

Similar, but not identical, equations give k' and R_S values for electrically charged solutes.

Optimization

The main parameters to be considered in the development of separations by MEKC are instrumental and electrolytic solution parameters.

Instrumental parameters

Voltage: Separation time is inversely proportional to applied voltage. However, an increase in voltage can cause excessive heat production that gives rise to temperature gradients and viscosity gradients of the buffer in the cross-section of the capillary. This effect can be significant with high conductivity buffers such as those containing micelles. Poor heat dissipation causes band broadening and decreases resolution.

Temperature: Variations in capillary temperature affect the partition coefficient of the solute between the buffer and the micelles, the critical micellar concentration and the

viscosity of the buffer. These parameters contribute to the migration time of the solutes. The use of a good cooling system improves the reproducibility of the migration time for the solutes.

Capillary: As in free solution capillary electrophoresis, the dimensions of the capillary (length and internal diameter) contribute to analysis time and efficiency of separations. Increasing both effective length and total length can decrease the electric fields (working at constant voltage), increase migration time and improve the separation efficiency. The internal diameter controls heat dissipation (for a given buffer and electric field) and consequently the sample band broadening.

Electrolytic solution parameters

Surfactant type and concentration: The type of surfactant, in the same way as the stationary phase in chromatography, affects the resolution since it modifies separation selectivity. Also, the $\log k'$ of a neutral compound increases linearly with the concentration of surfactant in the mobile phase. Since resolution in MEKC reaches a maximum when k' approaches the value of $\sqrt{t_m/t_0}$, modifying the concentration of surfactant in the mobile phase changes the resolution obtained.

Buffer pH: Although pH does not modify the partition coefficient of non-ionized solutes, it can modify the electro-osmotic flow in uncoated capillaries. A decrease in the buffer pH decreases the electro-osmotic flow and therefore increases the resolution of the neutral solutes in MEKC, resulting in a longer analysis time.

Organic solvents: To improve MEKC separation of hydrophobic compounds, organic modifiers (methanol, propanol, acetonitrile, etc.) can be added to the electrolytic solution. The addition of these modifiers usually decreases migration time and the selectivity of the separation. Since the addition of organic modifiers affects the critical micellar concentration, a given surfactant concentration can be used only within a certain percentage of organic modifier before the micellization is inhibited or adversely affected, resulting in the absence of micelles and, therefore, in the absence of partition. The dissociation of micelles in the presence of a high content of organic solvent does not always mean that the separation will no longer be possible; in some cases the hydrophobic interaction between the ionic surfactant monomer and the neutral solutes forms solvophobic complexes that can be separated electrophoretically.

Additives for chiral separations: For the separation of enantiomers using MEKC, a chiral selector is included in the micellar system, either covalently bound to the surfactant or added to the micellar separation electrolyte. Micelles that have a moiety with chiral discrimination properties include salts of *N*-dodecanoyl-L-amino acids, bile salts, etc. Chiral resolution can also be achieved using chiral discriminators, such as cyclodextrins, added to the electrolytic solutions which contain micellized achiral surfactants.

Other additives: Several strategies can be carried out to modify selectivity, by adding chemicals to the buffer. The addition of several types of cyclodextrins to the buffer can also be used to reduce the interaction of hydrophobic solutes with the micelle, thus increasing the selectivity for this type of compound.

The addition of substances able to modify solute-micelle interactions by adsorption on the latter, is used to improve the selectivity of the separations in MEKC. These additives

may be a second surfactant (ionic or non-ionic) which gives rise to mixed micelles or metallic cations which dissolve in the micelle and form co-ordination complexes with the solutes.

Quantification

Peak areas must be divided by the corresponding migration time to give the corrected area in order to:

- compensate for the shift in migration time from run to run, thus reducing the variation of the response,
- compensate for the different responses of sample constituents with different migration times.

Where an internal standard is used, verify that no peak of the substance to be examined is masked by that of the internal standard.

Calculations

From the values obtained, calculate the content of the component or components being examined. When prescribed, the percentage content of one or more components of the sample to be examined is calculated by determining the corrected area(s) of the peak(s) as a percentage of the total of the corrected areas of all peaks, excluding those due to solvents or any added reagents (normalization procedure). The use of an automatic integration system (integrator or data acquisition and processing system) is recommended.

System Suitability

In order to check the behavior of the capillary electrophoresis system, system suitability parameters are used. The choice of these parameters depends on the mode of capillary electrophoresis used. They are: retention factor (k') (only for micellar electrokinetic chromatography), apparent number of theoretical plates (N), symmetry factor (A_S) and resolution (R_S). In previous sections, the theoretical expressions for N and R_S have been described, but more practical equations that allow these parameters to be calculated from the electropherograms are given below.

Apparent Number of Theoretical Plates

The apparent number of theoretical plates (N) may be calculated using the expression:

$$N = 5.54 \left(\frac{t_R}{w_h} \right)^2$$

t_R : migration time or distance along the baseline from the point of injection to the perpendicular dropped from the maximum of the peak corresponding to the component,

w_h : width of the peak at half-height.

Resolution

The resolution (R_S) between peaks of similar height of two components may be calculated using the expression:

$$R_S = \left(\frac{1.18(t_{R2} - t_{R1})}{w_{h1} + w_{h2}} \right)$$

$$t_{R2} > t_{R1}$$

t_{R1} and t_{R2} : migration times or distances along the baseline from the point of injection to the perpendiculars dropped from the maxima of two adjacent peaks,

w_{h1} and w_{h2} : peak widths at half-height.

When appropriate, the resolution may be calculated by measuring the height of the valley (H_v) between two partly resolved peaks in a standard preparation and the height of the smaller peak (H_p) and calculating the peak-to-valley ratio:

$$p/v = \frac{H_p}{H_v}$$

Symmetry Factor

The symmetry factor (A_s) of a peak may be calculated using the expression:

$$A_s = \frac{w_{0.05}}{2d}$$

$w_{0.05}$: width of the peak at one-twentieth of the peak height,

d : distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

Tests for area repeatability (standard deviation of areas or of the area/migration-time ratio) and for migration time repeatability (standard deviation of migration time) are introduced as suitability parameters. Migration time repeatability provides a test for the suitability of the capillary washing procedures. An alternative practice to avoid the lack of repeatability of the migration time is to use migration time relative to an internal standard.

A test for the verification of the signal-to-noise ratio for a standard preparation (or the determination of the limit of quantification) may also be useful for the determination of related substances.

Signal-to-noise Ratio

The detection limit and quantification limit correspond to signal-to-noise ratios of 3 and 10 respectively. The signal-to-noise ratio (S/N) is calculated using the expression:

$$S/N = \frac{2H}{h}$$

H : height of the peak corresponding to the component concerned, in the electropherogram obtained with the prescribed reference solution, measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to twenty times the width at half-height,

h : range of the background in an electropherogram obtained after injection of a blank, observed over a distance equal to twenty times the width at the half-height of the peak in the electropherogram obtained with the prescribed reference solution and, if possible, situated equally around the place where this peak would be found.

5. Decision of Limit for Bacterial Endotoxins

The endotoxin limit for injections is to be decided as follows:

$$\text{Endotoxin limit} = K/M$$

where K is a minimum pyrogenic dose of endotoxin per kg body mass (EU/kg), and M is equal to the maximum dose of product per kg per hour.

M is expressed in mL/kg for products to be administered by volume, in mg/kg or mEq/kg for products to be administered by mass, and in Unit/kg for products to be administered by biological units. Depending on the administration route, values for K are set as in the following table.

Intended route of administration	K (EU/kg)
Intravenous	5.0
Intravenous, for radiopharmaceuticals	2.5
Intraspinal	0.2

Notes:

- 1) For products to be administered by mass or by units, the endotoxin limit should be decided based on the labeled amount of the principal drug.
- 2) Sixty kg should be used as the average body mass of an adult when calculating the maximum adult dose per kg.
- 3) The pediatric dose per kg body mass should be used when this is higher than the adult dose.
- 4) The K values for the intravenous route are applicable to drugs to be administered by any route other than those shown in the table.

6. Disinfection and Sterilization Methods

Disinfection and Sterilization Methods are applied to kill microorganisms in processing equipment/utensils and areas used for drug manufacturing, as well as to perform microbiological tests specified in the monographs, and so differ from "Terminal Sterilization" and "Filtration Method" described in "Terminal Sterilization and Sterilization Indicators". The killing effect on microorganisms or the estimated level of sterility assurance is greatly variable, so the conditions for disinfection and sterilization treatment must be chosen appropriately for each application. Generally, the following methods are to be used singly or in combination after appropriate optimization of operation procedures and conditions, in accordance with the kind and the degree of the contaminating microorganisms and the nature of the item to which the methods are applied.

The validation of sterilization in accordance with Terminal Sterilization and Sterilization Indicators is required when the methods are applied to the manufacturing processes of drug products.

1. Disinfection methods

These methods are used to reduce the number of living microorganisms, but do not always remove or kill all microorganisms present. Generally, disinfection is classified into chemical disinfection with the use of chemical drugs (disinfectants) and physical disinfection with the use of moist heat, ultraviolet light, and other agents.

1-1. Chemical disinfection

Microorganisms are killed with chemical drugs. The killing effect and mechanisms of a chemical drug differ depending on the type, applied concentration, action temperature, and action time of the chemical drug used, the degree of contamination on the object to be disinfected, and the species and state (e.g., vegetative bacteria or spore bacteria) of microorganisms.

Therefore, in applying the method, full consideration is required of the sterility and permissible storage period of the prepared chemical drug, the possibility of resistance of microorganisms at the site of application, and the effect of residual chemical drug on the product. In selecting a suitable chemical drug, the following items should be considered in relation to the intended use.

- 1) The antimicrobial spectrum
- 2) Action time for killing microorganisms
- 3) Action durability
- 4) Effect of the presence of proteins
- 5) Influence on the human body
- 6) Solubility in water
- 7) Influence on the object to be disinfected
- 8) Odor
- 9) Convenience of use
- 10) Easy disposability
- 11) Influence on the environment at disposal
- 12) Frequency of occurrence of resistance

1-2. Physical disinfection

Microorganisms are killed without a chemical drug.

(i) Steam flow method

Microorganisms are killed by direct application of steam. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is kept in flowing steam at 100°C for 30 – 60 minutes.

(ii) Boiling method

Microorganisms are killed by putting the object in boiling water. This method is used for a product which may be denatured by the moist heat method. As a rule, the product is put in boiling water for 15 minutes or more.

(iii) Intermittent method

Microorganisms are killed by heating for 30 – 60 minutes repeatedly, three to five times, once a day in water at 80 – 100°C or in steam. This method is used for a product which may be denatured by the moist heat method. There is another method called the low temperature intermittent method with repeated heating at 60 – 80°C. During the intermission periods between heating or warming, a suitable temperature for the growth of microorganisms of 20°C or higher, must be maintained.

(iv) Ultraviolet method

As a rule, microorganisms are killed by irradiation with ultraviolet rays at a wavelength of around 254 nm. This method is used for products which are resistant to ultraviolet rays, such as smooth-surfaced articles, facilities, and equipment, or water and air. This method does not suffer from the occurrence of resistance, which is observed in chemical disinfection, and shows a killing effect on bacteria, fungi, and viruses. It must be taken into consideration that direct ultraviolet irradiation of the human body can injure the eyes and skin.

2. Sterilization methods

2-1. Heating methods

In these methods, the heating time before the temperature or pressure reaches the prescribed value differs according to the properties of the product, the size of the container, and the conditions. The duration of heating in conducting these methods is counted from the time when all the parts of the product have reached the prescribed temperature.

(i) Moist heat method

Microorganisms are killed in saturated steam at a suitable

temperature and pressure. This method is generally used for heat-stable substances, such as glass, porcelain, metal, rubber, plastics, paper, and fiber, as well as heat-stable liquids, such as water, culture media, reagents, test solutions, liquid samples, etc. As a rule, one of the following conditions is used.

115 – 118°C for 30 minutes

121 – 124°C for 15 minutes

126 – 129°C for 10 minutes

(ii) Dry-heat method

Microorganisms are killed in dry-heated air. This method is generally used for heat-stable substances, such as glass, porcelain, and metal, as well as heat-stable products, such as mineral oils, fats and oils, powder samples, etc. This method is generally conducted in the way of direct heating by gas or electricity or circulating heated air. As a rule, one of the following conditions is used.

160 – 170°C for 120 minutes

170 – 180°C for 60 minutes

180 – 190°C for 30 minutes

2-2. Irradiation methods

(i) Radiation method

Microorganisms are killed by gamma-rays emitted from a radioisotope or electron beam and bremsstrahlung (X-ray) generated from an electron accelerator. This method is generally used for radiation-resistant substances such as glass, porcelain, metal, rubber, plastics, fiber, etc. The dose is decided according to the material properties, and the degree of contamination of the product to be sterilized. Special consideration is necessary of the possibility of qualitative change of the product after the application of the method.

(ii) Microwave method

Microorganisms are killed by the heat generated by direct microwave irradiation. This method is generally used for microwave-resistant products such as water, culture media, test solutions, etc. As a rule, microwave radiation with a wavelength of around 2450 ± 50 MHz is used.

2-3. Gas methods

Microorganisms are killed by a sterilizing gas. Suitable gases for killing microorganisms include ethylene oxide gas, formaldehyde gas, hydrogen peroxide gas, chlorine dioxide gas, etc. Temperature, humidity, the concentration of gas, and the exposure time differ in accordance with the species of gas used. As sterilizing gases are generally toxic to humans, full consideration is required of the environmental control for the use of gases and the concentration of residual gas. In some of the gas methods, it may be difficult to measure or estimate quantitatively the killing of microorganisms.

2-4. Filtration method

Microorganisms are removed by filtration with a suitable filtering device. This method is generally used for gas, water, or culture media and test solutions containing a substance that is water-soluble and unstable to heat. As a rule, a filter having a pore size of 0.22 μm or smaller is used for the sterilization. However, in this method, a filter with a pore size of 0.45 μm or smaller is permitted to be used.

7. Guideline for Residual Solvents, Residual Solvents Test, and Models for the Test in Monographs

1. Guideline for Residual Solvents

Refer to the Guideline for Residual Solvents in Pharmaceuticals (PAB/ELD Notification No.307; dated March 30, 1998).

The acceptable limits of residual solvents recommended in the Guideline were estimated to keep the safety of patients. The levels of residual solvents in pharmaceuticals should not exceed the limits, except for in a special case. Accordingly, pharmaceutical manufacturers should assure the quality of their products by performing the test with the products according to the Residual Solvents Test, to keep the limits recommended in the Guideline.

2. Residual Solvents Test

Method—Perform the test as directed in the Residual Solvents Test under the General Tests, Processes and Apparatus.

International harmonization—The test may also be performed according to the Residual solvents in the EP or the Organic volatile impurities in the USP. Even in this case, Monographs should be described in the JP style.

3. Models for the Test in Monographs

The following are typical examples for the test in Monographs, but these do not necessarily imply that other suitable operating conditions can not be used. It is important to prepare (draft) monographs according to the Guideline for the Preparation of the Japanese Pharmacopoeia.

1) A model for test item, amounts of test sample and reference standard (reference substance), preparation of the sample solution and the standard solution, injection amount for gas chromatography, calculation formula, and preparation of the internal standard solution

Residual solvents (or name of the solvent)—Weigh accurately about 0.200 g of $\triangle\triangle\triangle$ (name of the substance to be tested), add exactly 5 mL of the internal standard solution to dissolve, add water to make exactly 20 mL, and use this solution as the sample solution. If necessary filter or centrifuge. Separately, weigh exactly 0.10 g of $\circ\circ\circ$ reference substance (name of the solvent), put in a vessel containing 50 mL of water, and add water to make exactly 100 mL. Pipet 5 mL of this solution, and add water to make exactly 100 mL. Pipet 2 mL of this solution, add exactly 5 mL of the internal standard solution and 20 mL of water, and use this solution as the standard solution. Perform the test with 1 μ L each of the sample solution and standard solution as directed (in the head-space method) under Gas Chromatography according to the following conditions, and calculate the ratios, Q_T and Q_S , of the peak area of $\triangle\triangle\triangle$ (name of the substance to be tested) to that of the internal standard of each solution, respectively. The amount of $\triangle\triangle\triangle$ should be not more than $\times \times$ ppm.

$$\begin{aligned} &\text{Amount of } \circ\circ\circ \text{ (}\mu\text{g)} \\ &= \text{amount of } \circ\circ\circ \text{ reference substance (}\mu\text{g)} \times \frac{Q_T}{Q_S} \end{aligned}$$

Internal standard solution—A solution of $\triangle\triangle\triangle$ in $\nabla\nabla\nabla$ (name of solvent) (1 in 1000).

2) Models for operating conditions for a head-space sample injection device

Operating conditions (1) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 80°C
Equilibration time for inside vial	60 minutes
Transfer-line temperature	A constant temperature of about 85°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

Operating conditions (2) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 105°C
Equilibration time for inside vial	45 minutes
Transfer-line temperature	A constant temperature of about 110°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

Operating conditions (3) for the head-space sample injection device—

Equilibration temperature for inside vial	A constant temperature of about 80°C
Equilibration time for inside vial	45 minutes
Transfer-line temperature	A constant temperature of about 105°C
Carrier gas	Nitrogen
Pressurisation time	30 seconds
Injection volume of sample	1.0 mL

3) Models for operating conditions and system suitability

In the operating conditions, generally, items required for the test such as detector, column, column temperature, carrier gas, flow rate, and time span of measurement should be specified, and in the system suitability, items such as test for required detectability, system performance, and system repeatability should be specified.

The following are several models for the operating conditions and the system suitability:

Operating conditions—

Detector: Specify in the following manner: “Hydrogen flame-ionization detector”.

Column: Specify the inside diameter, length, material of column, name of stationary phase liquid and thickness of stationary phase in the following manner: “Coat the inside wall of a fused silica tube, 0.3 mm in inside diameter and 30 m in length, to 0.25 μ m thickness with polyethylene glycol 20M.” Describe the inside diameter and length of the column, and thickness or particle size of the stationary phase based on the data obtained for validation of the test method.

Column temperature: Specify in the following manner: “A constant temperature of about \times °C.” or “Maintain at 40°C for 20 minutes, then increase to 240°C at 10°C per minute, and keep at 240°C for 20 minutes.”

Carrier gas: Specify in the following manner: “Helium”.

Flow rate: Specify in the following manner: “Adjust the flow rate so that the retention time of $\blacktriangle\blacktriangle\blacktriangle$ is about \times minutes.” or “35 cm/second”. Describe the flow rate based on the data obtained for validation of the test method.

Time span of measurement: About \times times as long as the

retention time of $\triangle\triangle\triangle$ beginning after the air peak.

System suitability—

Test for required detectability: Specify in the following manner: "Measure exactly \times mL of the standard solution, and add $\square\square\square$ to make exactly \times mL. Confirm that the peak area of $\square\square\square$ obtained from \times μ L of this solution is equivalent to \times to $\times\times\%$ of that of $\square\square\square$ obtained from the standard solution". This item should be specified when, in Purity, the amount of an impurity is controlled by comparing the area of a specific peak from the sample solution with that of the peak of $\times\times\times$ from the standard solution and the system repeatability alone is not sufficient to check the system suitability.

System performance: Specify in the following manner: "Dissolve \times g of $\square\square\square$ and \times g of $\triangle\triangle\triangle$ in \times mL of $\circ\circ$ \circ . When the procedure is run with \times μ L of this solution under the above operating conditions, $\square\square\square$ and $\triangle\triangle\triangle$ are eluted in this order with the resolution between these peaks being not less than \times , and the number of theoretical plates and the symmetry factor of the peak of $\square\square\square$ are not less than $\times\times$ steps and not more than $\times\times\times$, respectively." This item should be specified in all of the test methods. Generally, the order of elution and the resolution, and in case of need (such as when the peak is asymmetrical) the symmetry factor, should be specified. The order of elution and the resolution may be replaced by the resolution and the number of theoretical plates. When no suitable reference substance is available to check separation, the number of theoretical plates and the symmetry factor of the peak of the substance to be tested may be specified.

System repeatability: Specify in the following manner: "When the test is repeated \times times with \times μ L of the solution for the system suitability test under the above operating conditions, the relative standard deviation of the peak areas of $\triangle\triangle\triangle$ is not more than $\times\%$." The system repeatability should be specified in any case, except in a qualitative test.

Examples of operating conditions and system suitability

Test conditions (1)

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.53 mm in inside diameter and 30 m in length, to 3 μ m thickness with 6% cyanopropylphenyl-methyl silicon polymer for gas chromatography. Use a guard column if necessary.

Column temperature: Maintain at 40°C for 20 minutes, then increase to 240°C at 10°C per minute if necessary, and keep at 240°C for 20 minutes.

Injection port temperature: A constant temperature of about 140°C

Detector temperature: A constant temperature of about 250°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: 1:5

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions,

the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

Test conditions (2)

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.53 mm in inside diameter and 30 m in length, to 5 μ m thickness with 5% phenyl-methyl silicon polymer for gas chromatography. If necessary use a guard column prepared by coating the inside wall of a fused silica tube, 0.53 mm in inside diameter and 5 m in length, to 5 μ m thickness with 5% phenyl-methyl silicon polymer for gas chromatography.

Column temperature: Maintain at 35°C for 5 minutes, then increase to 175°C at 8°C per minute, further increase to 260°C at 35°C per minute if necessary, and keep at 260°C for 16 minutes.

Injection port temperature: A constant temperature of about 70°C

Detector temperature: A constant temperature of about 260°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: Splitless

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

Test conditions (3)

Operating conditions—

Detector: Hydrogen flame-ionization detector

Column: Coat the inside wall of a fused silica tube, 0.32 mm in inside diameter and 30 m in length, to 0.25 μ m thickness with polyethylene glycol 20M for gas chromatography. Use a guard column if necessary.

Column temperature: Maintain at 50°C for 20 minutes, then increase to 165°C at 6°C per minute if necessary, and keep at 165°C for 20 minutes.

Injection port temperature: A constant temperature of about 140°C

Detector temperature: A constant temperature of about 250°C

Carrier gas: Helium

Flow rate: 35 cm/second

Split ratio: 1:5

System suitability—

System performance: When the procedure is run with the standard solution under the above operating conditions, the resolution between the peaks is not less than 1.0. (Note: In the case that the number of substances to be tested is two or more.)

System repeatability: When the test is repeated 3 times with the standard solution under the above operating conditions, the relative standard deviation of the peak areas of the substance to be tested is not more than 15%.

8. International Harmonization Implemented in the Japanese Pharmacopoeia Fifteenth Edition

Items for which harmonization has been agreed among the European Pharmacopoeia, the United States Pharmacopoeia and the Japanese Pharmacopoeia are implemented in the Japanese Pharmacopoeia Fifteenth Edition (JP 15). They are

shown in the tables below.

The column headed Harmonized items shows the harmonized items written in the Pharmacopoeial Harmonization Agreement Document, and the column headed JP 15 shows the items as they appear in JP 15. In the Remarks column, notes on any differences between JP 15 and the agreement are shown as occasion demands.

The date on which the agreement has been signed is shown on the top of each table. In the case where the harmonized items have been revised, this is indicated in parenthesis.

Oct. 2004 (Rev. 2)

Harmonized items	JP 15	Remarks
Residue on Ignition/Sulphated Ash Test (Introduction) Procedure	2.44 Residue on Ignition Test (Introduction) Procedure	JP's particular description: Explanation of the description in monograph, etc.

Nov. 2003

Harmonized items	JP 15	Remarks
Specific Surface Area (Introduction) Multi-point measurement Single-point measurement Sample preparation Outgassing Adsorbate Quantity of sample Method 1: The dynamic flow method Method 2: The volumetric method Reference materials Figure 1 Schematic diagram of the dynamic flow method apparatus Figure 2 Schematic diagram of the volumetric method apparatus	3.02 Specific Surface Area (Introduction) Multi-point measurement Single-point measurement Sample preparation Method 1: The dynamic flow method Method 2: The volumetric method Reference materials Fig. 3.02-1 Schematic diagram of the dynamic flow method apparatus Fig. 3.02-2 Schematic diagram of the volumetric method apparatus	JP's particular description: Explanation on this test.

June 2004

Harmonized items	JP 15	Remarks
(Introduction)	3.04 Particle Size Determination (Introduction)	JP's particular description: Explanation on this test.
Optical microscopy	Method 1. Optical microscopy	JP's particular description: Explanation on this test.
Apparatus	Apparatus	
Adjustment	Adjustment	
Illumination	Illumination	
Visual characterization	Visual characterization	JP's particular description: Explanation on the method of particle size measurement.
Photographic characterization	Photographic characterization	
Preparation of the mount	Preparation of the mount	
Crystallinity characterization	Crystallinity characterization	
Limit test of particle size by microscopy	Limit test of particle size by microscopy	
Particle size characterization	Particle size characterization	
Particle shape characterization	Particle shape characterization	
General observations	General observations	
Figure 1 Commonly used measurements of particle size	Fig. 3.04-1 Commonly used measurements of particle size	
Figure 2 Commonly used descriptions of particle shape	Fig. 3.04-2 Commonly used descriptions of particle shape	
Analytical sieving	Method 2. Analytical sieving method	JP's particular description: Explanation on this method.
Principles of analytical sieving	Principles of analytical sieving	
Test sieves	Test sieves	
Test specimen	Test specimen	
Agitation methods	Agitation methods	
Endpoint determination	Endpoint determination	
Sieving methods	Sieving methods	
1) Methanical agitation dry sieving method	Methanical agitation Dry sieving method	JP's particular description: How to treat the fine powder adherent on back surface of the sieve.
2) Air entrainment methods air jet and sonic sifter sieving	Air entrainment methods Air jet and sonic shifter sieving	
Table 1 Size of standard sieve series in range of interest	Table 3.04-1 Size of standard sieve series in range of interest	
Interpretation	Interpretation	

Jan. 2000

Harmonized items	JP 15	Remarks
<p>Bacterial Endotoxins Test</p> <p>(Introduction)</p> <p>Apparatus</p> <p>Preparation of standard endotoxin stock solution</p> <p>Preparation of standard endotoxin solution</p> <p>Preparation of sample solutions</p> <p>Determination of maximum valid dilution</p> <p>Gel-clot technique</p> <p>(1) Preparatory testing</p> <p>(i) Test for confirmation of labeled lysate sensitivity</p> <p>(ii) Test for interfering factors</p> <p>(2) Limit test</p> <p>(i) Procedure</p> <p>(ii) Interpretation</p> <p>(3) Assay</p> <p>(i) Procedure</p> <p>(ii) Calculation and interpretation</p> <p>Photometric techniques</p> <p>(1) Turbidimetric techniques</p> <p>(2) Chromogenic technique</p> <p>(3) Preparatory testing</p> <p>(i) Assurance of criteria for the standard curve</p> <p>(ii) Test for interfering factors</p> <p>(4) Assay</p> <p>(i) Procedure</p> <p>(ii) Calculation</p> <p>(iii) Interpretation</p>	<p>4.01 Bacterial Endotoxins Test</p> <p>(Introduction)</p> <p>Apparatus</p> <p>Preparation of standard endotoxin stock solution</p> <p>Preparation of standard endotoxin solution</p> <p>Preparation of sample solutions</p> <p>Determination of maximum valid dilution</p> <p>Gel-clot technique</p> <p>(1) Preparatory testing</p> <p>(i) Test for confirmation of labeled lysate sensitivity</p> <p>(ii) Test for interfering factors</p> <p>(2) Limit test</p> <p>(i) Procedure</p> <p>(ii) Interpretation</p> <p>(3) Assay</p> <p>(i) Procedure</p> <p>(ii) Calculation and interpretation</p> <p>Photometric techniques</p> <p>(1) Turbidimetric techniques</p> <p>(2) Chromogenic technique</p> <p>(3) Preparatory testing</p> <p>(i) Assurance of criteria for the standard curve</p> <p>(ii) Test for interfering factors</p> <p>(4) Assay</p> <p>(i) Procedure</p> <p>(ii) Calculation</p> <p>(iii) Interpretation</p>	<p>JP's particular description: Endotoxin 10000 Reference Standard and Endotoxin 100 Reference Standard.</p> <p>JP's particular description: Addition of the preparation of sample solutions for containers, and deletion of that for medical devices.</p> <p>JP's particular description: Addition of the concentration unit of sample solutions in the case where the endotoxin limit per equivalent is specified.</p> <p>JP's particular description: The test for assurance of criteria for the standard curve must be carried out for each lot of lysate reagent.</p> <p>JP's particular description: Two conditions which the test must meet are specified. Explanation of the test method where the interfering action is found. Explanation of the usual methods for eliminating the interference.</p> <p>JP's particular description: Addition of the alternative requirement to which solution D must meet for valid test.</p>

Regents, test solutions	Deletion
Amebocyte lysate	Deletion
Lysate TS	Deletion
Water for bacterial endotoxins test (BET)	Deletion

Oct. 2002

Harmonized items	JP 15	Remarks
Sterility (Introduction) Precautions against microbial contamination Culture media and incubation temperatures Fluid thioglycollate medium Soya-bean casein digest medium Sterility Growth promotion test of aerobes, anaerobes and fungi Validation test Membrane filtration Direct inoculation Test for sterility of the product to be examined Membrane filtration Aqueous solutions Soluble solids Oils and oily solutions Ointments and creams Direct inoculation of the culture medium Oily liquids Ointments and creams Catgut and other surgical sutures for veterinary use Observation and interpretation of results Application of the test to parenteral preparations, ophthalmic and other non-injectable preparations required to comply with the test for sterility Table 2.6.1.-1. Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Validation Test Table 2.6.1.-2. Minimum quantity to be used for each medium	4.06 Sterility Test (Introduction) Media and rinsing fluids Fluid thioglycollate medium Soybean-casein digest medium Sterility of media Growth promotion test Validation test Membrane filtration Direct inoculation Test for sterility of the products to be examined Membrane filtration a) Liquid medicines b) Solid medicines c) Oils and oily solutions d) Ointments and creams Direct inoculation of the culture medium a) Oily liquids b) Ointments and creams Cultivation and observation, Observation and interpretation of results Table 4.06-1. Microorganisms for growth promotion test and the validation test Table 4.06-3. Minimum quantity to be used for each medium	 The same statement appears in the introduction. Note 1 – 5 Note 6 Note 7 The items not included in JP. Note 8 – 9 A part of this is directed in the General Test.

Table 2.6.1.-3. Minimum number of items to be tested	Table 4.06-2. Number of items to be taken from the lot	Note 10 – 11
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- Note: 1) Non-pharmaceutical media: Not to be used.
 2) Water content of agar: Not being specified.
 3) Effective period of media: Unnecessary to be validated.
 4) Effective period of media stored in hermetic containers: Usable for maximum one year.
 5) Medium for sterility test of the products containing a mercurial preservative: Specified.
 6) Periodic growth promotion test for a ready-made powder medium: Specified.
 7) Amount of the rinsing fluid each time in the membrane-filtration method: 100 mL per filter.
 8) Transferring amount from turbid medium to fresh medium: A suitable amount.
 9) Requirements for the retesting in the case when the evidence of microbial growth is found: Specified.
 10) The table of 'Number of items to be taken from the lot': Specified as a part of the General Test.
 11) Number of large-volume products (more than 100 mL) to be taken from the lot: Maximum 10 containers.

Feb. 2004

Harmonized items	JP 15	Remarks
Uniformity of Dosage Units (Introduction)	6.02 Uniformity of Dosage Units (Introduction)	JP's particular description: Additional explanation on Liquids. Additional explanation for the part not containing drug substance.
Content uniformity	Content uniformity	
Solid dosage forms	Solid dosage forms	
Liquid dosage forms	Liquid dosage forms	
Calculation of acceptance value	Calculation of acceptance value	
Mass variation	Mass variation	JP's particular description: Assuming that the concentration of drug substance is uniform in each lot.
Uncoated or film-coated tablets	Uncoated or film-coated tablets	
Hard capsules	Hard capsules	
Soft capsules	Soft capsules	
Solid dosage forms other than tablets and capsules	Solid dosage forms other than tablets and capsules	
Liquid dosage forms	Liquid dosage forms	The phrase "in conditions of normal use. If necessary, compute the equivalent volume after determining the density." is deleted.
Calculation of acceptance value	Calculation of acceptance value	
Criteria	Criteria	
Solid and liquid dosage forms	Solid and liquid dosage forms	
Table 1	Table 6.02-1	JP's particular description: Addition of "(divided forms, lyophilized forms)" and "(true solution)".
Application of content uniformity (CU) and mass variation (MV) test for dosage forms	Application of content uniformity (CU) and mass variation (MV) test for dosage forms	
Table 2	Table 6.02-2	The phrases "at time of manufacture" and "For purposes of this Pharmacopoeia" are deleted

June 2004 (Rev.1)

Harmonized items	JP 15	Remarks
Test for Extractable Volume of Parenteral Preparations (Introduction)	6.05 Test for Extractable Volume of Parenteral Preparations (Introduction)	JP's particular description: Explanation of this Test
Single-dose containers	(1) Single-dose containers	
Multi-dose containers	(2) Multi-dose containers	
Cartridges and prefilled syringes	(3) Cartridges and prefilled syringes	
Parenteral infusions	(4) Parenteral infusions	

June 2004 (Rev.1)

Harmonized items	JP 15	Remarks
Particulate Matter in Injectables (Introduction)	6.07 Insoluble Particulate Matter Test for Injections (Introduction)	The phrase "other than gas bubbles" is deleted.
Method 1. Light obscuration particle count test	Method 1. Light obscuration particle count test	JP's particular description: Description of evaluation frequency.
	Apparatus	JP's particular description
	Calibration	JP's particular description
	Manual methods	JP's particular description
	Electronic methods	JP's particular description
	Automated methods	JP's particular description
	Sample volume accuracy	JP's particular description
	Sample flow rate	JP's particular description
	Sensor	JP's particular description
	Particle resolution	JP's particular description
	Particle counting accuracy	JP's particular description
	Threshold accuracy	JP's particular description
	Reagents	JP's particular description
General precautions	General precautions	
Method	Method	
Evaluation	Evaluation	JP's particular description: "equal to or more than 100 mL".
Method 2. Microscopic particle count test	Method 2. Microscopic particle count test	
	Apparatus	
General precautions	General precautions	
Method	Method	
Evaluation	Evaluation	JP's particular description: "equal to or more than 100 mL".
1. Circular diameter graticule	Fig. 6.07-1 Circular diameter graticule	

June 2004

Harmonized items	JP 15	Remarks
Disintegration	6.09 Disintegration	JP's particular description: This test is applied also to Granules and Pills.
Apparatus	Apparatus	
Basket-rack assembly	Basket-rack assembly	JP's particular description: The apparatus may be varied somewhat provided the specifications.
Disk	Disk	
	Auxiliary tube	JP's particular description
Procedure	Procedure	JP's particular description: Making use of water also as the test fluid. Setting each intervals of the immersion. Making a definition of complete disintegration. Setting a procedure for Granules.
	(1) Immediate-release preparation	JP's particular description: Setting a test for Granules and Pills.
	(2) Enteric coated preparations	JP's particular description: Setting a test for enteric coated preparations.
Figure 1 Disintegration apparatus	Fig. 6.09-1 Disintegration apparatus Fig. 6.09-2 Auxiliary tube	JP's particular description.

June 2004

Harmonized items	JP 15	Remarks
Dissolution	6.10 Dissolution Test	JP's particular description: The test also aims at preventing significant bioinequivalence.
Apparatus	Apparatus	
Apparatus 1 (Basket apparatus)	Apparatus for Basket Method (Apparatus 1)	
Apparatus 2 (Paddle apparatus)	Apparatus for Paddle Method (Apparatus 2)	JP's particular description: The sinker is allowed to use in case only when specified in the monograph.
Apparatus 3 (Reciprocating cylinder)		
Apparatus 4 (Flow-through cell)	Apparatus for Flow-Through Cell Method (Apparatus 3)	JP's particular description: A pump without the pulsation may also be used.
Procedure	Procedure	
Apparatus 1 or 2	Basket Method or Paddle Method	
Immediate-release dosage forms	Immediate-release dosage forms	
Procedure	Procedure	
Dissolution medium	Dissolution medium	
Time	Time	
Extended-release dosage forms	Extended-release dosage forms	
Procedure	Procedure	
Dissolution medium	Dissolution medium	
Time	Time	

Delayed-release dosage forms Procedure Method A Method B Time	Delayed-release dosage forms Procedure Dissolution medium Time not specified	In the Harmonized text: Alternative usage of Method A or B. JP's particular description. JP's particular description: Time is specified each for the 1st and 2nd fluids.
Apparatus 3 Immediate-release dosage forms Procedure Dissolution medium Time Extended-release dosage forms Procedure Dissolution medium Time Delayed-release dosage forms Procedure Time		
Apparatus 4 Immediate-release dosage forms Procedure Dissolution medium Time Extended-release dosage forms Procedure Dissolution medium Time Delayed-release dosage forms Procedure Time	Flow-Through Cell Method Immediate-release dosage forms Procedure Dissolution medium Time Extended-release dosage forms Procedure Dissolution medium Time	
Interpretation	Interpretation	JP's particular description: Follow Interpretation 1 when the value Q is specified in the individual monograph, otherwise follow Interpretation 2.
Immediate-release dosage forms	Immediate-release dosage forms Interpretation 1 Interpretation 2	JP's particular description: Setting Interpretation 2.
Extended-release dosage forms	Extended-release dosage forms Interpretation 1 Interpretation 2	JP's particular description: Setting Interpretation 2.
Delayed-release dosage forms	Delayed-release dosage forms	Non-harmonized items: Different dissolution medium. Deletion of disharmonized part on the value Q.

	<p>Interpretation 1</p> <p>Interpretation 2</p> <p>Acceptance Table 6.10-1</p> <p>Acceptance Table 6.10-2</p> <p>Acceptance Table 6.10-3</p> <p>Acceptance Table 6.10-4</p> <p>Fig. 6.10-1 Apparatus 1. Basket stirring element</p> <p>Fig. 6.10-2 Paddle stirring element</p> <p>Fig. 6.10-2 a Alternative sinker</p> <p>Figure 3 Apparatus not specified</p> <p>Fig. 6.10-3 Apparatus 3 (top) large cell tablets and capsules (bottom) tablet holder for the large cell</p> <p>Fig. 6.10-4 Apparatus 3 (top) small cell tablets and capsules (bottom) tablet holder for the small cell</p>	<p>JP's particular description: Setting Interpretation 2.</p> <p>The value Q is specified in the individual monograph.</p>
<p>Acceptance Table 1</p> <p>Acceptance Table 2</p> <p>Acceptance Table 3</p> <p>Acceptance Table 4</p> <p>Figure 1 Apparatus 1. Basket stirring element</p> <p>Figure 2 Paddle stirring element</p> <p>Figure 2 a Alternative sinker</p> <p>Figure 3 Apparatus</p> <p>Figure 4 Apparatus 4 (top) large cell tablets and capsules (bottom) tablet holder for the large cell</p> <p>Figure 5 Apparatus 4 (top) small cell tablets and capsules (bottom) tablet holder for the small cell</p>		

Sep. 2002 (Rev. 1)

Harmonized items	JP 15	Remarks
Ethanol	Ethanol	
Identification A	not specified as Identification	Setting Specific gravity as specification.
Identification B	Identification	
Relative density	Specific gravity	Setting Specific gravity at 15°C.
Appearance	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acid or alkali	
Volatile impurities	Purity (3) Volatile impurities	
Absorbance	Purity (4) Other impurities	
Residue on evaporation	Purity (5) Residue on evaporation	
Storage	Containers and storage	

Sep. 2002 (Rev. 1)

Harmonized items	JP 15	Remarks
Ethanol, Anhydrous	Anhydrous Ethanol	
Definition	limits of content	
Identification A	not specified as Identification	Setting Specific gravity as specification.
Identification B	Identification	
Relative density	Specific gravity	Setting Specific gravity at 15°C.

Appearance	Purity (1) Clarity and color of solution
Acidity or alkalinity	Purity (2) Acid or alkali
Volatile impurities	Purity (3) Volatile impurities
Absorbance	Purity (4) Other impurities
Residue on evaporation	Purity (5) Residue on evaporation
Storage	Containers and storage

Nov. 2003 (Rev. 2)

Harmonized items	JP 15	Remarks
Sodium Chloride	Sodium Chloride	
Definition	limits of the content	
Identification A	Identification (1)	
Identification B	Identification (2)	
Acidity or alkalinity	Purity (2) Acidity or alkalinity	
Sulphates	Purity (3) Sulfates	
Phosphates	Purity (4) Phosphates	
Bromides	Purity (5) Bromides	
Iodides	Purity (6) Iodides	
Ferrocyanides	Purity (7) Ferrocyanides	
Iron	Purity (9) Iron	
Barium	Purity (10) Barium	
Magnesium and alkaline-earth metals	Purity (11) Magnesium and alkaline-earth materials	
Aluminium	not specified	
Nitrites	not specified	
Potassium	not specified	
Loss on drying	Loss on drying	
Assay	Assay	

July 2003 (Rev. 1)

Harmonized items	JP 15	Remarks
Carboxymethylcellulose Calcium	Carmellose Calcium	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
Identification D	Identification (4)	
Alkalinity	Purity (1) Alkali	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Limit of chloride	Purity (2) Chloride	
Limit of sulfate	Purity (3) Sulfate	

Nov. 2003 (Rev. 1)

Harmonized items	JP 15	Remarks
Citric Acid, Anhydrous	Anhydrous Citric Acid	
Definition	limits of the content	
Appearance of solution	Purity (1) Clarity and color of solution	
Sulphates	Purity (2) Sulfates	
Oxalic acid	Purity (3) Oxalate	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

Nov. 2003 (Rev. 1)

Harmonized items	JP 15	Remarks
Citric Acid Monohydrate	Citric Acid Hydrate	
Definition	limits of the content	
Appearance of solution	Purity (1) Clarity and color of solution	
Sulphates	Purity (2) Sulfates	
Oxalic acid	Purity (3) Oxalate	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Aluminium	not specified	
Water	Water	
Sulphated ash	Residue on ignition	
Assay	Assay	

Oct. 2001

Harmonized items	JP 15	Remarks
Croscarmellose Sodium	Definition	
Croscarmellose Sodium	origin	
Identification A	Identification (1)	
B	Identification (2)	
C	Identification (3)	
pH	pH	
Settling volume	Precipitation test	
Degree of substitution	Degree of substitution	
Loss on drying	Residue on ignition	
Loss on drying	Residue on ignition	
Packaging and storage	Containers and storage	

Oct. 2001

Harmonized items	JP 15	Remarks
Wheat Starch	Wheat Starch	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Total protein	not specified	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	

Feb. 2003

Harmonized items	JP 15	Remarks
Saccharin	Saccharin	
Definition	limits of content	
Limit of benzoate and salicylate	Purity (3) Benzoate and salicylate	
Readily carbonisable substances	Purity (5) Readily carbonizable substances	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	
Packaging and storage	Containers and storage	

Feb. 2004 (Rev. 1)

Harmonized items	JP 15	Remarks
Saccharin Sodium	Saccharin Sodium Hydrate	
Definition	limits of content	
Identification A, B	Identification (2)	
Acidity or alkalinity	Purity (2) Acid or alkali	
Limit of benzoate and salicylate	Purity (4) Benzoate and salicylate	
Readily carbonisable substances	Purity (6) Readily carbonizable substances	
Water	Water	
Assay	Assay	

Oct. 2001

Harmonized items	JP 15	Remarks
Cellacefate	Cellulose Acetate Phthalate	
Definition	content of the acetyl and carboxybenzoyl groups	
Identification	Identification	
Viscosity	Viscosity	
Limit of free acid	Purity (2) Free acids	
Water	Water	
Residue on ignition	Residue on ignition	
Phthalyl content	Assay (1) Carboxybenzoyl group	
Content of acetyl	Assay (2) Acetyl group	
Packaging and storage	Containers and storage	

May 2005 (Rev. 1)

Harmonized items	JP 15	Remarks
Microcrystalline Cellulose	Microcrystalline Cellulose	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (3)	
pH	pH	
Water-soluble substances	Purity (2) Water-soluble substances	
Ether-soluble substances	Purity(3) Diethyl ether-soluble substances	
Conductivity	Conductivity	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Bulk density	Bulk density	

May 2005 (Rev. 1)

Harmonized items	JP 15	Remarks
Powdered Cellulose	Powdered Cellulose	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (3)	
pH	pH	
Water-soluble substances	Purity (2) Water-soluble substances	
Ether-soluble substances	Purity (3) Diethyl ether-soluble substances	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	

Feb. 2004 (Rev. 1)

Harmonized items	JP 15	Remarks
Corn Starch	Corn Starch	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Limit of iron	Purity (1) Iron	
Limit of oxidizing substances	Purity (2) Oxidizing substances	
Sulfur dioxide determination	Purity (3) Sulfur dioxide	

Feb. 2003 (Rev. 1)

Harmonized items	JP 15	Remarks
Anhydrous Lactose	Anhydrous Lactose	
Definition	origin	
Labeling	labeling of relative quantities of α and β lactose	
Identification	Identification	
Specific rotation	Optical rotation	
Clarity and color of solution	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acid and alkali	
Protein and light-absorbing impurities	Purity (4) Proteins and light absorbing substances	
Loss on drying	Loss on drying	
Water	Water	
Residue on ignition	Residue on ignition	
Content of alpha and beta anomers	Isomer ratio	

Sep. 2002

Harmonized items	JP 15	Remarks
Lactose Monohydrate	Lactose Hydrate	
Definition	origin	
Identification	Identification	
Specific rotation	Specific rotation	
Clarity and color of solution	Purity (1) Clarity and color of solution	
Acidity or alkalinity	Purity (2) Acid and alkali	
Protein and light-absorbing impurities	Purity (4) Protein and light absorbing substances	
Water	Water	
Residue on ignition	Residue on ignition	

Feb. 2004

Harmonized items	JP 15	Remarks
Ethyl Parahydroxybenzoate Definition Identification A Appearance of solution Acidity Related substances Sulphated ash Assay	Ethyl Parahydroxybenzoate limits of content Identification (1) Purity (1) Clarity and color of solution Purity (2) Acid Purity (4) Related substances Residue on ignition Assay	System suitability is not specified.

Feb. 2004

Harmonized items	JP 15	Remarks
Butyl Parahydroxybenzoate Definition Identification A Appearance of solution Acidity Related substances Sulphated ash Assay	Butyl Parahydroxybenzoate limits of content Identification Purity (1) Clarity and color of solution Purity (2) Acid Purity (4) Related substances Residue on ignition Assay	System suitability is not specified.

Feb. 2004

Harmonized items	JP 15	Remarks
Propyl Parahydroxybenzoate Definition Identification A Appearance of solution Acidity Related substances Sulphated ash Assay	Propyl Parahydroxybenzoate limits of content Identification (1) Purity (1) Clarity and color of solution Purity (2) Acid Purity (4) Related substances Residue on ignition Assay	System suitability is not specified.

Feb. 2004

Harmonized items	JP 15	Remarks
Methyl Parahydroxybenzoate Definition Identification A Appearance of solution	Methyl Parahydroxybenzoate limits of content Identification (1) Purity (1) Clarity and color of solution	

Acidity	Purity (2) Acid	System suitability is not specified.
Related substances	Purity (4) Related substances	
Sulphated ash	Residue on ignition	
Assay	Assay	

Oct. 2001

Harmonized items	JP 15	Remarks
Potato Starch	Potato Starch	
Definition	origin	
Identification A	Identification (1)	
Identification B	Identification (2)	
Identification C	Identification (3)	
pH	pH	
Iron	Purity (1) Iron	
Oxidising substances	Purity (2) Oxidizing substances	
Sulphur dioxide	Purity (3) Sulfur dioxide	
Loss on drying	Loss on drying	
Sulphated ash	Residue on ignition	

Nov. 2003

Harmonized items	JP 15	Remarks
Hydroxypropyl Methylcellulose	Hypromellose	
Definition	limits of content of methoxy group and hydroxypropoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Heavy metals	Purity Heavy metals	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

July, 2000

Harmonized items	JP 15	Remarks
Benzyl Alcohol	Benzyl Alcohol	
Definition	limits of the content	
Identification	Identification	
Appearance of solution	Purity (1) Clarity and color of solution	
Refractive index	Refractive index	
Acidity	Purity (2) Acid	
Benzaldehyde and other related substances	Purity (3) Benzaldehyde and other related substances	
Peroxide value	Purity (4) Peroxide value	
Residue on evaporation	Purity (5) Residue on evaporation	
Assay	Assay	

Nov. 2003

Harmonized items	JP 15	Remarks
Methylcellulose	Methylcellulose	
Definition	limits of content of methoxy group	
Labeling	labeling of viscosity	
Identification (1)	Identification (1)	
Identification (2)	Identification (2)	
Identification (3)	Identification (3)	
Identification (4)	Identification (4)	
Identification (5)	Identification (5)	
Viscosity	Viscosity	
Method 1	Method I	
Method 2	Method II	
pH	pH	
Heavy metals	Purity Heavy metals	
Loss on drying	Loss on drying	
Residue on ignition	Residue on ignition	
Assay	Assay	

Sep. 2002

Harmonized items	JP 15	Remarks
Amino Acid Analysis	General Information Amino Acid Analysis	
Apparatus	Apparatus	
General Precautions	General Precautions	
Reference Standard Material	Reference Standard Material	
Calibration of Instrumentation	Calibration of Instrumentation	
Repeatability	Repeatability	
Sample Preparation	Sample Preparation	
Internal Standards	Internal Standards	

Protein Hydrolysis	Protein Hydrolysis
Method 1	Method 1
Hydrolysis Solution	Hydrolysis Solution
Procedure	Procedure
Method 2	Method 2
Hydrolysis Solution	Hydrolysis Solution
Vapor Phase Hydrolysis	Vapor Phase Hydrolysis
Method 3	Method 3
Hydrolysis Solution	Hydrolysis Solution
Vapor Phase Hydrolysis	Vapor Phase Hydrolysis
Method 4	Method 4
Oxidation Solution	Oxidation Solution
Procedure	Procedure
Method 5	Method 5
Hydrolysis Solution	Hydrolysis Solution
Liquid Phase Hydrolysis	Liquid Phase Hydrolysis
Method 6	Method 6
Hydrolysis Solution	Hydrolysis Solution
Vapor Phase Hydrolysis	Vapor Phase Hydrolysis
Method 7	Method 7
Reducing Solution	Reducing Solution
Procedure	Procedure
Method 8	Method 8
Stock Solutions	Stock Solutions
Reducing Solution	Reducing Solution
Procedure	Procedure
Method 9	Method 9
Stock Solutions	Stock Solutions
Carboxymethylation Solution	Carboxymethylation Solution
Buffer Solution	Buffer Solution
Procedure	Procedure
Method 10	Method 10
Reducing Solution	Reducing Solution
Procedure	Procedure
Method 11	Method 11
Reducing Solutions	Reducing Solutions
Procedure	Procedure
Methodologies of amino acid analysis general principles	Methodologies of Amino Acid Analy- sis General Principles
Method 1-Postcolumn ninhydrin detection general principle	Method 1-Postcolumn Ninhydrin Detection General Principle
Method 2-Postcolumn OPA fluoro- metric detection general principle	Method 2-Postcolumn OPA Fluoro- metric Detection General Principle
Method 3-Precolumn PITC derivati- zation general principle	Method 3-Precolumn PITC Derivati- zation General Principle
Method 4-Precolumn AQC derivati- zation general principle	Method 4-Precolumn AQC Derivati- zation General Principle

Method 5-Precolumn OPA derivatization general principle	Method 5-Precolumn OPA Derivatization General Principle
Method 6-Precolumn DABS-Cl derivatization general principle	Method 6-Precolumn DABS-Cl Derivatization General Principle
Method 7-Precolumn FMOC-Cl derivatization general principle	Method 7-Precolumn FMOC-Cl Derivatization General Principle
Method 8-Precolumn NBD-F derivatization general principle	Method 8-Precolumn NBD-F Derivatization General Principle
Data calculation and analysis	Data Calculation and Analysis
Calculations	Calculations
Amino acid mole percent	Amino Acid Mole Percent
Unknown protein samples	Unknown Protein Samples
Known protein samples	Known Protein Samples

Oct. 1999

Harmonized items	JP 15	Remarks
Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	General Information	
Characteristics of polyacrylamide gels	1. Characteristics of Polyacrylamide Gels	
Denaturing polyacrylamide gel electrophoresis	2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions	
Reducing conditions	1) Reducing conditions	
Non-reducing conditions	2) Non-reducing conditions	
Characteristics of discontinuous buffer system gel electrophoresis	3. Characteristics of Discontinuous Buffer System Gel Electrophoresis	
Preparing vertical discontinuous buffer SDS polyacrylamide gels	4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels	
Assembling of the gel moulding cassette	1) Assembling of the gel moulding cassette	
Preparation of the gel	2) Preparation of the gel	
Mounting the gel in the electrophoresis apparatus and electrophoretic separation	3) Mounting the gel in the electrophoresis apparatus and electrophoretic separation	
Detection of protein in gels	5. Detection of Proteins in Gels	
Coomassie staining	1) Coomassie staining	
Silver staining	2) Silver staining	
Drying of stained SDS polyacrylamide gels	6. Drying of Stained SDS-Polyacrylamide Gels	
Molecular-mass determination	7. Molecular-Mass Determination	
Validation of the test	8. Suitability of the Test	
Quantification of impurities	9. Quantification of Impurities	
Reagents, test solutions	Test Solutions	
Blocking solution	Blocking TS	
Coomassie staining solution	Coomassie staining TS	
Destaining solution	Destaining TS	
Developer solution	Developer TS	

Fixing solution	Fixing TS
Silver nitrate reagent	Silver nitrate TS for silver staining
Trichloroacetic acid reagent	Trichloroacetic acid TS for fixing
Table 1 – Preparation of resolving gel	Table 1. Preparation of resolving gel
Table 2 – Preparation of stacking gel	Table 2. Preparation of stacking gel

Sep. 2002

Harmonized items	JP 15	Remarks
Capillary Electrophoresis	General Information	
Apparatus	Capillary Electrophoresis	
Capillary zone electrophoresis	Apparatus	
Optimisation	1. Capillary Zone Electrophoresis	
Instrumental parameters	Optimization	
Voltage	Instrumental parameters	
Polarity	Voltage	
Temperature	Polarity	
Capillary	Temperature	
Electrolytic solution parameters	Capillary	
Buffer type and concentration	Electrolytic solution parameters	
Buffer pH	Buffer type and concentration	
Organic solvents	Buffer pH	
Additives for chiral separations	Organic solvents	
Capillary gel electrophoresis	Additives for chiral separations	
Characteristics of gels	2. Capillary Gel Electrophoresis	
Capillary isoelectric focusing	Characteristics of Gels	
Loading step	3. Capillary Isoelectric Focusing	
loading in one step	Loading step	
sequential loading	loading in one step	
Focusing step	sequential loading	
Mobilisation step	Focusing step	
Optimisation	Mobilization step	
Voltage	Optimization	
Capillary	Voltage	
Solutions	Capillary	
Micellar electrokinetic chromatography (MEKC)	Solutions	
Optimisation	4. Micellar Electrokinetic Chromatography (MEKC)	
Instrumental parameters	Optimization	
Voltage	Instrumental parameters	
Temperature	Voltage	
Capillary	Temperature	
Electrolytic solution parameters	Capillary	
Surfactant type and concentration	Electrolytic solution parameters	
Buffer pH	Surfactant type and concentration	
	Buffer pH	

Organic solvents	Organic solvents	
Additives for chiral separations	Additives for chiral separations	
Other additives	Other additives	
Quantification	Quantification	
Calculations	Calculations	
System Suitability	System Suitability	
Apparent number of theoretical plates	Apparent Number of Theoretical Plates	
Resolution	Resolution	
Symmetry factor	Symmetry Factor	
Signal-to-noise ratio	Signal-to-noise Ratio	

Feb. 2004

Harmonized items	JP 15	Remarks
Tablet Friability	General Information Tablet Friability Test	

Sep. 2002

Harmonized items	JP 15	Remarks
Total Protein Assay	General Information Total Protein Assay	
Method 1	Method 1	
Standard Solution	Standard Solution	
Test Solution	Test Solution	
Procedure	Procedure	
Light-scattering	Light-Scattering	
Calculations	Calculations	
Method 2	Method 2	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents and solutions	Reagents and Solutions	
Copper sulfate reagent	Copper Sulfate Reagent	
SDS Solution	5% SDS TS	
Sodium Hydroxide Solution	Sodium Hydroxide Solution	
Alkaline copper reagent	Alkaline Copper Reagent	
Diluted Folin-Ciocalteu's Phenol Reagent	Diluted Folin's TS	
Procedure	Procedure	
Calculations	Calculations	
Interfering substances	Interfering Substances	
Sodium deoxycholate reagent	Sodium Deoxycholate Reagent	
Trichloroacetic acid reagent	Trichloroacetic Acid Reagent	
Procedure	Procedure	
Method 3	Method 3	
Standard solutions	Standard Solutions	

Explanatory footnote "Example: the Minimum Requirements for Biological Products and individual monograph of JP" is added.

Test solution	Test Solution	
Blank	Blank	
Coomassie reagent	Coomassie Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 4	Method 4	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents	Reagents and Solutions	
BCA Reagent	BCA Reagent	
Copper sulfate reagent	Copper Sulfate Reagent	
Copper-BCA reagent	Copper-BCA Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 5	Method 5	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Biuret reagent	Biuret Reagent	
Procedure	Procedure	
Calculations	Calculations	
Interfering substances	Interfering Substances	
Comments	Comments	
Method 6	Method 6	
Standard solutions	Standard Solutions	
Test solution	Test Solution	
Blank	Blank	
Reagents	Reagents and Solutions	
Borate buffer	Borate Buffer	
Stock OPA reagent	Stock OPA Reagent	
OPA reagent	OPA Reagent	
Procedure	Procedure	
Calculations	Calculations	
Method 7	Method 7	
Procedure A	Procedure A	
Procedure B	Procedure B	
Calculations	Calculations	

Sep. 2002

Harmonized items	JP 15	Remarks
Isoelectric Focusing	General Information	
Theoretical aspects	Isoelectric Focusing	
Practical aspects	Theoretical Aspects	
	Practical Aspects	

Apparatus Isoelectric Focusing in polyacrylamide gels: detailed procedure Preparation of the gels 1) 7.5 per cent polyacrylamide gel 2) Preparation of the mould Method Variations to the detailed procedure (subject to validation) Validation of iso-electric focusing procedures Specified variation to the general method Point to consider Figure-Mould Reagents Fixing solution for isoelectric focusing in polyacrylamide gel	Apparatus Isoelectric Focusing in Polyacrylamide Gels: Detailed Procedure Preparation of the Gels 7.5% Polyacrylamide gel Preparation of the mould Method Variations to the Detailed Procedure (Subject to Validation) Validation of Iso-Electric Focusing Procedures Specified Variations to the General Method Points to Consider Figure. Mould Reagents and Solutions Fixing solution for isoelectric focusing in polyacrylamide gel Coomassie staining TS Destaining solution	Coomassie staining TS and destaining solution are specified.
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June 2004

Harmonized items	JP 15	Remarks
Powder Flow	General Information	
Angel of repose	1. Angel of repose	
Basic methods for angel of repose	1.1 Basic methods for angel of repose	
Variations in angel of repose methods	1.2 Variations in angel of repose methods	
Angel of repose general scale of flowability	1.3 Angel of repose general scale of flowability	
Experimental considerations for angle of repose	1.4 Experimental considerations for angle of repose	
Recommended procedure for angle of repose	1.5 Recommended procedure for angle of repose	
Compressibility index and Hausner ratio	2. Compressibility index and Hausner ratio	
Basic methods for compressibility index and Hausner ratio	2.1 Basic methods for compressibility index and Hausner ratio	
Experimental considerations for the compressibility index and Hausner ratio	2.2 Experimental considerations for the compressibility index and Hausner ratio	
Recommended procedure for compressibility index and Hausner ratio	2.3 Recommended procedure for compressibility index and Hausner ratio	
Flow through an orifice	3. Flow through an orifice	
Basic methods for flow through an orifice	3.1 Basic methods for flow through an orifice	

Variations in methods for flow through an orifice	3.2 Variations in methods for flow through an orifice
General scale of flowability for flow through an orifice	3.3 General scale of flowability for flow through an orifice
Experimental considerations for flow through an orifice	3.4 Experimental considerations for flow through an orifice
Recommended procedure for flow through an orifice	3.5 Recommended procedure for flow through an orifice
Shear cell methods	4. Shear cell methods
Basic methods for shear cell	4.1 Basic methods for shear cell
Recommendation for shear cell	4.2 Recommendation for shear cell
Table 1 Flow properties and corresponding angle repose	Table 1 Flow properties and corresponding angle repose
Table 2 Scale of flowability	Table 2 Scale of flowability

Sep. 2002

Harmonized items	JP 15	Remarks
Peptide Mapping	General Information	
Purpose and scope	Peptide Mapping Purpose and Scope	
The peptide map	The Peptide Map	
Isolation and purification	Isolation and Purification	
Selective cleavage of peptide bonds	Selective Cleavage of Peptide Bonds	
Pretreatment of sample	Pretreatment of Sample	
Pretreatment of the cleavage agent	Pretreatment of the Cleavage Agent	
Pretreatment of the protein	Pretreatment of the Protein	
Establishment of optimal digestion conditions	Establishment of Optimal Digestion Conditions	
pH	pH	
Temperature	Temperature	
Time	Time	
Amount of cleavage agent	Amount of Cleavage Agent	
Chromatographic separation	Chromatographic Separation	
Chromatographic column	Chromatographic Column	
solvent	Solvent	
Mobile phase	Mobile Phase	
Gradient selection	Gradient Selection	
Isocratic selection	Isocratic Selection	
Other parameters	Other Parameters	
Validation	Validation	
Analysis and identification of peptides	Analysis and Identification of Peptides	
Table 1. Examples of cleavage agents.	Table 1. Examples of Cleavage Agents	
Table 2. Techniques used for the separation of peptides.	Table 2. Techniques Used for the Separation of Peptides	

9. Isoelectric Focusing

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ◆).

General Principles

Isoelectric focusing (IEF) is a method of electrophoresis that separates proteins according to their isoelectric point. Separation is carried out in a slab of polyacrylamide or agarose gel that contains a mixture of amphoteric electrolytes (ampholytes). When subjected to an electric field, the ampholytes migrate in the gel to create a pH gradient. In some cases gels containing an immobilized pH gradient, prepared by incorporating weak acids and bases to specific regions of the gel network during the preparation of the gel, are used. When the applied proteins reach the gel fraction that has a pH that is the same as their isoelectric point (pI), their charge is neutralized and migration ceases. Gradients can be made over various ranges of pH, according to the mixture of ampholytes chosen.

Theoretical Aspects

When a protein is at the position of its isoelectric point, it has no net charge and cannot be moved in a gel matrix by the electric field. It may, however, move from that position by diffusion. The pH gradient forces a protein to remain in its isoelectric point position, thus concentrating it; this concentrating effect is called "focusing". Increasing the applied voltage or reducing the sample load result in improved separation of bands. The applied voltage is limited by the heat generated, which must be dissipated. The use of thin gels and an efficient cooling plate controlled by a thermostatic circulator prevents the burning of the gel whilst allowing sharp focusing. The separation is estimated by determining the minimum pI difference (ΔpI), which is necessary to separate 2 neighboring bands:

$$\Delta pI = 3 \sqrt{\frac{D(dpH/dx)}{E(-d\mu/dpH)}}$$

D : Diffusion coefficient of the protein

dpH/dx : pH gradient

E : Intensity of the electric field, in volts per centimeter

$-d\mu/dpH$: Variation of the solute mobility with the pH in the region close to the pI

Since D and $-d\mu/dpH$ for a given protein cannot be altered, the separation can be improved by using a narrower pH range and by increasing the intensity of the electric field. Resolution between protein bands on an IEF gel prepared with carrier ampholytes can be quite good. Improvements in resolution may be achieved by using immobilized pH gradients where the buffering species, which are analogous to carrier ampholytes, are copolymerized within the gel matrix. Proteins exhibiting pIs differing by as little as 0.02 pH units may be resolved using a gel prepared with carrier ampholytes while immobilized pH gradients can resolve proteins differing by approximately 0.001 pH units.

Practical Aspects

Special attention must be paid to sample characteristics and/or preparation. Having salt in the sample can be prob-

lematic and it is best to prepare the sample, if possible, in deionized water or 2 per cent ampholytes, using dialysis or gel filtration if necessary.

The time required for completion of focusing in thin-layer polyacrylamide gels is determined by placing a colored protein (e.g. hemoglobin) at different positions on the gel surface and by applying the electric field: the steady state is reached when all applications give an identical band pattern. In some protocols the completion of the focusing is indicated by the time elapsed after the sample application.

The IEF gel can be used as an identity test when the migration pattern on the gel is compared to a suitable standard preparation and IEF calibration proteins, the IEF gel can be used as a limit test when the density of a band on IEF is compared subjectively with the density of bands appearing in a standard preparation, or it can be used as a quantitative test when the density is measured using a densitometer or similar instrumentation to determine the relative concentration of protein in the bands subject to validation.

Apparatus

An apparatus for IEF consists of:

- a controllable generator for constant potential, current and power. Potentials of 2500 V have been used and are considered optimal under a given set of operating conditions. Supply of up to 30 W of constant power is recommended,
- a rigid plastic IEF chamber that contains a cooled plate, of suitable material, to support the gel,
- a plastic cover with platinum electrodes that are connected to the gel by means of paper wicks of suitable width, length and thickness, impregnated with solutions of anodic and cathodic electrolytes.

Isoelectric Focusing in Polyacrylamide Gels: Detailed Procedure

The following method is a detailed description of an IEF procedure in thick polyacrylamide slab gels, which is used unless otherwise stated in the monograph.

Preparation of the Gels

Mould The mould (see Figure) is composed of a glass plate (A) on which a polyester film (B) is placed to facilitate handling of the gel, one or more spacers (C), a second glass plate (D) and clamps to hold the structure together.

7.5% Polyacrylamide gel Dissolve 29.1 g of acrylamide and 0.9 g of *N,N'*-methylenebisacrylamide in 100 mL of water. To 2.5 volumes of this solution, add the mixture of ampholytes specified in the monograph and dilute to 10

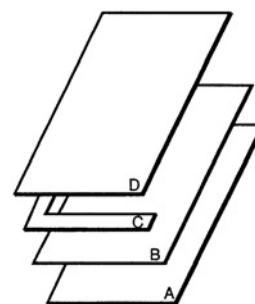


Figure. Mould

volumes with water. Mix carefully and degas the solution.

Preparation of the mould Place the polyester film on the lower glass plate, apply the spacer, place the second glass plate and fit the clamps. Place 7.5% polyacrylamide gel prepared before use on a magnetic stirrer, and add 0.25 volumes of a solution of ammonium persulfate (1 in 10) and 0.25 volumes of *N,N,N',N'*-tetramethylethylenediamine. Immediately fill the space between the glass plates of the mould with the solution.

Method

Dismantle the mould and, making use of the polyester film, transfer the gel onto the cooled support, wetted with a few millilitres of a suitable liquid, taking care to avoid forming air bubbles. Prepare the test solutions and reference solutions as specified in the monograph. Place strips of paper for sample application, about 10 mm × 5 mm in size, on the gel and impregnate each with the prescribed amount of the test and reference solutions. Also apply the prescribed quantity of a solution of proteins with known isoelectric points as pH markers to calibrate the gel. In some protocols the gel has pre-cast slots where a solution of the sample is applied instead of using impregnated paper strips. Cut 2 strips of paper to the length of the gel and impregnate them with the electrolyte solutions: acid for the anode and alkaline for the cathode. The compositions of the anode and cathode solutions are given in the monograph. Apply these paper wicks to each side of the gel several millimetres from the edge. Fit the cover so that the electrodes are in contact with the wicks (respecting the anodic and cathodic poles). Proceed with the isoelectric focusing by applying the electrical parameters described in the monograph. Switch off the current when the migration of the mixture of standard proteins has stabilized. Using forceps, remove the sample application strips and the 2 electrode wicks. Immerse the gel in fixing solution for isoelectric focusing in polyacrylamide gel. Incubate with gentle shaking at room temperature for 30 minutes. Drain off the solution and add 200 mL of destaining solution. Incubate with shaking for 1 hour. Drain the gel, add coomassie staining TS. Incubate for 30 minutes. Destain the gel by passive diffusion with destaining solution until the bands are well visualized against a clear background. Locate the position and intensity of the bands in the electropherogram as prescribed in the monograph.

Variations to the Detailed Procedure (Subject to Validation)

Where reference to the general method on isoelectric focusing is made, variations in methodology or procedure may be made subject to validation. These include:

- the use of commercially available pre-cast gels and of commercial staining and destaining kits,
- the use of immobilized pH gradients,
- the use of rod gels,
- the use of gel cassettes of different dimensions, including ultra-thin (0.2 mm) gels,
- variations in the sample application procedure, including different sample volumes or the use of sample application masks or wicks other than paper,
- the use of alternate running conditions, including variations in the electric field depending on gel dimensions and equipment, and the use of fixed migration times rather than subjective interpretation of band stability,
- the inclusion of a pre-focusing step,
- the use of automated instrumentation,
- the use of agarose gels.

Validation of Iso-Electric Focusing Procedures

Where alternative methods to the detailed procedure are employed they must be validated. The following criteria may be used to validate the separation:

- formation of a stable pH gradient of desired characteristics, assessed for example using colored pH markers of known isoelectric points,
- comparison with the electropherogram provided with the chemical reference substance for the preparation to be examined,
- any other validation criteria as prescribed in the monograph.

Specified Variations to the General Method

Variations to the general method required for the analysis of specific substances may be specified in detail in monographs. These include:

- the addition of urea in the gel (3 mol/L concentration is often satisfactory to keep protein in solution but up to 8 mol/L can be used): some proteins precipitate at their isoelectric point. In this case, urea is included in the gel formulation to keep the protein in solution. If urea is used, only fresh solutions should be used to prevent carbamylation of the protein,
- the use of alternative staining methods,
- the use of gel additives such as non-ionic detergents (e.g. octylglucoside) or zwitterionic detergents (e.g., CHAPS or CHAPSO), and the addition of ampholyte to the sample, to prevent proteins from aggregating or precipitating.

Points to Consider

Samples can be applied to any area on the gel, but to protect the proteins from extreme pH environments samples should not be applied close to either electrode. During method development the analyst can try applying the protein in 3 positions on the gel (i.e. middle and both ends); the pattern of a protein applied at opposite ends of the gel may not be identical.

A phenomenon known as cathodic drift, where the pH gradient decays over time, may occur if a gel is focused too long. Although not well understood, electroendosmosis and absorption of carbon dioxide may be factors that lead to cathodic drift. Cathodic drift is observed as focused protein migrating off the cathode end of the gel. Immobilized pH gradients may be used to address this problem.

Efficient cooling (approximately 4°C) of the bed that the gel lies on during focusing is important. High field strengths used during isoelectric focusing can lead to overheating and affect the quality of the focused gel.

Reagents and Solutions—

Fixing solution for isoelectric focusing in polyacrylamide gel Dissolve 35 g of 5-sulfosalicylic acid dihydrate and 100 g of trichloroacetic acid in water to make 1000 mL.

♦**Coomassie staining TS** Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

♦**Destaining solution** A mixture of water, methanol and acetic acid (100) (5:4:1).♦

10. Laser Diffraction Measurement of Particle Size

Particle size is one of the important factors concerning the powder characteristics of solid-state drugs and pharmaceutical excipients, and therefore prompt and highly accurate determination method is necessary. The laser diffraction method is considered as a potent candidate, but due to the limit of kind of particles and the range of particle diameter that can be measured, the optical microscopy method should be sometimes applied in parallel. In the pharmaceutical field, the reliable and reproducible particle size determination method is required for quality control of the composition of the formulation, especially, of the pharmaceutical excipients that are difficult to apply the analytical sieving method due to the particle diameter of not larger than 100 μm .

In the Japanese Pharmacopoeia, the optical microscopy (Method 1) for the particles ranging from approximately 1 μm to 100 μm and the analytical sieving method (Method 2) for the particles of not smaller than 75 μm are listed as the powder particle size determination.

This document describes the laser diffraction technique for particle size determination as the method for analyzing the particles having the diameter ranging from approximately 1 μm to 1000 μm by utilizing the diffraction phenomenon against the laser beam (Fraunhofer diffraction). There are methods utilizing the principles such as the Mie scattering other than the Fraunhofer diffraction, which can be also used for particle size determination of the particles in sub-micron range (not larger than 1 μm).

1. Principle of laser diffraction technique

The laser diffraction technique is based upon the beam diffraction phenomenon (Fraunhofer diffraction), in which particle size-dependent intensity patterns exhibit when the powder particles are exposed to the parallel rays, and accurate and reproducible particle size distribution image can be obtained by mathematical analysis of the diffraction pattern. In other words, this apparatus is designed to detect the beams diffracted to each angle by the particles when the laser beam is radiated to the powder sample dispersed in a liquid or gas, in a way that the beam passes over the specimen and, the diffraction pattern is obtained and recorded for data analysis. For the analysis of data, the particles are qualified as spherical form. In this technique, at every particle diameter interval that is provided in the apparatus, distribution of the spherical equivalent diameter in the standard volume is calculated by inverse operation from the optical model to attain the highest coherence to the diffraction intensity pattern.

For the particles in sub-micron region, it is not easy to obtain an accurate particle size distribution due to difficulty in distinction of the particle diameter-dependent scattering pattern because of weakness of the forward light scattering intensity, and it is generally considered that the Fraunhofer diffraction can not be applied for the evaluation of particle diameter in the sub-micron range. Therefore, for the particles in sub-micron region, the method that applies the Mie scattering theory is introduced here. The particle diameter is measured by the analysis of the scattered lights toward back and side that are easy to catch the signals of transmitted and

refracted light. However, since the scattering patterns of the non-spherical particles vary depending on the particle shape, the correct particle diameter can not be obtained even if the accurate physical properties (refraction index or transmittance) of the dispersing medium are given. Therefore, the Mie scattering theory can be effectively applied if the particles are nearly spherical, and for the other cases, evaluation of the data is in general difficult due to strongly be affected by the physical properties or the particle concentration.

2. Apparatus

A typical set-up for a laser diffraction instrument is given in Figure 1. As the light source, the laser beam of a single wavelength is generally used. A beam expander is equipped as the laser beam processing unit H for irradiation to the particle ensemble dispersed. When the sample (particle ensemble F) passes through the laser beam, a part of the laser beam is scattered by the particles, and the diffraction images given by the scattered and transmitted beams are transcribed by the detector J placed at the focal length of the Fourier lens D.

A representative sample is dispersed in a liquid or gas, and the recirculating system consisting of the optical measurement cell, a dispersion bath (usually equipped with the stirrer and ultrasonic elements), a pump and tubing are commonly most used.

(1) Calibration of apparatus

According to the description in ISO standard 13320-1 (1999): Particle size analysis-Laser diffraction methods-Part 1: General principles, apparatus should be calibrated by using the plural standard particles for particle size measurement having a known particle size distribution and over at least one decade of size.

(2) Particle size determination in sub-micron region

In order to extend the measuring range of particle diameter to sub-micron region, apparatuses based on the Mie scattering theory have been developed and used. This type of instrument is designed so that the scattered lights toward back and side are detected and analyzed, but there are large variations in the analytical results depending on the type of instrument. However, this type of apparatus can produce reproducible data even in a sub-micron region, if the apparatus used and the sample tested are restricted under the specific conditions in which the input number for data analysis, e.g., physical properties, are fixed for the apparatus.

(3) Particle size determination of emulsion

In case of particle size determination of emulsion, the method utilizing dynamic light scattering or static light scattering is generally used. Since the emulsion particles transmit the light, the laser diffraction method is not very appropriate to apply. However, it is possible to evaluate emulsion particle diameter when the apparatus has been appropriately calibrated using the standard particles for particle size measurement.

3. Measurement

In case of the laser diffraction method, the particle concentration affects the accuracy of measurement. Therefore, for the sample having the size near the upper measuring limit of particle diameter, the particle concentration should be high enough to attain the static analysis. On the contrary, for the sample having smaller particle diameter, it is necessary for accurate measurement to adjust the particle concentration lower to some extent to prevent the multiple scattering.

(1) Conditions of measurement

·Location of the apparatus: It is important that the appara-

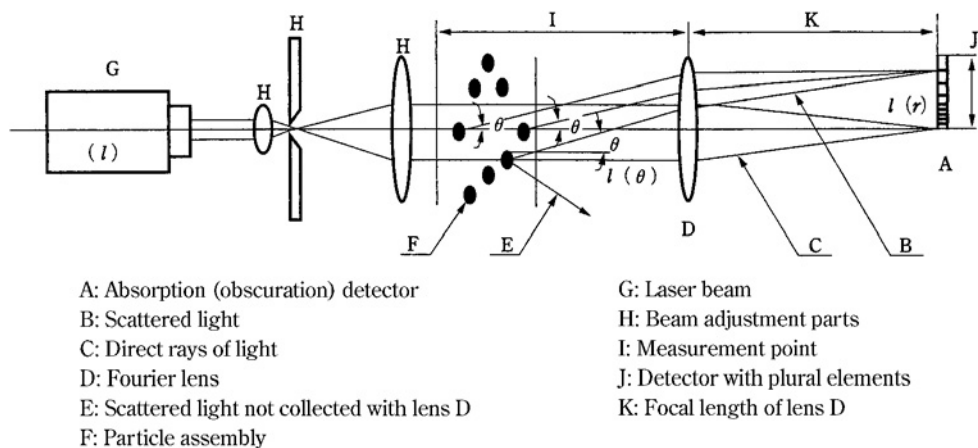


Fig. 1 Construction of the laser diffraction apparatus

tus is not affected by electrical noise and mechanical vibrations. It is also desirable that the apparatus is located under the environment where there is no variation of temperature and humidity to the extent that affects the measurement and is no exposure of direct sun light.

- Blank measurement: After selection of proper alignment of the optical part of the instrument, a blank measurement performed in a pure dispersing medium using the same method as that for measurement of the sample.

- Preliminary observation of the sample: Prior to the measurement, the powder sample should be observed in advance by visual/microscopic inspection to confirm the approximate particle diameter range and particle shape.

- Establishment of the measurement conditions: The time for measurement, the reading time of detector and the number of repetition should be experimentally decided in accordance with required measurement accuracy.

- Measurement of the sample: The sample is measured under the established measurement conditions. Data analysis is performed based on the pulse of dispersed particles from the difference of diffraction intensity between the sample and the blank.

(2) Preparation of the sample

The dry powder sample can be dispersed in a liquid or gas, and the appropriate method is selected depending on the purpose of measurement. There are two types of dispersion method, wet type using a liquid as the dispersion medium and dry type using a gas as the dispersion medium. It is advisable that the powder sample for measurement is divided to an appropriate apparent volume using a sample splitting technique.

The result of the particle size distribution much varies depending on the conditions of preparation of the sample. Therefore, the procedures and conditions of sample preparation should be defined in advance.

(3) Sampling

The amount of the sample necessary for the laser diffraction for powder particle size measurement has decreased due to technical advancement of the apparatus, but the sample for the measurement should represent the whole sample. Therefore, how to sample a small amount for actual measurement from a sample of large volume is an important problem. It is general that a large amount of the sample is divided/reduced to reach the amount necessary for measure-

ment, and the divider or the cone and quartering method, etc. are available for this purpose. The cone and quartering method is used for reduction of a small amount of sample with poor flowability, and is a simple method without using any divider.

For sampling of the powder particles dispersed in a liquid, the liquid is first stirred uniformly and mixed, and it should be confirmed that no precipitate remains at the bottom of the vessel. Then, the amount necessary for measurement is quickly sampled using a pipette, etc. In case of the sample that contains larger particles, it is easy to cause precipitates, and they are precipitated at the bottom after once stopping agitation. Therefore, the sampling of the suspension should be made under agitation.

(4) Pretreatment of the sample for measurement

If the sample contains the particles or agglomerates over the upper limit of measurable particle diameter range of the laser diffraction instrument, they should be removed by sieving in advance, and the mass and percent of such particles or agglomerates removed should be recorded.

(5) Liquids used as the dispersion medium

A variety of liquids is available for the dispersion of the powder samples, and at selection of the dispersion liquids, the followings should be taken into account:

- be compatible with the materials used in the instrument (O-ring, tubing, etc.)
- not dissolve or alter the size of particular materials,
- favour easy and stable dispersion of the particulate materials,
- have suitable viscosity in order to enable recirculation,
- not be hazardous to health and should meet safety requirements.

A low-foaming surfactant and dispersant may be used to facilitate the wetting of the particles, and to stabilize the dispersion. A preliminary check on the dispersion quality can be made by visual/microscopic inspection of the suspension.

(6) Dispersion of the agglomerated particles by ultrasonication

For the powder sample containing fine particles, intense physical power should be given to disperse the agglomerated particles. The ultrasonication is generally used for this purpose. In this case, since the ultrasonic output, irradiation time and the volumes of both the suspension and the vessel

used significantly affect the intensified dispersion effect, the optimum irradiation conditions should be established taking account of these factors. In case that an ultrasonic bath is used for dispersion, kinetic condition of the dispersant and dispersion effect may change depending on the altitude of its surface. Therefore, it is necessary to confirm the resonance point where the ultrasonic wave is intensely irradiated. Further, in case of stronger ultrasonic output or longer irradiation time, the particles for measurement may be destructed. Therefore, the change of the particle size distribution depending on the variation of the irradiation conditions should be checked to determine the appropriate ultrasonic output and irradiation time.

(7) Dispersion gases as the dispersion medium

An appropriate dry type dispersion apparatus using compressed gas is used for dispersion of the powder sample in a gas. Any substance that may affect the measurement, such as oil, water and particulate matter, should not be contaminated in the dispersion medium gas, and such substances should be removed, if necessary, by a filter, etc. In case of the dry dispersion method, it is advisable to measure all the samples reduced. A larger amount of sample diminishes the statistical error at a rough and large particle size area even if the particle size distribution is broad. It is also important that the particles are dispersed uniformly. It can be confirmed by comparing the results obtained by dry and wet dispersion methods. It is desirable that the equivalent results are obtained by dry and wet measurements.

4. Data analysis

Various softwares have been developed to analyze the data utilizing some mathematical methods, and the particle diameter and particle size distribution can be calculated from the data to meet its object. As for the accuracy of measurement, the reproducibility required for each apparatus is attained when measured on the independent triplicate samplings of the same batch.

(1) Calculation of the particle size distribution

The instrument manufacturers may use different algorithms for calculation of the particle size distribution from the intensity of diffraction (or scattering) beams obtained by the laser diffraction instrument, but the principle of estimating particle diameter from the intensity of light diffraction obtained by the detector under the diffraction (or scattering) theory is fundamentally the same. Since this method is based upon the optical model that assumes spherical particle, the sphere equivalent diameter is obtained for the non-spherical particles. The laser diffraction method can not distinguish between the diffraction of a single particle and that of the agglomerate or of the primary particle cluster that constitutes an aggregates or agglomerates.

(2) Weaving of the results

For the samples having particle size distributions, the median diameter or the modal diameter are used as the characteristic diameters, and the particle size distribution is expressed either as the cumulative size distribution or the frequency size distribution. The cumulative size distribution is expressed as a quantitative proportion of the particles smaller than the specified particle diameter among the whole sample. The analytical sieving method is generally used for the particle size distribution determination, and this cumulative distribution is represented as the cumulative undersize distribution. On the other hand, the frequency size distribution is ex-

pressed as the frequency that is shared by the particles that belong to the specified particle diameter fraction among the whole sample.

· Mathematical expression of the particle size distribution

The particle size distribution is expressed by a function of either the normal distribution or the lognormal distribution. The lognormal distribution is characterized by distribution pattern that spreads out toward large particle size range, and is generally applied for the samples having a large amount of larger particles and a small amount of smaller particles.

5. Experimental considerations on the measurement

Accurate particle diameter measurement can not be expected for the laser diffraction method in case of the samples having wide range of particle size distribution. Therefore, the setting the measurement range is essential for the measurement of the particle diameter by the laser diffraction method, because the scattering intensity is much influenced by the lower limit diameter (1 μm) and upper limit diameter (1000 μm) of sample particles. It is desirable in this method that the measurement diameter range is confirmed by interpolation using the standard sample having a known particle size distribution considering the accuracy of measurement and the sensitivity of instrument used. For the particles of not larger than 10 μm , it is difficult to obtain accurate particle diameter, because the difference of diffraction pulse detected by the detector is small. Particularly, for the particles in sub-micron region, there is almost no difference of the scattered light forward, and it is hard to distinguish them. Further, in addition to the error caused by recognizing the non-spherical particles as spherical particles, decrease of the scattering intensity, which is much bigger than that estimated from the reduction of geometrical particle diameter, occurs as the particle diameter decreases, and securing the accuracy of measurement comes to be difficult. Therefore, the lower limit for measurement of the particle diameter by the laser diffraction technique is approximately 1 μm , similar to that by the optical microscopic method. In case of the laser diffraction technique, since the scattering intensity is related to the volume concentration of the particles, and the maximum measurement range of particle diameter should be decided taking account of the relative variation of the scattering intensity. A particle of 10 times larger in diameter has a volume of 1000 times larger. This means that in case of 1 μm for lower limit measurement of particle diameter, the particles of 1000 μm have a volume of 1 billion times larger, and consequently the sample necessary for measurement is 10 billion times larger, similar to the case of volume. Therefore, approximately 1000 μm is appropriate for the maximum particle diameter for measurement.

Example of the standard reference particles

Use the standard reference particles for SAP 10-03, the Specification of Association of Powder Process Industry and Engineering, Japan. Either of MBP1-10 or MBP10-100 is used to meet the particle size measurement range (1 - 10 μm or 10 - 100 μm).

11. Media Fill Test

The media fill test (MFT) is one of the processing validations employed to evaluate the propriety of the aseptic

Table 1. Alert and action levels for large numbers of media filled units

Number of units*1	Number of contaminated units		
	Acceptance levels	Alert levels	Action levels
3,000	0	not applicable	≧ 1
4,750	0	1	≧ 2
6,300	0	1 – 2	≧ 3
7,760	0	1 – 3	≧ 4
9,160	0	1 – 4	≧ 5
10,520	1	2 – 5	≧ 6
11,850	1	2 – 6	≧ 7
13,150	1 – 2	3 – 7	≧ 8
14,440	1 – 2	3 – 8	≧ 9
15,710	1 – 3	4 – 9	≧ 10
16,970	1 – 3	4 – 10	≧ 11

*1 It is not necessary to relate the number of units with lot size of actual products.

Table 2. Upper 95% confidence limit of a Poisson variable for numbers of contaminated units

Observed numbers of contaminated units (k)	Upper 95% confidence limit (U)
0	2.9957
1	4.7439
2	6.2958
3	7.7537
4	9.1537
5	10.5130
6	11.8424
7	13.1481
8	14.4346
9	15.7052
10	16.9622

Using the 95% confidence limit (U) in Table 2, the contamination rate (P) of observed numbers of contaminated units (k) per filled units (n) can be calculated as $P = U/n$ (equation 1). For example:

If 5,000 units were filled and two contaminated units were observed, $n = 5,000$ and $U = 6.30$ ($k = 2$) are substituted in the equation 1; $P = 6.30/5,000 = 0.0013$. The upper 95% confidence limit for the contamination rate would be 0.13%.

processing of pharmaceutical products using sterile media, etc. instead of actual products. Therefore, media fill tests should be conducted with the manipulations normally performed in actual processing, e.g. filling and closing operation, operating environment, processing operation, number of personnel involved, etc., and conducted under processing conditions that include “worst case” conditions. Refer to GMP (1), WHO/GMP for pharmaceutical products (2), and ISO 13408 (3), etc. for necessary information to conduct this test.

1. Frequency of media fills

1.1 Initial performance qualification

Initial performance qualification should be conducted for each new facility, item of equipment, filling line, and container design (except for multiple sizes of the same container design), etc. For production batch sizes exceeding 3,000 units, a minimum of three media fill runs should be conducted on separate days. For production batch sizes of less than 3,000 units, see Table 3.

Table 3. Initial performance qualification: Media fills

Production lot size	Numbers of media fill runs	Alert level and action required	Action level and action required
< 500	A minimum of 10 media fill runs using the maximum lot size of the product	One contaminated unit in any run. Investigate cause.	Two contaminated units in single run, or one each in two runs. Investigate cause and repeat initial qualification media fills.
500 – 2,999	A minimum of 3 media fill runs using the maximum lot size of the product	The same as above	The same as above
≧ 3,000	A minimum of 3 media fill runs using at least 3,000 units	When any of the media fill runs exceeds the alert level shown in Table 1, take the action set out in 2.1.1.	When any of the media fill runs exceeds the action level shown in Table 1, take the action set out in 2.1.1

Table 4. Periodic performance requalification: Media fills

Production lot size	Numbers of media fill runs	Alert level and action required	Action level and action required
< 500	A minimum of 3 media fill runs using the maximum lot size of the product		One contaminated unit in any run. Investigate cause and repeat initial qualification media fill runs.
500 – 2,999	One media fill run using the maximum lot size of the product		One contaminated unit. Investigate cause and repeat initial qualification media fill runs.
≧ 3,000	One media fill run using at least 3,000 units	When the media fill run exceeds the alert level shown in Table 1, take the action set out in 2.1.1.	When the media fill run exceeds the action level shown in Table 1, take the action set out in 2.1.1

1.2 Periodic performance requalification

1) Conduct media fill requalifications periodically on each working shift for the filling line. Employees working in the aseptic processing area should be trained for aseptic processing operations and take part in media fills.

2) When filling lines have not been used for over six months, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to resumption of use of the filling lines.

3) In cases of facility and equipment modification (interchanging parts may not require requalification), changes in personnel working in critical aseptic processing (e.g. new crews), anomalies in environmental testing results, or a

product sterility test showing contaminated products, conduct appropriate numbers of media fill runs in the same way as for the initial performance qualification prior to the scheduled media fills.

2. Acceptance criteria of media fills

A large number of media filled units is required to detect 0.1% contamination rate. The alert level is more than 0.05% but less than 0.1%, and the action level is more than 0.1% contamination rate at the upper 95% confidence level. Table 1 shows alert and action levels for media filled units. Table 2 is to be used for the calculation of contamination rate of contaminated units found in media filled units. The contamination rate of 0.05% in media fills is the minimum acceptable level, and manufacturers should make efforts to achieve lower contamination rate than this. Table 3 shows the alert and action levels for initial performance qualification of an aseptic processing line, and actions required for each level. Table 4 shows the alert and action levels for requalification of an aseptic processing line, and actions required for each level. The alert and action levels for production batch sizes of less than 3,000 units take semiautomatic or manual operation into consideration.

2.1 Actions required for each level

2.1.1 Initial performance qualification

1) When the result of the media fill run is less than the alert level, the media fill run meets the requirement of the MFT.

2) When the results of any media fill run done with at least three replicate runs reach the alert or the action level, an investigation regarding the cause is required, and initial qualification media fills are to be repeated. When the result of each media fill run is less than the alert level, the initial qualification media fills meet the requirement of the MFT.

2.1.2 Requalification

1) When the result of the media fill run is less than the alert level, the media fill run meets the requirement of the MFT.

2) When the result of the media fill run exceeds the alert level, an investigation regarding the cause is required, and one more media fill run is to be done. If the result is less than the alert level, the media fill run meets the requirement of the MFT.

3) When the result of the media fill run exceeds the action level, a prompt review of all appropriate records relating to aseptic production between the current media fill and the last successful one, and an investigation regarding the cause must be conducted simultaneously. If necessary, appropriate action to sequester stored and/or distributed products should be taken. After investigation regarding the cause, repeat three serial media fill runs. If the results are less than the alert level, the media fill runs meet the requirement of the MFT.

2.2 Parameters which affect sterility

When media fill alert and action levels are exceeded, an investigation should be conducted regarding the cause, taking into consideration the following points:

- 1) Microbial environmental monitoring data
- 2) Particulate monitoring data
- 3) Personnel monitoring data (microbial monitoring data on gloves, gowns, etc. at the end of work)
- 4) Sterilization cycles for media, commodities, equipment, etc.
- 5) Calibration of sterilization equipment

- 6) Storage conditions of sterile commodities
- 7) HEPA filter evaluation (airborne particulate levels, DOP test, velocity measurements, etc.)
- 8) Pre and post filter integrity test data (including filter housing assembly)
- 9) Room air flow patterns and pressures
- 10) Unusual events that occurred during the media fill run
- 11) Characterization of contaminants
- 12) Hygienic control and training programs
- 13) Gowning procedures and training programs
- 14) Aseptic processing technique and training programs
- 15) Operator's health status (especially coughing, sneezing, etc., due to respiratory diseases)
- 16) Other factors that affect sterility

3. Data guidance for media fills

Each media fill run should be fully documented and the following information recorded:

- 1) Data and time of media fill
- 2) Identification of filling room and filling line used
- 3) Container/closure type and size
- 4) Volume filled per container
- 5) Filling speed
- 6) Filter lot and catalogue number
- 7) Type of media filled
- 8) Number of units filled
- 9) Number of units not incubated and reason
- 10) Number of units incubated
- 11) Number of units positive
- 12) Incubation time and temperature
- 13) Procedures used to simulate any step of a normal production fill (e.g., mock lyophilization or substitution of vial headspace gas)
- 14) Microbiological monitoring data obtained during the media fill set-up and run
- 15) List of personnel who took part in the media fill
- 16) Growth promotion results of the media (in case of powder fill, an antimicrobial activity test for the powder is necessary)
- 17) Characterization of the microorganisms from any positive units
- 18) Review

4. Media fill procedures

Methods to validate aseptic processing of liquid, powder and freeze-dried products are described. Basically, it is possible to apply media fill procedures for liquid products to other dosage forms and container configurations.

4.1 Media selection and growth promotion

Soybean-casein digest medium or other suitable media are used. As growth promotion testing microorganisms, strains listed in the Sterility Test and, if necessary, one to two representative microorganisms which are frequently isolated in environmental monitoring should be used. The media inoculated with 10 to 100 viable microorganisms of each strain should show obvious growth when incubated at the predetermined temperature for 5 days.

4.2 Sterile medium preparation

The medium is sterilized according to the pre-validated method.

4.3 Incubation and inspection of media filled units

Leaking or damaged media fill evaluation units should be removed and recorded prior to incubation of media filled units. Incubate at 20 – 25°C for 1 week, and then at 30 –

35°C for 1 week (or at 30 – 35°C for 1 week, and then at 20 – 25°C for 1 week), or at 30 – 35°C for 2 weeks. Observe the media filled units for growth of microorganisms at least once between the third day and seventh day and on the last day of the test period, twice in total. Microorganisms present in contaminated units should be characterized.

A. Liquid products

Media fill procedure

Media fill should include normal facility/equipment operations and clean-up routines. Containers, closures, parts of the filling machine, trays, etc. are washed and sterilized according to the standard operating procedures. Media fills should be conducted under processing conditions that include “worst case” conditions, e.g., correction of line stoppage, repair or replacement of filling needles/tubes, replacement of on-line filters, permitted interventions, duration and size of run, number of personnel involved, etc.

A predetermined volume of medium is filled into sterilized containers at a predetermined filling speed and the containers are sealed. The media are contacted with all product contact surfaces in the containers by an appropriate method, and then incubated at the predetermined temperature.

B. Powder products

B.1 Powder selection and antimicrobial activity test

Actual products or placebo powder are used. In general, lactose monohydrate, D-mannitol, polyethylene glycol 6,000, carboxymethyl cellulose salts or media powder, etc. are used as placebo powders. Prior to employing any of the powders, evaluate whether the powder has antimicrobial activity. Media powders are dissolved in water and other powders in liquid medium, and the solutions are inoculated with 10 to 100 viable microorganisms of each kind, shown in 4.1, for the growth promotion test. If obvious growth appears in the medium incubated at the predetermined temperature for 5 days, the powder has no antimicrobial activity and is available for the media fill test.

B.2 Sterilization of powders

Dry powders are bagged in suitable containers (e.g. double heat-sealed polyethylene bags), and are subjected to radiation sterilization.

B.3 Sterility of filling powders

The powders must pass the Sterility Test. However, if the sterilization is fully validated, sterility testing of the powders can be omitted.

B.4 Media fill procedures

Chose a suitable procedure from among the following procedures.

1) Fill sterilized liquid media into containers by suitable methods, and then fill actual products or sterilized placebo powder with the powder filling machine. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

2) Distribute liquid media into containers, and then sterilize them in an autoclave. Remove the containers to the filling area, and then fill actual products or sterilized placebo powder into the containers with the powder filling machine.

3) Fill actual products or sterilized placebo powder into containers with the powder filling machine, and then fill sterilized liquid media into the containers by appropriate methods. If sterilized powder media are used as a placebo powder, fill sterilized water instead of sterilized liquid media.

C. Lyophilized products

In the case of lyophilized products, it may be impossible to conduct a media fill run in the same way as used for actual processing of lyophilized products. The process of freezing and lyophilization of the solution may kill contaminant organisms and change the characteristics of the media too. The use of inert gas as a blanket gas may inhibit the growth of aerobic bacteria and fungi. Therefore, in general, the actual freezing and lyophilization process should be avoided and air used as the blanket gas.

Media fill procedures

Use the following method or other methods considered to be equivalent to these methods.

1) After filling of the media into containers by the filling machine, cap the containers loosely and collect them in pre-sterilized trays.

2) After placing the trays in the lyophilizer, close the chamber door, and conduct lyophilization according to the procedures for production operation. Hold them without freezing under weak vacuum for the predetermined time.

3) After the vacuum process, break the vacuum, and seal the stoppers.

4) Contact the media with all product contact surfaces in the containers by appropriate methods, and then cultivate them at the predetermined temperature.

References

- 1) Good manufacturing practices for pharmaceutical products (WHO-GMP, 1992)
- 2) ISO 13408-1 (Aseptic processing of health care products: Generals)

12. Microbial Attributes of Nonsterile Pharmaceutical Products

The presence of microbial contaminants in nonsterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the product and has the potential to affect adversely the health of patients. Manufacturers, therefore, should ensure as low as possible a contamination level for finished dosage forms, raw materials and packaging components to maintain appropriate quality, safety and efficacy of nonsterile pharmaceutical products. This chapter provides guidelines for acceptable limits of viable microorganisms (bacteria and fungi) existing in raw materials and nonsterile pharmaceutical products. Testing methods for the counting of total viable microorganisms and methods for the detection and identification of specified microorganisms (*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, etc.) are given under the “Microbial Limit Test”. When these tests are carried out, a microbial control program must be established as an important part of the quality management system of the product. Personnel responsible for conducting the tests should have specialized training in microbiology and in the interpretation of the testing results.

1. Definitions

- 1.1 Nonsterile pharmaceutical products: Nonsterile drugs shown in monographs of the JP and nonsterile products including intermediate products and finished dosage

forms.

- 1.2 Raw materials: All materials, including raw ingredients and excipients, used for the preparation of drugs, except for water and gases.
- 1.3 Bioburden: Number and type of viable microorganisms existing in nonsterile pharmaceutical products.
- 1.4 Action levels: Established bioburden levels that require immediate follow-up and corrective action if they are exceeded.
- 1.5 Alert levels: Established bioburden levels that give early warning of a potential drift from normal bioburden level, but which are not necessary grounds for definitive corrective action, though they may require follow-up investigation.
- 1.6 Quality management system: The procedures, operation methods and organizational structure of a manufacturer (including responsibilities, authorities and relationships between these) needed to implement quality management.

2. Scope

In general, the tests for total viable aerobic count and for the detection of specified microorganisms are not applied to antibiotic or bacteriostatic drugs. However, the tests should be done for microorganisms that are not affected by the drug. The test for total viable aerobic count is not applied to drugs containing viable microorganisms as an active ingredient.

3. Sampling plan and frequency of testing

3.1 Sampling methods

Microbial contaminants are usually not uniformly distributed throughout the batches of non-sterile pharmaceutical products or raw materials. A biased sampling plan, therefore, cannot be used to estimate the real bioburden in the product. A sampling plan which can properly reflect the status of the product batch should be established on the basis of the bioburden data obtained by retrospective validation and/or concurrent validation. In general, a mixture of samples randomly taken from at least different three portions, almost the same amount for each portion, is used for the tests of the product. When the sampling is difficult in a contamination-controlled environment, special care is required during sampling to avoid introducing microbial contamination into the product or affecting the nature of the product bioburden. If it is confirmed that the product bioburden is stable for a certain period, as in the case of nonaqueous or dried products, it is not necessary to do the tests for total viable aerobic counts and for the detection of specified microorganisms, immediately after the sampling.

3.2 Testing frequency

The frequency of the tests should be established on the basis of a variety of factors unless otherwise specified. These factors include:

- a) Dosage forms of non-sterile pharmaceutical products (dosage directions);
- b) Manufacturing processes;
- c) Manufacturing frequency;
- d) Characteristics of raw materials (natural raw material, synthetic compound, etc.);
- e) Batch sizes;
- f) Variations in bioburden estimates (changes in batches, seasonal variations, etc.);
- g) Changes affecting the product bioburden (changes in manufacturing process, supplier of raw materials, lot

number of raw materials, etc.);

h) Others.

In general, the tests may be performed at a high frequency during the initial production of a drug to get information on the microbiological attributes of the product or raw materials used for the production. However, this frequency may be reduced as bioburden data are accumulated through retrospective validation and/or concurrent validation. For example, the tests may be performed at a frequency based on time (e.g., weekly, monthly or seasonally), or on alternate batches.

4. Microbial control program

When the "Microbial Limit Test" is applied to a nonsterile pharmaceutical product, the methods for the recovery, cultivation and estimation of the bioburden from the product must be validated and a "Microbial control program" covering the items listed below must be prepared.

- a) Subject pharmaceutical name (product name);
- b) Frequency of sampling and testing;
- c) Sampling methods (including responsible person, quantity, environment, etc. for sampling);
- d) Transfer methods of the samples to the testing area (including storage condition until the tests);
- e) Treatment of the samples (recovery methods of microbial contaminants);
- f) Enumeration of viable microorganisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
- g) Detection of specified microorganisms (including testing quantity, culture media, growth-supporting test of the media, culturing methods, etc.);
- h) Estimation of the number of and characterization of microbial contaminants;
- i) Establishment of "Microbial contamination limits" (including alert level and action level);
- j) Actions to be taken when the levels exceed "Microbial contamination limits";
- k) Persons responsible for the testing and evaluation, etc.;
- l) Other necessary items

5. Microbial contamination limits for nonsterile pharmaceutical products

By establishing "Microbial contamination limits", it is possible to evaluate at the initial processing stage of the product whether the microbiological quality of the raw materials is adequate or not. Furthermore, it is then possible to implement appropriate corrective action as needed to maintain or improve the microbiological quality of the product. The target limits of microbial levels for raw materials (synthetic compounds and minerals) are shown in Table 1.

In general, synthetic compounds have low bioburden levels due to the high temperatures, organic solvents, etc., used in their manufacturing processes. Raw materials originated from plants and animals in general have higher bioburdens than synthetic compounds.

The microbial quality of the city water or purified water used in the processing of active ingredients or nonsterile pharmaceuticals may have a direct effect on the quality of the finished dosage form. This means it is necessary to keep the level of microbial contaminants in the water as low as possible.

The microbial contamination limits for nonsterile finished dosage forms are shown in Table 2. These microbial limits

are based primarily on the type of dosage form, water activity, and so on. For oral liquids and pharmaceutical products having a high water activity, in general, low microbial contamination limits are given. In this guideline, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* are shown as specified microorganisms, but it is also necessary to test certain pharmaceutical products for other microorganisms (for example, certain species of *Clostridia*, *Pseudomonas*, *Burkholderia*, *Aspergillus*, and *Enterobacter species*) that may have the potential to present a microbial risk to patients. The selection of the specified microorganisms was based on following criteria; indicator for poor hygienic practices, pathogenic potential for route of administration, and survival profile of the microorganism and recoverability in the product. Due to the inherent precision limitations of the enumeration methods, a value exceeding the target limit by not more than 2 times.

6. Microbial contamination limits for herbal drugs

Target limits of microbial contamination for herbal drugs and herbal drug containing preparations are shown in Table 3. Category 1 indicates herbal drugs and their preparations to which boiling water is added before use, and category 2 indicates other herbal drugs and their preparations. In this guideline, enterobacteria and other gram-negative bacteria, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* are mentioned as specified microorganisms, but other microorganisms such as certain species of *Bacillus cereus*, *Clostridia*, *Pseudomonas*, *Burkholderia*, *Aspergillus* and *Enterobacter species* are also necessary to be tested depending on the origin of the herbal drug raw materials or the preparation method of the preparations.

Table 1. Microbial enumeration limits for raw materials

Microorganisms	Target limit (cfu/g or cfu/mL)
Total aerobic microbial count (TAMC)	≤ 1000
Total combined yeasts/molds count (TYMC)	≤ 100

Table 2. Microbial enumeration limits for nonsterile finished dosage forms

Route of administration	TAMC (cfu/g or cfu/mL)	TYMC (cfu/g or cfu/mL)	Examples of objectionable microorganisms
Inhalation (liquid)	≤ 20	≤ 20	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>
Inhalation (powder)	≤ 100	≤ 50	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>
Nasal	≤ 100	≤ 50	<i>Staphylococcus aureus</i>

			<i>Pseudomonas aeruginosa</i>
Varginal	≤ 100	≤ 50	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Candida albicans</i>
Otic or Topical (including transdermal patches)	≤ 100*	≤ 50*	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>
Rectal	≤ 1000	≤ 100	Not specified
Oral (solid)	≤ 1000	≤ 100	<i>Escherichia coli</i>
Oral (liquid)	≤ 100	≤ 50	<i>Escherichia coli</i>

* For transdermal patches, the limits are expressed as cfu per transdermal patch.

Table 3. Microbial enumeration limits for herbal drugs and their preparations

Microorganisms	Category 1 (cfu/g or cfu/mL)	Category 2 (cfu/g or cfu/mL)
Aerobic bacteria	10 ⁷	10 ⁵
Molds and yeasts	10 ⁴	10 ³
Enterobacteria and other gram-negative bacteria	*	10 ³
<i>Escherichia coli</i>	10 ²	not detected
<i>Salmonella</i>	not detected	not detected
<i>Staphylococcus aureus</i>	*	*

* The limits are not specified.

13. Microbiological Evaluation of Processing Areas for Sterile Pharmaceutical Products

This chapter describes the methods for the control and evaluation of microbial contamination in areas used for the processing of sterile pharmaceutical products. Such processing areas are classified into critical areas and clean areas according to the required levels of air-cleanliness. A critical area is a defined space in which the airborne particulate and microorganism levels are controlled to meet grade A. The cleanliness requirements for such a space extend to the surfaces of the facilities and equipment which form or are located within the space, as well as to the supplied raw materials, chemicals, water, etc. Environmental conditions, such as temperature, humidity, and air pressure, are also controlled in this space when required. A clean area is a controlled space such that the levels of contaminants (particulates and microorganisms) in air, gases and liquids are maintained within specified limits, which are less stringent than those of grade A. When sterile pharmaceutical products are manufac-

Table 1. Air-cleanliness requirements for processing of sterile pharmaceutical products

Air cleanliness Grade* ¹	Maximum number of airborne particulates per m ³	
	at rest	in operation
	≥ 0.5 μm	≥ 0.5 μm
A (Laminar-airflow zone)	3,530	3,530
B (Non laminar-airflow zone)	3,530	353,000
C	353,000	3,530,000
D	3,530,000	—* ²

*1 The maximum permitted number of particles in the “in operation” condition corresponds to the standards described under USP <1116> as follows.

Grade A: Class 100 (M3.5); Grade B: Class 10,000 (M5.5); Grade C: Class 100,000 (M6.5);

Grade D: no corresponding standard.

*2 The limit for this area will depend on the nature of the operation carried out there.

Table 2. Suggested frequency of environmental monitoring

Processing area	Frequency of monitoring
Critical area (Grade A)	Each shift
Clean area adjacent to critical area (Grade B)	Each shift
Other clean areas (Grade C, D)	
Potential product/container contact areas	Twice a week
Non-product/container contact areas	Once a week

Table 3. Media and culture conditions

Microorganisms to be detected	Media* ¹	Culture conditions
Aerobes	Soybean-casein digest agar (or fluid) medium Brain-heart infusion agar (or fluid) medium Nutrient agar (or fluid) medium	30 – 35°C* ² More than 5 days* ³
Yeast and fungi	Soybean-casein digest agar (or fluid) medium Sabouraud dextrose agar (or fluid) medium Potato-dextrose agar (or fluid) medium Glucose peptone agar (or fluid) medium	20 – 25°C* ² More than 5 days* ³
Anaerobes* ⁴	Soybean-casein digest agar medium Fluid cooked meat medium Reinforced clostridial agar (or fluid) medium Thioglycolate medium I (or thioglycolate agar medium) for sterility test	30 – 35°C More than 5 days* ³

*1 If necessary, antibiotics may be added to media in an appropriate concentration (see Microbial Limit Test). If the existence of disinfectants that may interfere with the test on the surface of the specimen is suspected, add a substance to inactivate them.

*2 When soybean-casein digest agar medium is used for the detection of aerobes, yeast and fungi, incubation at 25 to 30°C for more than 5 days is acceptable.

*3 If a reliable count is obtained in a shorter incubation time than 5 days, this may be adopted.

*4 Generally, anaerobes are not targets for the monitoring. For the detection of anaerobes, agar medium is incubated in an appropriate anaerobic jar.

Table 4. Recommended limits for environmental microorganisms*¹

Grade	Airborne microorganisms* ² (CFU/m ³)	Minimum air sample (m ³)	CFU on a surface	
			instruments/facilities	gloves
			(CFU/24–30 cm ²)* ³	
A	< 1	0.5	< 1	< 1
B	10	0.5	5	5
C	100	0.2	25	—
D	200	0.2	50	—

*1 Maximum acceptable average numbers of microorganisms under each condition.

*2 These values are by using a slit sampler or equivalent.

*3 Viable microbe cell number per contact plate (5.4–6.2 cm in diameter). When swabbing is used in sampling, the number of microorganisms is calculated per 25 cm². For gloves, usually, put their all fingers on the plate.

tured, the environment, facilities/equipment, and personnel should be routinely monitored to ensure appropriate microbiological control in the processing areas. The detection of microorganisms should be performed under normal operational conditions, using an appropriate sampling device, according to an environmental control program established previously. The sampling, cultivation, counting, and evaluation methods for airborne microorganisms, as well as those found on surfaces, should also be chosen appropriately, depending on the monitoring purpose, monitoring items, and microorganisms being detected. Sampling devices, measurement methods, media, culture conditions, frequency of monitoring, and recommended limits for environmental microorganisms shown in this chapter are for information only, and are not requirements.

1. Definitions

For the purposes of this chapter, the following definitions apply.

1) Processing areas: Areas in which actions such as cultivation, extraction/purification, weighing of raw materials, washing and drying of containers and stoppers, preparation of solutions, filling, sealing and packaging are performed, including the gowning area.

2) Action levels: Established microbial levels (and type of microorganisms, if appropriate) that require immediate follow-up and corrective action if they are exceeded.

3) Alert levels: Established microbial levels (and type of microorganisms if appropriate) that give early warning of a potential drift from normal operating conditions, but which are not necessarily grounds for definitive corrective action, though they may require follow-up investigation.

4) Contaminants: Particulates and microorganisms causing contamination by adhering to surfaces or by being incorporated into materials.

5) Cleanliness: A quantity which indicates the condition of cleanliness of a monitored item, expressed as mass or number of contaminants contained in a certain volume or area.

6) Contamination control: The planning, establishment of systems and implementation activities performed in order to maintain the required cleanliness of a specified space or surface.

7) Shift: Scheduled period of work or production, usually less than 12 hours in length, during which operations are conducted by a single defined group of workers.

8) Characterization of contaminants: Procedures for classifying contaminants so that they can be differentiated. In routine control, classification to the genus level is sufficient; as required, identification to the species level is performed.

2. Air-cleanliness of processing areas for sterile pharmaceutical products

Airborne particulates in areas used for the processing of pharmaceutical products may act physically as a source of insoluble particles in the products, and biologically as a carrier of microorganisms. So, it is necessary to control strictly the number of particles in the air. The air-cleanliness criteria are shown in Table 1.

2.1 Terminally sterilized products

Solutions should generally be prepared in a grade C environment. Solution preparation may be permitted in a grade D environment if additional measures are taken to minimize contamination. For parenterals, filling should be done in a grade A workstation in a grade B or C environment. The re-

quirements during the preparation and filling of other sterile products are generally similar to those for parenterals.

2.2 Sterile products prepared aseptically after filtration

The handling of starting materials and the preparation of solutions should be done in a grade C environment. These activities may be permitted in a grade D environment if additional measures are taken to minimize contamination, such as the use of closed vessels prior to filtration. After sterile filtration, the product must be handled and filled into containers under grade A aseptic conditions.

2.3 Sterile products prepared aseptically from sterile starting materials

The handling of starting materials and all further processing should be done under grade A conditions.

3. Microbiological environmental monitoring program

Environmental monitoring is especially important in sterility assurance for sterile pharmaceutical products that are manufactured by aseptic processing. The major purpose of environmental monitoring is to predict potential deterioration of the processing environment before it occurs, and to produce high-quality, sterile pharmaceutical products under appropriate contamination control.

3.1 Monitoring of environmental microorganisms

a) An environmental control program document is prepared for each area used for the processing of sterile pharmaceutical products. The procedures in the document include: 1) items to be monitored, 2) type of microorganisms to be monitored, 3) frequency of monitoring, 4) methods of monitoring, 5) alert and action levels, and 6) actions to be taken when specified levels are exceeded.

b) The aseptic processing areas and other processing areas maintained under controlled conditions are monitored on a routine basis. The critical processing areas where sterile products are in contact with environmental air are monitored during every operational shift. The items to be monitored include the air, floor, walls, equipment surfaces, and the gowns and gloves of the personnel. Table 2 shows suggested frequencies of the environmental monitoring.

c) The sampling devices used for monitoring environmental microorganisms, as well as the methods and culture media, should be suitable to detect microorganisms that may be present (aerobic bacteria, anaerobic bacteria, molds, yeast, etc.). The cultivation conditions, such as incubation temperature and time, are selected to be appropriate for the specific growth requirements of microorganisms to be detected. Table 3 shows the culture media and cultivation conditions that are generally used in testing for environmental microorganisms.

d) The number of microorganisms in the samples is estimated by using the Membrane Filtration, Pour Plating, Spread Plating, or Serial Dilution (Most Probable Number) Methods described in the Microbial Limit Test.

e) Table 4 shows recommended limits for environmental microorganisms. The alert and action levels may be adjusted if necessary after sufficient data have been accumulated. The most important point in environmental monitoring is to confirm that an acceptable value of each monitoring item is maintained consistently.

f) Microorganisms isolated are characterized if necessary. In addition, analysis of hourly or daily variation of airborne particulate numbers will provide data to assist in the control of the cleanliness of processing areas.

3.2. Evaluation of environmental monitoring data

a) The data from the environmental monitoring are evaluated on a routine basis for each area and location. The source of any discrepancy should be investigated immediately and the investigation should be documented in a report. After corrective action has been taken, follow-up monitoring should be done to demonstrate that the affected area is once again within specification.

b) The report is reviewed and approved by personnel responsible for quality control and distributed to all key personnel associated with the aseptic processing operation.

4. Sampling devices and measuring methodology

Various types of sampling devices and measurement methods are available for the sampling and measurement of microorganisms in the air and on surfaces, and appropriate samplers and measuring methodology are selected according to the purpose of monitoring and the items to be monitored.

4.1 Evaluation of airborne microorganisms

a) Settle plates

Petri dishes of a specified diameter containing a suitable culture medium are placed at the measurement location and the cover is removed there. The plates are exposed for a given time and the microorganisms deposited from the air onto the agar surface are enumerated after incubation. This method is not effective for quantitative monitoring of total airborne microorganisms because it does not detect microorganisms that do not settle onto the surface of the culture media, and the settling velocity of aggregates of microorganisms is affected by air currents and disturbances in airflow. Although the results obtained by the settle plate method are only qualitative or semi-quantitative, this method is suitable for long-term evaluation of possible contamination of products or devices by airborne microorganisms.

b) Active microbial sampling methods

1) Measuring methods

Methods in which a fixed volume of air is aspirated include filtration-type sampling devices and impact-type sampling devices. With the filter-type sampling devices the desired volume of air can be collected by appropriately changing the air intake rate or the filter size. However, care must be taken to ensure that sterility is maintained while the filter is placed in and removed from the holder. When air sampling devices are used in critical areas, care must be taken to avoid disturbance of the airflow around the products. There are two types of filters; wet-type used gelatin filters and dry-type used membrane filters. With the dry-type filters, static electricity effects can make it impossible to collect quantitatively microorganisms on the filter. When an impact-type sampling device is used, the following points are important: 1) The speed at which the collected air strikes the culture medium surface must be sufficient to capture the microorganisms, but must not have an adverse effect on the collected microorganisms. 2) A sufficient volume of air must be sampled so that even extremely low levels of microbiological contaminants are detected, but the procedure must not cause a significant change in the physical or chemical properties of the culture medium. 3) When the device is used in critical areas, care must be exercised to ensure that the processing of the sterile pharmaceutical products is not adversely affected by the air disturbance.

2) Sampling devices

The most commonly used samplers are as follows: Slit sampler, Andersen sampler, pinhole sampler, centrifugal

sampler and filtration-type sampler. Each sampler has specific characteristics. The slit sampler is a device to trap microorganisms in a known volume of air passed through a standardized slit. The air is impacted on a slowly revolving Petri dish containing a nutrient agar. The rotation rate of the Petri dish and the distance from the slit to the agar surface are adjustable and it is possible to estimate the number of microorganisms in the air passed through the device for a period of up to 1 hr. The Andersen sampler consists of a perforated cover and several pieces of Petri dishes containing a nutrient agar, and a known volume of air passed through the perforated cover impacts on the agar medium in the Petri dishes. The sampler is suitable for the determination of the distribution of size ranges of microorganism particulates in the air. The pinhole sampler resembles the slit of the slit sampler, but has pinholes in place of the slit. A known volume of air passed through several pinholes impacts on agar medium in a slowly revolving Petri dish. The centrifugal sampler consists of a propeller that pulls a known volume of air into the device and then propels the air outward to impact on a tangentially placed nutrient agar strip. The sampler is portable and can be used anywhere, but the sampling volume of air is limited.

See above 1) on the characteristics of the filtration-type sampler.

4.2 Measurement methods for microorganisms on surfaces

a) Contact plates

Use a contact plate with an appropriate contact surface. The culture medium surface should be brought into contact with the sampling site for several seconds by applying uniform pressure without circular or linear movement. After contact and removal, the plates are covered and, as soon as possible, incubated using appropriate culture conditions. After a contact plate has been used, the site to which the plate was applied must be wiped aseptically to remove any adherent culture medium.

b) Swabs

A piece of sterilized gauze, absorbent cotton, cotton swab, or other suitable material premoistened with an appropriate rinse fluid is stroked in closely parallel sweeps or slowly rotated over the defined sampling area. After sampling, the swab is agitated in a specified amount of an appropriate sterilized rinse fluid, and the rinse fluid is assayed for viable organisms.

5. Test methods for collection performance of a sampling device for airborne microorganisms

The testing of the collection performance of sampling devices for airborne microorganisms is performed in accordance with JIS K 3836 (Testing methods for collection efficiency of airborne microbe samplers) or ISO 14698 - 1 (Cleanrooms and associated controlled environments. Biocontamination control. General principles).

6. Growth-promotion test of media and confirmation of antimicrobial substances

This test and confirmation are performed according to "Effectiveness of culture media and confirmation of antimicrobial substances" in the Microbial Limit Test.

Media

Brain-heart infusion agar medium or Fluid brain-heart infusion medium

Bovine brain extract powder*1

An amount equivalent to 200 g of calf brain
Bovine heart extract powder*2

An amount equivalent to 250 g of the material	
Peptone	10.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Disodium hydrogenphosphate dodecahydrate	2.5 g
Agar	15.0 g
Water	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.2 – 7.6.

Fluid cooked meat medium

Bovine heart extract powder*2

An amount equivalent to 450 g of the material	
Peptone	20.0 g
Glucose	2.0 g
Sodium chloride	5.0 g
Water	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.2 – 7.6.

Glucose peptone agar medium or Fluid glucose peptone medium

See Microbial Limit Test. Antibiotic is added if necessary.

Nutrient agar medium or Fluid nutrient medium

Meat extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
Water	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 6.6 – 7.0.

Potato-dextrose agar medium or Fluid potato-dextrose medium

See Microbial Limit Test. Antibiotic is added if necessary.

Reinforced clostridial agar medium or Fluid reinforced clostridial medium

Meat extract	10.0 g
Peptone	10.0 g
Yeast extract	3.0 g
Soluble starch	1.0 g
Glucose	5.0 g
L-Cystein hydrochloride monohydrate	0.5 g
Sodium chloride	5.0 g
Sodium acetate trihydrate	3.0 g
Agar	15.0 g
Water	1,000 mL

For fluid medium, add 0.5 g of agar. Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 6.7 – 6.9.

Sabouraud dextrose agar medium or Fluid sabouraud dextrose medium

See Microbial Limit Test. Antibiotic is added if necessary.

Soybean-casein digest agar medium or Fluid soybean-casein digest medium

See Microbial Limit Test.

Thioglycolate agar medium or thioglycolate medium I for sterility test

See Sterility Test. The agar concentration of Thioglycolate agar medium is about 1.5%.

*1 **Bovine brain extract powder** Dried extract of bovine fresh brain. A yellow-brown powder having a characteristic odor.

Loss on drying: not more than 5%.

*2 **Bovine heart extract powder** Dried extract of bovine fresh heart. A yellow-brown powder having a characteristic odor.

Loss on drying: not more than 5%.

Rinsing Liquids

Buffered sodium chloride-peptone solution (pH 7.0)

See Microbial Limit Test.

LP liquid

Casein peptone	1.0 g
Soybean lecithin	0.7 g
Polysorbate 80	1.0 – 20.0 g
Water	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.2.

Phosphate buffered solution (pH 7.2)

See Microbial Limit Test.

Ringer's solution, 1/4 concentration

Sodium chloride	2.25 g
Potassium chloride	0.105 g
Calcium chloride dihydrate	0.16 g
Water	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 7.0.

Thiosulfate-Ringer's solution

Sodium thiosulfate pentahydrate	0.8 g
Ringer's solution, 1/4 concentration	1,000 mL

Sterilize by heating in an autoclave at 121°C for 15 to 20 min. pH after sterilization: 6.6.

14. Mycoplasma Testing for Cell Substrates used for the Production of Biotechnological/Biological Products

This document describes the currently available methods of mycoplasma testing that should be performed for cell substrates that are used in the manufacture of biotechnological/biological products.

Methods suggested for detection of mycoplasma are, A. culture method, B. indicator cell culture method, and C. polymerase chain reaction (PCR) method.

Mycoplasma testing should be performed on the master cell bank (MCB) and the working cell bank (WCB), as well as on the cell cultures used during the manufacturing process of

the product. For the assessment of these cells, mycoplasma testing should be performed using both methods A and B. Method B, however, does not detect only DNA derived from mycoplasma. Therefore, if a positive result is obtained only from method B, method C can be used to determine whether mycoplasma is actually present. When method C is used, it is necessary to demonstrate the rationale for determining a negative result. In such a case, the sensitivity and specificity of the method, the appropriateness of the sample preparation, and the suitability of the selection of the test method, including selection of reagents, reaction conditions and primers should be taken into account.

Prior to mycoplasma testing, the sample should be tested to detect the presence of any factors inhibiting the growth of mycoplasma. If such growth-inhibiting factors are detected they should be neutralized or eliminated by an appropriate method, such as centrifugation or cell passage.

If the test will be performed within 24 hours of obtaining the sample, the sample should be stored at a temperature between 2°C and 8°C. If more than 24 hours will elapse before the test is performed, the sample should be stored at -60°C or lower.

If mycoplasma is detected, additional testing to identify the species may be helpful in determining the source of contamination.

A. Culture Method

1. Culture Medium

Both agar plates and broth are used. Each lot of agar and broth medium should be free of antibiotics except for penicillin. Refer to the Minimum Requirements for Biological Products regarding selection of the culture media. Other culture media may be used if they fulfill the requirements described in the following section 2.

2. Suitability of Culture Medium

Each lot of medium should be examined for mycoplasma growth-promoting properties. To demonstrate the capacity of the media to detect known mycoplasma, each test should include control cultures of at least two known species or strains of mycoplasma, one of which should be a dextrose fermenter (i.e., *M. pneumoniae* strain FH or equivalent species or strains) and one of which should be an arginine hydrolyser (i.e., *M. orale* CH 19299 or equivalent species or strains). The mycoplasma strains used for the positive control tests should be obtained from an official or suitably accredited agency, and handled appropriately. Inoculate the culture medium with no more than 100 colony-forming units (CFU).

3. Culture and Observation

1) Inoculate no less than 0.2 mL of test sample (cell suspension) in evenly distributed amounts over the surface of each of four or more agar plates. After the surfaces of the inoculated plates are dried, one half of the plates should be incubated under aerobic conditions in an atmosphere of air containing 5 to 10 percent carbon dioxide and adequate humidity, and the other half under anaerobic conditions in an atmosphere of nitrogen containing 5 to 10 percent carbon dioxide and adequate humidity at $36 \pm 1^\circ\text{C}$ for no less than 14 days.

2) Inoculate no less than 10 mL of the test sample (cell suspension) into each of two vessels containing 100 mL of broth medium. One vessel should be incubated under aerobic conditions, and the other under anaerobic conditions.

If the culture medium for the sample cells contains any

growth-inhibiting factors, such as antibiotics, these factors must be removed. A method such as centrifugation is recommended for this purpose.

3) Subculture 0.2 mL of broth culture from each vessel on the 3rd, 7th, and 14th days of incubation onto two or more agar plates. The plates inoculated with aerobic or anaerobic broth cultures should be incubated aerobically or anaerobically, respectively at $36 \pm 1^\circ\text{C}$ for no less than 14 days.

4) Examination of all plates for mycoplasma colonies should be done microscopically on the 7th and 14th day at 100 times magnification or greater.

B. Indicator Cell Culture Method

Using Vero cell culture substrate, pretest the suitability of the method using an inoculum of no more than 100 CFU of *M. hyorhinis* DBS 1050 or *M. orale* CH 19299.

An equivalent indicator cell substrate and suitable mycoplasma strains may be acceptable if data demonstrate at least equal sensitivity for the detection of known mycoplasma contaminants. The mycoplasma strains should be obtained from an official or suitably accredited agency, and handled appropriately. The cell substrate used should be obtained from a qualified cell bank and certified to be mycoplasma free. The acquired cells should be carefully cultured and propagated, and sufficient volumes of seed stock should be prepared with the proper precautions to avoid mycoplasma contamination. The stock should be tested for mycoplasma contamination using at least one of the methods described in this document, then frozen for storage. For each test a new container from the stock should be thawed and used within 6 passages.

Indicator cell cultures should be grown on cover slips submerged in culture dishes or equivalent containers for one day. Inoculate no less than 1 mL of the test sample (cell culture supernatant) into two or more of the culture dishes.

The test should include a negative (non-infected) control and two positive mycoplasma controls, such as *M. hyorhinis* DBS 1050 or *M. orale* CH 19299. Use an inoculum of no more than 100 CFU for the positive controls.

Incubate the cell cultures for 3 to 6 days at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide.

Examine the cell cultures after fixation for the presence of mycoplasma by epifluorescence microscopy (400 to 600 times magnification or greater) using a DNA-binding fluorochrome, such as bisbenzimidazole or an equivalent stain. Compare the microscopical appearance of the test cultures with that of the negative and positive controls.

Procedure

1) Aseptically place a sterilized glass cover slip into each cell culture dish (35 mm diameter).

2) Prepare Vero cell suspension in Eagle's minimum essential medium containing 10 percent bovine calf serum at a concentration of 1×10^4 cells per 1 mL. The bovine calf serum should be tested and confirmed to be free from mycoplasma prior to use.

3) Inoculate aliquots of 2 mL of the Vero cell suspension into each culture dish. Ensure that the cover slips are completely submerged, and not floating on the surface of the culture medium. Incubate the cultures at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide for one day, so that the cells are attached to the glass cover slip.

4) Replace 2 mL of the culture medium with fresh medium, then add 0.5 mL of the test sample (cell culture super-

nant) to each of two or more culture dishes. Perform the same procedure for the positive (2 types of mycoplasma, such as *M. hyorhinitis* and *M. orale*) and negative controls.

5) Incubate the cultures for 3 to 6 days at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide.

6) Remove the culture medium from the culture dishes, and add 2 mL of a mixture of acetic acid (100) and methanol (1:3) (fixative) to each dish; then, allow them to stand for 5 minutes.

7) Remove the fixative from each dish, then add the same amount of fixative again, and leave the dishes to stand for 10 minutes.

8) Remove the fixative and then completely air-dry all the dishes.

9) Add 2 mL of bisbenzamide fluorochrome staining solution to each culture dish. Cover the dishes and let them stand at room temperature for 30 minutes.

10) Aspirate the staining solution and rinse each dish with 2 mL of distilled water 3 times. Take out the glass cover slips and dry them.

11) Mount each cover slip with a drop of a mounting fluid. Blot off surplus mounting fluid from the edges of the cover slips.

12) Examine by epifluorescence microscopy at 400 to 600 times magnification or greater.

13) Compare the microscopic appearance of the test sample with that of the negative and positive controls.

14) The test result is judged to be positive if there are more than 5 cells per 1000 (0.5%) that have minute fluorescent spots that appear to surround, but are outside, the cell nucleus.

C. Polymerase Chain Reaction (PCR) Detection Method

The PCR method is a highly specific method that enables the detection of trace amounts of mycoplasma DNA, and has come to be widely used in recent years as a means of detecting mycoplasma contamination. However, the sensitivity and specificity depend on the procedure employed, and a positive result from PCR does not always indicate the presence of viable mycoplasma.

The PCR method is based on amplifying DNA extracted from the cell culture with specific primers so that the presence of the target DNA is detected. A two-step PCR (nested PCR) is recommended in order to increase sensitivity and specificity. The tests should include both a positive control (such as *M. hyorhinitis* of 100 CFU or less) and a negative control.

Mycoplasma DNA from the sample of cells or cell cultures is amplified using primers which should be able to amplify some common conserved mycoplasma DNA sequence. The amplification should be performed using an appropriate heat-resistant DNA polymerase, and suitable conditions. The amplified DNA can be identified after agarose gel electrophoresis, followed by ethidium bromide staining and UV irradiation of the gel.

For this method, it is important to use primers that are specific to mycoplasma by choosing base sequences that are well-conserved for a wide range of mycoplasma species, for example, the spacer region between the 16S-23S ribosome genes.

It is recommended that a two-step PCR using nested primers should be performed to increase the sensitivity and specificity, if the one-step PCR is negative.

The primers to be selected for the second stage of a two-

step PCR are nested primers from the inner portion of the sequence. The outer and inner primers should have proven effectiveness and specificity as described in publications or be validated experimentally.

It is possible to increase the accuracy of the detection of mycoplasma DNA by performing PCR tests after cultivation of mycoplasma that may be present in samples using Vero cells.

The following is an example of a two-step PCR procedure. The reagents and reaction conditions in this example are not exclusive. If the suitability of other reagents and conditions is verified, they may be used. If another procedure is used, the procedure should be justified and documented in detail, and the information provided should include the sensitivity and specificity of the method.

Example Procedure

1. Preparation of template

1) Place 600 μL of the test cell suspension (if necessary, subcultured with Vero cells) in a tube and dissolve the cells with 0.1% SDS or an equivalent. Add an equal volume (600 μL) of TE (10 mmol/L tris-hydrochloric acid (pH 8.0), 1 mmol/L EDTA) buffer-saturated phenol, and mix.

2) Centrifuge at $15,000 \text{ min}^{-1}$ for 5 minutes at room temperature.

3) Transfer 400 μL of the supernatant to another tube, and add 10 μL of 3 mol/L sodium acetate.

4) Add 1 mL (2.5 volumes) of ethanol (95) and stir thoroughly. Ice the mixture for 15 minutes, then centrifuge at $15,000 \text{ min}^{-1}$ for 10 minutes at 4°C .

5) Discard the supernatant and rinse the precipitate once or twice with 200 to 300 μL of 80% ethanol. Remove the rinse solution using a pipette. Centrifuge at $15,000 \text{ min}^{-1}$ for 10 minutes at 4°C , then remove the supernatant thoroughly and dry up the precipitate.

6) Dissolve the precipitate in 40 μL of distilled water.

2. Perform the same procedure for the positive and negative controls

3. First stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, outer primer, and reaction buffer solution (including Mg ions), and place 90 μL in each tube.

2) Add 10 μL of the template prepared as above to each tube containing the first stage PCR solution (90 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94°C for 30 seconds, annealing at an appropriate temperature for the primer (55°C for the primer in this example), and elongation at 72°C for 2 minutes. Add drops of mineral oil or suitable equivalent as needed during the reaction to prevent evaporation.

4. Second stage of a two-step PCR

1) Make a mixture of the heat-resistant DNA polymerase, dNTP solution, inner primer, and reaction buffer solution (including Mg ions), and place 99 μL in each tube.

2) Add 1 μL of the first stage PCR product from each tube to a tube containing the second stage PCR solution (99 μL).

3) Perform the DNA amplification by repeating 30 cycles of denaturation at 94°C for 30 seconds, annealing at an appropriate temperature for the primer (55°C for the primer in this example), and elongation at 72°C for 2 minutes. Add drops of mineral oil or suitable equivalent as needed during the reaction to prevent evaporation.

5. Agarose gel electrophoresis

- 1) Mix 10 μL of each of the first stage and second stage PCR products with 2 μL of an appropriate dye as a migration marker, and perform 1% agarose gel electrophoresis.
- 2) Stain the gel with ethidium bromide and take a photograph under UV irradiation.
- 3) The test is judged to be positive if a DNA band is detected.

[An Example of Primer]

For mycoplasma detection

Outer primer

F1: 5'-ACACCATGGGAG(C/T)TGGTAAT-3'

R1: 5'-CTTC(A/T)TCGACTT(C/T)CAGACCCAAGG-CAT-3'

Inner primer

F2: 5'-GTG(G/C)GG(A/C)TGGATCACCTCCT-3'

R2: 5'-GCATCCACCA(A/T)A(A/T)AC(C/T)CTT-3'

() indicates a mixture.

[PCR reaction solution]

	[First stage]	[Second stage]
dNTP solution (each 1.25 mol)	16 μL	16 μL
Primer (10 pmol/ μL)	F1 2 μL	F2 2 μL
Primer (10 pmol/ μL)	R1 2 μL	R2 2 μL
Heat-resistant DNA polymerase (1 U/ μL)	2 μL	2 μL
Reaction buffer solution	68 μL	77 μL
25 mmol/L magnesium chloride hexahydrate	8 μL	8 μL
10-fold buffer solution*	10 μL	10 μL
Sterile distilled water	50 μL	59 μL

*Composition of 10-fold buffer solution

2-amino-2-hydroxymethyl-1,3-propanediol-hydrochloric acid (pH 8.4)	100 mmol/L
Potassium chloride	500 mmol/L
Magnesium chloride hexahydrate	20 mmol/L
Gelatin	0.1 g/L

[Method of cultivating mycoplasma within Vero cells]

- 1) Use at least two cell culture dishes for each of the test sample, positive control and negative control.
- 2) Into each cell culture dish (diameter 35 mm), inoculate 2 mL of the Vero cell suspension (1×10^4 cells per 1 mL) in Eagle's minimum essential medium containing 10 percent bovine calf serum (tested in advance using the PCR method to verify that it does not contain any detectable mycoplasma DNA). Incubate the cultures at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide for one day.
- 3) Replace the culture media with fresh media, and add 0.5 mL of the test sample (cell culture supernatant) to each of two or more Vero cell culture dishes. Perform the same procedure for the positive (such as 100 CFU or less *M. hyorhinis*) and negative controls.
- 4) Incubate the Vero cell culture dishes for the test sample, positive and negative controls for 3 to 6 days at $36 \pm 1^\circ\text{C}$ in an atmosphere of air containing 5 percent carbon dioxide.

15. Peptide Mapping

This test is harmonized with the European Pharmacopoeia

and the U.S. Pharmacopoeia.

Purpose and Scope

Peptide mapping is an identity test for proteins, especially those obtained by r-DNA technology. It involves the chemical or enzymatic treatment of a protein resulting in the formation of peptide fragments followed by separation and identification of the fragments in a reproducible manner. It is a powerful test that is capable of identifying single amino acid changes resulting from events such as errors in the reading of complementary DNA (cDNA) sequences or point mutations. Peptide mapping is a comparative procedure because the information obtained, compared to a reference standard or reference material similarly treated, confirms the primary structure of the protein, is capable of detecting whether alterations in structure have occurred, and demonstrates process consistency and genetic stability. Each protein presents unique characteristics which must be well understood so that the scientific and analytical approaches permit validated development of a peptide map that provides sufficient specificity.

This chapter provides detailed assistance in the application of peptide mapping and its validation to characterize the desired protein product, to evaluate the stability of the expression construct of cells used for recombinant DNA products and to evaluate the consistency of the overall process, to assess product stability as well as to ensure the identity of the protein product, or to detect the presence of protein variant.

The Peptide Map

Peptide mapping is not a general method, but involves developing specific maps for each unique protein. Although the technology is evolving rapidly, there are certain methods that are generally accepted. Variations of these methods will be indicated, when appropriate, in specific monographs.

A peptide map may be viewed as a fingerprint of a protein and is the end product of several chemical processes that provide a comprehensive understanding of the protein being analyzed. Four major steps are necessary for the development of the procedure: isolation and purification of the protein, if the protein is part of a formulation; selective cleavage of the peptide bonds; chromatographic separation of the peptides; and analysis and identification of the peptides. A test sample is digested and assayed in parallel with a reference standard or a reference material. Complete cleavage of peptide bonds is more likely to occur when enzymes such as endoproteases (e.g., trypsin) are used, instead of chemical cleavage reagents. A map should contain enough peptides to be meaningful. On the other hand, if there are too many fragments, the map might lose its specificity because many proteins will then have the same profiles.

Isolation and Purification

Isolation and purification are necessary for analysis of bulk drugs or dosage forms containing interfering excipients and carrier proteins and, when required, will be specified in the monograph. Quantitative recovery of protein from the dosage form should be validated.

Selective Cleavage of Peptide Bonds

The selection of the approach used for the cleavage of peptide bonds will depend on the protein under test. This selection process involves determination of the type of cleavage to be employed—enzymatic or chemical—and the type of

cleavage agent within the chosen category. Several cleavage agents and their specificity are shown in Table 1. This list is not all-inclusive and will be expanded as other cleavage agents are identified.

Table 1. Examples of Cleavage Agents

Type	Agent	Specificity
Enzymatic	Trypsin (EC 3.4.21.4)	C-terminal side of Arg and Lys
	Chymotrypsin (EC 3.4.21.1)	C-terminal side of hydrophobic residues (e.g., Leu, Met, Ala, aromatics)
	Pepsin (EC 3.4.23.1 & 2)	Nonspecific digest
	Lysyl endopeptidase (Lys-C Endopeptidase) (EC 3.4.21.50)	C-terminal side of Lys
	Glutamyl endopeptidase (from <i>S. aureus</i> strain V8) (EC 3.4.21.19)	C-terminal side of Glu and Asp
	Peptidyl-Asp metallo endopeptidase (Endoproteinase Asp-N) (EC 3.24.33)	N-terminal side of Asp
Chemical	Cyanogen bromide	C-terminal side of Met
	2-Nitro-5-thio-cyanobenzoic acid	N-terminal side of Cys
	<i>o</i> -Iodosobenzoic acid	C-terminal side of Trp and Tyr
	Dilute acid	Asp and Pro
	BNPS-skatole	Trp

Pretreatment of Sample Depending on the size or the configuration of the protein, different approaches in the pretreatment of samples can be used. For monoclonal antibodies the heavy and light chains will need to be separated before mapping. If trypsin is used as a cleavage agent for proteins with a molecular mass greater than 100,000 Da, lysine residues must be protected by citraconylation or maleylation; otherwise, many peptides will be generated.

Pretreatment of the Cleavage Agent Pretreatment of cleavage agents—especially enzymatic agents—might be necessary for purification purposes to ensure reproducibility of the map. For example, trypsin used as a cleavage agent will have to be treated with tosyl-L-phenylalanine chloromethyl ketone to inactivate chymotrypsin. Other methods, such as purification of trypsin by HPLC or immobilization of enzyme on a gel support, have been successfully used when only a small amount of protein is available.

Pretreatment of the Protein Under certain conditions, it might be necessary to concentrate the sample or to separate the protein from added substances and stabilizers used in formulation of the product, if these interfere with the mapping procedure. Physical procedures used for pretreatment can include ultrafiltration, column chromatography, and lyophilization.

Other pretreatments, such as the addition of chaotropic agents (e.g., urea) can be used to unfold the protein prior to mapping. To allow the enzyme to have full access to cleavage sites and permit some unfolding of the protein, it is often necessary to reduce and alkylate the disulfide bonds prior to digestion.

Digestion with trypsin can introduce ambiguities in the tryptic map due to side reactions occurring during the digestion reaction, such as nonspecific cleavage, deamidation, disulfide isomerization, oxidation of methionine residues, or formation of pyroglutamic groups created from the deamidation of glutamine at the N-terminal side of a peptide. Furthermore, peaks may be produced by autohydrolysis of trypsin. Their intensities depend on the ratio of trypsin to protein. To avoid autohydrolysis, solutions of proteases may be prepared at a pH that is not optimal (e.g., at pH 5 for trypsin), which would mean that the enzyme would not become active until diluted with the digest buffer.

Establishment of Optimal Digestion Conditions Factors that affect the completeness and effectiveness of digestion of proteins are those that could affect any chemical or enzymatic reactions.

pH: The pH of the digestion mixture is empirically determined to ensure the optimization of the performance of the given cleavage agent. For example, when using cyanogen bromide as a cleavage agent, a highly acidic environment (e.g., pH 2, formic acid) is necessary; however, when using trypsin as a cleavage agent, a slightly alkaline environment (pH 8) is optimal. As a general rule, the pH of the reaction milieu should not alter the chemical integrity of the protein during the digestion and should not change during the course of the fragmentation reaction.

Temperature: A temperature between 25°C and 37°C is adequate for most digestions. The temperature used is intended to minimize chemical side reactions. The type of protein under test will dictate the temperature of the reaction milieu, because some proteins are more susceptible to denaturation as the temperature of the reaction increases. For example, digestion of recombinant bovine somatotropin is conducted at 4°C, because at higher temperatures it will precipitate during digestion.

Time: If sufficient sample is available, a time course study is considered in order to determine the optimum time to obtain a reproducible map and avoid incomplete digestion. Time of digestion varies from 2 to 30 hours. The reaction is stopped by the addition of an acid which does not interfere in the tryptic map or by freezing.

Amount of Cleavage Agent: Although excessive amounts of cleavage agent are used to accomplish a reasonably rapid digestion time (i.e., 6 to 20 hours), the amount of cleavage agent is minimized to avoid its contribution to the chromatographic map pattern. A protein to protease ratio between 20:1 and 200:1 is generally used. It is recommended that the cleavage agent can be added in two or more stages to optimize cleavage. Nonetheless, the final reaction volume remains small enough to facilitate the next step in peptide mapping—the separation step. To sort out digestion artifacts that might be interfering with the subsequent analysis, a blank determination is performed, using a digestion control with all the reagents, except the test protein.

Chromatographic Separation

Many techniques are used to separate peptides for

mapping. The selection of a technique depends on the protein being mapped. Techniques that have been successfully used for separation of peptides are shown in Table 2. In this section, a most widely used reverse-phase High Performance Liquid Chromatographic (RP-HPLC) method is described as one of the procedures of chromatographic separation.

Table 2. Techniques Used for the Separation of Peptides

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)
Ion-Exchange Chromatography (IEC)
Hydrophobic Interaction Chromatography (HIC)
Polyacrylamide Gel Electrophoresis (PAGE), nondenaturing
SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)
Capillary Electrophoresis (CE)
Paper Chromatography-High Voltage (PCHV)
High-Voltage Paper Electrophoresis (HVPE)

The purity of solvents and mobile phases is a critical factor in HPLC separation. HPLC-grade solvents and water that are commercially available, are recommended for RP-HPLC. Dissolved gases present a problem in gradient systems where the solubility of the gas in a solvent may be less in a mixture than in a single solvent. Vacuum degassing and agitation by sonication are often used as useful degassing procedures. When solid particles in the solvents are drawn into the HPLC system, they can damage the sealing of pump valves or clog the top of the chromatographic column. Both pre- and post-pump filtration is also recommended.

Chromatographic Column The selection of a chromatographic column is empirically determined for each protein. Columns with 100 Å or 300 Å pore size with silica support can give optimal separation. For smaller peptides, octylsilane chemically bonded to totally porous silica articles, 3 to 10 μm in diameter (L7) and octadecylsilane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 μm in diameter (L1) column packings are more efficient than the butyl silane chemically bonded to totally porous silica particles, 5 to 10 μm in diameter (L26) packing.

Solvent The most commonly used solvent is water with acetonitrile as the organic modifier to which less than 0.1% trifluoroacetic acid is added. If necessary, add isopropyl alcohol or n-propyl alcohol to solubilize the digest components, provided that the addition does not unduly increase the viscosity of the components.

Mobile Phase Buffered mobile phases containing phosphate are used to provide some flexibility in the selection of pH conditions, since shifts of pH in the 3.0 to 5.0 range enhance the separation of peptides containing acidic residues (e.g., glutamic and aspartic acids). Sodium or potassium phosphates, ammonium acetate, phosphoric acid, and a pH between 2 and 7 (or higher for polymer-based supports) have also been used with acetonitrile gradients. Acetonitrile containing trifluoroacetic acid is used quite often.

Gradient Selection Gradients can be linear, nonlinear, or include step functions. A shallow gradient is recommended in order to separate complex mixtures. Gradients are optimized to provide clear resolution of one or two peaks that will become "marker" peaks for the test.

Isocratic Selection Isocratic HPLC systems using a single

mobile phase are used on the basis of their convenience of use and improved detector responses. Optimal composition of a mobile phase to obtain clear resolution of each peak is sometimes difficult to establish. Mobile phases for which slight changes in component ratios or in pH significantly affect retention times of peaks in peptide maps should not be used in isocratic HPLC systems.

Other Parameters Temperature control of the column is usually necessary to achieve good reproducibility. The flow rates for the mobile phases range from 0.1 to 2.0 mL per minute, and the detection of peptides is performed with a UV detector at 200 to 230 nm. Other methods of detection have been used (e.g., postcolumn derivatization), but they are not as robust or versatile as UV detection.

Validation This section provides an experimental means for measuring the overall performance of the test method. The acceptance criteria for system suitability depend on the identification of critical test parameters that affect data interpretation and acceptance. These critical parameters are also criteria that monitor peptide digestion and peptide analysis. An indicator that the desired digestion endpoint was achieved is by the comparison with a Reference Standard, which is treated exactly as the article under test. The use of a reference standard or reference material in parallel with the protein under test is critical in the development and establishment of system suitability limits. In addition a specimen chromatogram should be included with the Reference Standard or Reference Material for additional comparison purposes. Other indicators may include visual inspection of protein or peptide solubility, the absence of intact protein, or measurement of responses of a digestion-dependent peptide. The critical system suitability parameters for peptide analysis will depend on the particular mode of peptide separation and detection and on the data analysis requirements.

When peptide mapping is used as an identification test, the system suitability requirements for the identified peptides covers selectivity and precision. In this case, as well as when identification of variant protein is done, the identification of the primary structure of the peptide fragments in the peptide map provides both a verification of the known primary structure and the identification of protein variants by comparison with the peptide map of the reference standard/reference material for the specified protein. The use of a digested reference standard or reference material for a given protein in the determination of peptide resolution is the method of choice. For an analysis of a variant protein, a characterized mixture of a variant and a reference standard or reference material can be used, especially if the variant peptide is located in a less-resolved region of the map. The index of pattern consistency can be simply the number of major peptides detected. Peptide pattern consistency can be best defined by the resolution of peptide peaks. Chromatographic parameters—such as peak-to-peak resolution, maximum peak width, peak area, peak tailing factors, and column efficiency—may be used to define peptide resolution. Depending on the protein under test and the method of separation used, single peptide or multiple peptide resolution requirements may be necessary.

The replicate analysis of the digest of the reference standard or reference material for the protein under test yields measures of precision and quantitative recovery. Recovery of the identified peptides is generally ascertained by the use of internal or external peptide standards. The

precision is expressed as the relative standard deviation (RSD). Differences in the recovery and precision of the identified peptides are expected; therefore, the system suitability limits will have to be established for both the recovery and the precision of the identified peptides. These limits are unique for a given protein and will be specified in the individual monograph.

Visual comparison of the relative retention times, the peak responses (the peak area or the peak height), the number of peaks, and the overall elution pattern is completed initially. It is then complemented and supported by mathematical analysis of the peak response ratios and by the chromatographic profile of a 1:1 (v/v) mixture of sample and reference standard or reference material digest. If all peaks in the sample digest and in the reference standard or reference material digest have the same relative retention times and peak response ratios, then the identity of the sample under test is confirmed.

If peaks that initially eluted with significantly different relative retention times are then observed as single peaks in the 1:1 mixture, the initial difference would be an indication of system variability. However, if separate peaks are observed in the 1:1 mixture, this would be evidence of the nonequivalence of the peptides in each peak. If a peak in the 1:1 mixture is significantly broader than the corresponding peak in the sample and reference standard or reference material digest, it may indicate the presence of different peptides. The use of computer-aided pattern recognition software for the analysis of peptide mapping data has been proposed and applied, but issues related to the validation of the computer software preclude its use in a compendial test in the near future. Other automated approaches have been used that employ mathematical formulas, models, and pattern recognition. Such approaches are, for example, the automated identification of compounds by IR spectroscopy and the application of diode-array UV spectral analysis for identification of peptides. These methods have limitations due to inadequate resolutions, co-elution of fragments, or absolute peak response differences between reference standard or reference material and sample fragments.

The numerical comparison of the retention times and peak areas or peak heights can be done for a selected group of relevant peaks that have been correctly identified in the peptide maps. Peak areas can be calculated using one peak showing relatively small variation as an internal reference, keeping in mind that peak area integration is sensitive to baseline variation and likely to introduce error in the analysis. Alternatively, the percentage of each peptide peak height relative to the sum of all peak heights can be calculated for the sample under test. The percentage is then compared to that of the corresponding peak of the reference standard/reference material. The possibility of auto-hydrolysis of trypsin is monitored by producing a blank peptide map, that is, the peptide map obtained when a blank solution is treated with trypsin.

The minimum requirement for the qualification of peptide mapping is an approved test procedure that includes system suitability as a test control. In general, early in the regulatory process, qualification of peptide mapping for a protein is sufficient. As the regulatory approval process for the protein progresses, additional qualifications of the test can include a partial validation of the analytical procedure to provide assurance that the method will perform as intended in the de-

velopment of a peptide map for the specified protein.

Analysis and Identification of Peptides

This section gives guidance on the use of peptide mapping during development in support of regulatory applications.

The use of a peptide map as a qualitative tool does not require the complete characterization of the individual peptide peaks. However, validation of peptide mapping in support of regulatory applications requires rigorous characterization of each of the individual peaks in the peptide map. Methods to characterize peaks range from N-terminal sequencing of each peak followed by amino acid analysis to the use of mass spectroscopy (MS).

For characterization purposes, when N-terminal sequencing and amino acids analysis are used, the analytical separation is scaled up. Since scale-up might affect the resolution of peptide peaks, it is necessary, using empirical data, to assure that there is no loss of resolution due to scale-up. Eluates corresponding to specific peptide peaks are collected, vacuum-concentrated, and chromatographed again, if necessary. Amino acid analysis of fragments may be limited by the peptide size. If the N-terminus is blocked, it may need to be cleared before sequencing. C-terminal sequencing of proteins in combination with carboxypeptidase and MALDITOF-MS can also be used for characterization purposes.

The use of MS for characterization of peptide fragments is by direct infusion of isolated peptides or by the use of on-line LC-MS for structure analysis. In general, it includes electrospray and matrix-assisted laser desorption ionization coupled to time-of-flight analyzer (MALDITOF) as well as fast atom bombardment (FAB). Tandem MS has also been used to sequence a modified protein and to determine the type of amino acid modification that has occurred. The comparison of mass spectra of the digests before and after reduction provides a method to assign the disulfide bonds to the various sulfhydryl-containing peptides.

If regions of the primary structure are not clearly demonstrated by the peptide map, it might be necessary to develop a secondary peptide map. The goal of a validated method of characterization of a protein through peptide mapping is to reconcile and account for at least 95% of the theoretical composition of the protein structure.

16. pH Test for Gastrointestinal Medicine

In this test, medicine for the stomach and bowels, which is said to control stomach acid, is stirred in a fixed amount of the 0.1 mol/L hydrochloric acid for a fixed duration, and the pH value of this solution is obtained. The pH value of a stomach medicine will be based on the dose and the dosage of the medicine (when the dosage varies, a minimum dosage is used) and expressed in the pH value obtained from the test performed by the following procedure.

Preparation of Sample

Solid medicine which conforms to the general regulations for medicine (the powdered medicine section) can be used as a sample. When the medicine is in separate packages, the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and mixed evenly to

make a sample. For granules and similar types in separate packages, among the solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), the content of 20 or more packages is accurately weighed to calculate the average mass for one dose and is then powdered to make sample. For granules and similar types not in separate packages, among solid medicine which does not conform to the general regulations for medicine (the powdered medicine section), 20 doses or more are powdered to make a sample. For capsules and tablets, 20 doses or more are weighed accurately to calculate the average mass for one dose or average mass and then powdered to make a sample.

Liquid medicine is generously mixed to make a sample.

Procedure

Put 50 mL of the 0.1 mol/L hydrochloric acid with the molarity coefficient adjusted to 1.000, or equivalent 0.1 mol/L hydrochloric acid with its volume accurately measured in a 100-mL beaker. Stir this solution with a magnetic stirrer and a magnetic stirrer rotator (35 mm length, 8 mm diameter) at the speed of about 300 revolutions per minute. While stirring, add the accurately weighed one-dose sample. After 10 minutes, measure the pH value of the solution using the pH Determination. The solution temperature should be maintained at $37 \pm 2^\circ\text{C}$ throughout this operation.

17. Plastic Containers for Pharmaceutical Products

Various kinds of plastics are used in the manufacture of containers for pharmaceutical products. Such plastics should not alter the efficacy, safety or stability of the pharmaceutical products. In selecting a suitable plastic container, it is desirable to have full information on the manufacturing processes of the plastic container including the substances added. Since each plastic has specific properties and a wide variety of pharmaceutical products may be stored in containers made from it, the compatibility of plastic containers with pharmaceutical products should be judged for each combination of container and the specific pharmaceutical product to be contained therein. This judgement should be carried out by verifying that a type sample of the container for the pharmaceutical preparation fulfills the essential requirements, i.e., the design specifications, according to experiments and/or scientific documentation, etc. In addition, the compatibility must be ensured based upon an appropriate quality assurance system.

Furthermore, in introducing a plastic container, it is desirable that proper disposal after use is taken into consideration.

Essential Requirements in Designing Plastic Containers for Pharmaceutical Products

The plastic material for the container should be of high quality. Therefore, recycled plastic materials, which are of unknown constitution, must not be used.

The leachables or migrants from the container should not alter the efficacy or stability of the pharmaceutical products contained therein. In addition, the possible toxic hazards of the leachables or migrants should not exceed a given level. Furthermore, the amounts of leachable or migratable chemical substances, such as monomers and additives, from the containers to the pharmaceutical products contained therein

must be sufficiently small from the viewpoint of safety.

The container should have a certain level of physical properties such as hardness, flexibility, shock resistance, tensile strength, tear strength, bending strength, heat resistance and the like, in accordance with the intended usage.

The quality of the pharmaceutical products contained in the container must not deteriorate during storage. For example, in the case of pharmaceutical products which are unstable to light, the container should provide a sufficient level of light shielding. In the case of pharmaceutical products which are easily oxidized, the container material should not allow the permeation of oxygen. In the case of aqueous pharmaceutical products and pharmaceutical products that must be kept dry, the container material should not allow the permeation of water vapor. In addition, care should be taken that the container is impermeable to the solvent in the case of solvents other than water. The concentration of the pharmaceuticals must not be decreased by more than a certain level due to the absorption of the pharmaceuticals on the surface of the container, the migration of the pharmaceuticals into the inside of the material of the container, or the loss of pharmaceuticals through the container. Also, the pharmaceutical products contained therein must not be degraded by an interaction with the material of the container.

The container should not be deformed, should not deteriorate and should not be degraded by the pharmaceutical products contained therein. Unacceptable loss of function of the container should not result from possible high temperature or low temperature or cycles thereof encountered during storage or transportation.

The container should be of a required level of transparency, when it is necessary to examine foreign insoluble matter and/or turbidity of the pharmaceutical products by visual observation.

In the case of pharmaceutical products which must be sterilized, it is required to satisfy the above-mentioned essential requirements of the container after the sterilization if there is a possibility that the quality of the container may change after the sterilization. There should not be any residue or generation of new toxic substances of more than certain risk level after the sterilization. In addition, the container should not have any inappropriate structure and/or material that might result in any bacterial contamination of the pharmaceutical products contained therein during storage and transportation after sterilization.

Toxicity Evaluation of Container at Design Phase

For design verification, the toxicity of the container should be evaluated. For the toxicity evaluation, it is desirable to select appropriate test methods and criteria for the evaluation, and to clarify the rationales for the selection. The tests should be conducted using samples of the whole or a part of the prototype container. If the container consists of plural parts of different materials, each part should be tested separately. Such materials as laminates, composites, and like are regarded as a single material. To test containers made of such materials, it is recommended to expose the inner surface of the container, which contacts the pharmaceutical products contained therein, to the extraction media used in the tests as far as possible.

The tests required for the toxicity evaluation of the container are different depending upon the tissue to which the pharmaceutical products contained therein are to be applied.

The following tests are required for containers for

- 1) preparations contacting blood:
Acute toxicity test, cytotoxicity test, sensitization test and hemolysis test
- 2) preparations contacting skin or mucous membranes:
Cytotoxicity test and sensitization test
- 3) liquid orally administered preparations:
Cytotoxicity test

It is recommended to conduct the tests in accordance with the latest versions of the standard test methods on medical devices and materials published in Japan and other countries.

Those standard test methods are listed for information:

(A) Selection of Tests

· Guidelines for Basic Biological Tests of Medical Devices and Materials (PAB Notification, YAKU-KI NO.99, June 27, 1995), Principles and selection of tests

· ISO 10993-1: Biological evaluation of medical devices—Evaluation and testing

(B) Acute Toxicity Test

· ASTM F750-82: Standard practice for evaluating material extracts by systemic injection in the mice

· BS5736: Part 3 Method of test for systemic toxicity; assessment of acute toxicity of extracts from medical devices

· USP 24 <88> Biological reactivity tests, in vivo

(C) Cytotoxicity Test

· Guidelines for Basic Biological Tests of Medical Devices and Materials, I. Cytotoxicity Test 10. Cytotoxicity test using extract of medical device or material

· ISO 10993-5: Biological evaluation of medical devices—Tests for cytotoxicity : in vitro methods

· USP 24 <87> Biological reactivity tests, in vitro

(D) Hemolysis Test

· Guidelines for Basic Biological Tests of Medical Devices and Materials, VII. Hemolysis Test

· ISO 10993-4: Biological evaluation of medical devices—Selection of tests for interaction with blood. Annex D

· ASTM F756-82: Standard practice for assessment of hemolytic properties of materials

(E) Sensitization Test

· Guidelines for Basic Biological Tests of Medical Devices and Materials, II. Sensitization Test

· ISO 10993-10: Biological evaluation of medical devices—Tests for irritation and sensitization

Test Results to be Recorded per Production Unit

At the line production phase, it is required to establish the values of acceptable limits on at least the test items mentioned below and to record the test results of each production unit of plastic containers for pharmaceutical products. In addition, it is desirable to clarify the rationales for setting the values of limits. However, these requirements should not be applied to orally administered preparations except liquid ones.

1) Combustion Tests: Residue of ignition, heavy metals. If necessary, the amounts of the specified metals (lead, cadmium, etc.)

2) Extraction Tests: pH, ultraviolet absorption spectra, potassium permanganate-reducing substances, foaming, non-volatile residue

3) Cytotoxicity Test

4) Any other necessary tests for the specific container for aqueous infusions.

18. Powder Flow

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

Four commonly reported methods for testing powder flow are (1) angle of repose, (2) compressibility index of Hausner ratio, (3) flow rate through an orifice, and (4) shear cell. In addition, numerous variations of each of these basic methods are available.

In general, any method of measuring powder flow should be practical, useful, reproducible, sensitive, and yield meaningful results. It bears repeating that no one simple powder flow method will adequately or completely characterize the wide range of flow properties. An appropriate strategy may well be the use of multiple standardized test methods to characterize the various aspects of powder flow as needed by the pharmaceutical scientist.

1. Angle of Repose

The angle of repose is the constant, three-dimensional angle (relative to the horizontal base) assumed by a cone-like pile of material formed by any of several different methods.

The angle of repose has been used in several branches of science to characterize the flow properties of solids. Angle of repose is a characteristic related to interparticulate friction, or resistance to movement between particles. Angle of repose test results are reported to be very dependent upon the method used. Experimental difficulties arise due to segregation of material and consolidation or aeration of the powder as the cone is formed.

1.1 Basic Methods for Angle of Repose

A variety of angle of repose test methods are reported in the literature. The most common methods for determining the static angle of repose can be classified based on two important experimental variables:

(1) The height of the “funnel” through which the powder passes may be fixed relative to the base, or the height may be varied as the pile forms.

(2) The base upon which the pile forms may be of fixed diameter or the diameter of the powder cone may be allowed to vary as the pile forms.

1.2 Variations in Angle of Repose Methods

In addition to the above methods, variations of them have been used to some extent.

· Drained angle of repose. This is determined by allowing an excess quantity of material positioned above a fixed diameter base to “drain” from the container. Formation of a cone of powder on the fixed diameter base allows determination of the drained angle of repose.

· Dynamic angle of repose. This is determined by filling a cylinder (with a clear, flat cover on one end) and rotating it at a specified speed. The dynamic angle of repose is the angle (relative to the horizontal) formed by the flowing powder. The internal angle of kinetic friction is defined by the plane separating those particles sliding down the top layer of the powder and those particles that are rotating with the drum (with roughened surface).

1.3 Angle of Repose General Scale of Flowability

While there is some variation in the qualitative description of powder flow using the angle of repose, much of the pharmaceutical literature appears to be consistent with the classification by Carr¹, which is shown in Table 1. There are examples of formulations with an angle of repose in the range of 40 to 50 degrees that manufactured satisfactorily. When the angle of repose exceeds 50 degrees, the flow is rarely acceptable for manufacturing purposes.

Table 1 Flow Properties and Corresponding Angles of Repose¹⁾

Flow Property	Angle of Repose (degrees)
Excellent	25 – 30
Good	31 – 35
Fair	36 – 40
Passable	41 – 45
Poor	46 – 55
Very poor	56 – 65
Very, very poor	>66

1.4 Experimental Considerations for Angle of Repose

Angle of repose is not an intrinsic property of the powder, that is to say, it is very much dependent upon the method used to form the cone of powder. On this subject, the existing literature raises these important considerations:

- The peak of the cone of powder can be distorted by the impact of powder from above. By carefully building the powder cone, the distortion caused by impact can be minimized.
- The nature of the base upon which the powder cone is formed influences the angle of repose. It is recommended that the powder cone be formed on a "common base", which can be achieved by forming the cone of powder on a layer of powder. This can be done by using a base of fixed diameter with a protruding outer edge to retain a layer of powder upon which the cone is formed.

1.5 Recommended Procedure for Angle of Repose

Form the angle of repose on a fixed base with a retaining lip to retain a layer of powder on the base. The base should be free of vibration. Vary the height of the funnel to carefully build up a symmetrical cone of powder. Care should be taken to prevent vibration as the funnel is moved. The funnel height should be maintained approximately 2 – 4 cm from the top of the powder pile as it is being formed in order to minimize the impact of falling powder on the tip of the cone. If a symmetrical cone of powder cannot be successfully or reproducibly prepared, this method is not appropriate. Determine the angle of repose by measuring the height of the cone of powder and calculating the angle of repose, α , from the following equation:
 $\tan \alpha = \text{height}/0.5 \text{ base}$

2. Compressibility Index and Hausner Ratio

In recent years the compressibility index and the closely related Hausner ratio have become the simple, fast and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of bulk density, size and shape surface area, moisture content, and cohesiveness of materials because all of these can influence the observed compressibility index. The compressibility index and the Hausner ratio are determined by measuring both the bulk volume and tapped volume of a

powder.

2.1 Basic Methods for Compressibility Index and Hausner Ratio

While there are some variations in the method of determining the compressibility index and Hausner ratio, the basic procedure is to measure (1) the unsettled apparent volume, V_o , and (2) the final tapped volume, V_f , of the powder after tapping the material until no further volume changes occur (refer to the General Test Method, 3.01 Determination of Bulk and Tapped Densities). The compressibility index and the Hausner ratio are calculated as follows:

$$\text{Compressibility Index} = 100 \times \{(V_o - V_f)/V_o\}$$

$$\text{Hausner Ratio} = V_o/V_f$$

Alternatively, the compressibility index and Hausner ratio may be calculated using measured values for bulk density (ρ_B) and tapped density (ρ_T) as follows:

$$\text{Compressibility Index} = 100 \times \{(\rho_T - \rho_B) / \rho_T\}$$

$$\text{Hausner Ratio} = \rho_T / \rho_B$$

In a variation of these methods, the rate of consolidation is sometimes measured rather than, or in addition to, the change in volume that occurs on tapping. For the compressibility index and the Hausner ratio, the generally accepted scale of flowability is given in Table 2.

Table 2. Scale of Flowability¹⁾

Compressibility Index (%)	Flow Character	Hausner Ratio
≤ 10	Excellent	1.00 – 1.11
11 – 15	Good	1.12 – 1.18
16 – 20	Fair	1.19 – 1.25
21 – 25	Passable	1.26 – 1.34
26 – 31	Poor	1.35 – 1.45
32 – 37	Very poor	1.46 – 1.59
≥ 38	Very, very poor	> 1.60

2.2 Experimental Considerations for the Compressibility Index and Hausner Ratio

Compressibility index and Hausner ratio are not intrinsic properties of the powder, that is to say, they are dependent upon the methodology used. The existing literature points out several important considerations affecting the determination of the (1) unsettled apparent volume, V_o , (2) the final tapped volume, V_f , (3) the bulk density, ρ_B , and (4) the tapped density, ρ_T :

- The diameter of the cylinder used
- The number of times the powder is tapped to achieve the tapped density
- The mass of material used in the test
- Rotation of the sample during tapping

2.3 Recommended Procedure for Compressibility Index and Hausner Ratio

Use a 250-mL volumetric cylinder with a test sample weight of 100 grams. Smaller weights and volumes may be used, but weight of the test sample and volume of the cylinder used should be described with the results. An average of three determinations is recommended.

3. Flow through an Orifice

The flow rate of a material depends upon many factors, some of which are particle-related and some related to the process. Monitoring the rate of flow of material through an orifice has been proposed as a better measure of powder flowability. Of particular significance is the utility of monitoring flow continuously since pulsating flow patterns have been observed even for free flowing materials. Changes in flow rate as the container empties can also be observed. Empirical equations relating flow rate to the diameter of the opening, particle size, and particle density have been determined. However, determining the flow rate through an orifice is useful only with free-flowing materials.

The flow rate through an orifice is generally measured as the mass per time flowing from any of a number of types of containers (cylinders, funnels, hoppers). Measurement of the flow rate can be in discrete increments or continuous.

3.1 Basic Methods for Flow through an Orifice

There are a variety of methods described in the literature. The most common for determining the flow rate through an orifice can be classified based on three important experimental variables:

(1) The type of container used to contain the powder. Common containers are cylinders, funnels and hoppers from production equipment.

(2) The size and shape of the orifice used. The orifice diameter and shape are critical factors in determining powder flow rate.

(3) The method of measuring powder flow rate. Flow rate can be measured continuously using an electronic balance and with some sort of recording device (strip chart recorder, computer). It can also be measured in discrete samples (for example, the time it takes for 100 grams of powder to pass through the orifice to the nearest tenth of a second or the amount of powder passing through the orifice in 10 seconds to the nearest tenth of a gram).

3.2 Variations in Methods for Flow through an Orifice

Either mass flow rate or volume flow rate can be determined. Mass flow rate is the easier of the methods, but it biases the results in favor of high-density materials. Since die fill is volumetric, determining volume flow rate may be preferable.

3.3 General Scale of Flowability for Flow through an Orifice

No general scale is available because flow rate is critically dependent on the method used to measure it.

3.4 Experimental Considerations for Flow through an Orifice

Flow rate through an orifice is not an intrinsic property of the powder. It is very much dependent upon the methodology used. The existing literature points out several important considerations affecting these methods:

- The diameter and shape of the orifice
- The type of container material (metal, glass, plastic)
- The diameter and height of the powder bed.

3.5 Recommended Procedure for Flow through an Orifice

Flow rate through an orifice can be used only for materials that have some capacity to flow. It is not useful for cohesive materials. Provided that the height of the powder bed (the

'head' of powder) is much greater than the diameter of the orifice, the flow rate is virtually independent of the powder head. Use a cylinder as the container because the cylinder material should have little effect on flow. Powder flow rate often increases when the height of the powder column is less than two times the diameter of the column. The orifice should be circular and the cylinder should be free of vibration. General guidelines for dimensions of the cylinder are as follows:

- Diameter of opening > 6 times the diameter of the particles
- Diameter of the cylinder > 2 times the diameter of the opening

Use of a hopper as the container may be appropriate and representative of flow in a production situation. It is not advisable to use a funnel, particularly one with a stem, because flow rate will be determined by the size and length of the stem as well as the friction between the stem and the powder. A truncated cone may be appropriate, but flow will be influenced by the powder-wall friction coefficient, thus, selection of an appropriate construction material is important.

For the opening in the cylinder, use a flat-faced bottom plate with the option to vary orifice diameter to provide maximum flexibility and better ensure a powder-over-powder flow pattern. Rate measurement can be either discrete or continuous. Continuous measurement using an electronic balance can more effectively detect momentary flow rate variations.

4. Shear Cell Methods

A variety of powder shear testers and methods that permit more thorough and precisely defined assessment of powder flow properties have been developed. Shear cell methodology has been used extensively in the study of pharmaceutical materials. From these methods, a wide variety of parameters can be obtained, including the yield loci representing the shear stress-shear strain relationship, the angle of internal friction, the unconfined yield strength, the tensile strength, and a variety of derived parameters such as the flow factor and other flowability indices. Because of the ability to more precisely control experimental parameters, flow properties can also be determined as a function of consolidation load, time, and other environmental conditions.

4.1 Basic Methods for Shear Cell

One type of shear cell is the cylindrical shear cell which is split horizontally, forming a shear plane between the lower stationary base and the upper movable portion of the shear cell ring. After powder bed consolidation in the shear cell (using a well-defined procedure), the force necessary to shear the powder bed by moving the upper ring is determined. Annular shear cell designs offer some advantages over the cylindrical shear cell design, including the need for less material. A disadvantage, however, is that because of its design, the powder bed is not sheared as uniformly because material on the outside of the annulus is sheared more than material in the inner region. A third type of shear cell (plate-type) consists of a thin sandwich of powder between a lower stationary rough surface and an upper rough surface that is moveable.

All of the shear cell methods have their advantages and disadvantages. A significant advantage of shear cell methodology in general is a greater degree of experimental control. The methodology generally is rather time-consuming and requires significant amounts of material and a well-trained operator.

4.2 Recommendations for Shear Cell

Because of the diversity of available equipment and experimental procedures, no specific recommendations regarding methodology are presented in this chapter. It is recommended that the results of powder flow characterization using shear cell methodology include a complete description of equipment and methodology used.

References

- 1) Carr R.L.: Evaluating flow properties of solids. *Chem. Eng.*, 72: 163-168(1965).

19. Preservatives-Effectiveness Tests

The purpose of the Preservatives-Effectiveness Tests is to assess microbiologically the preservative efficacy, either due to the action of product components themselves or any added preservative(s), for multi-dose containers. The efficacy of the preservatives is assessed by direct inoculation and mixing of the test strains in the product, and titration of survival of the test strains with time.

Preservatives must not be used solely to comply with GMP for drugs or to reduce viable aerobic counts. In addition, preservatives themselves are toxic substances. Therefore, preservatives must not be added to products in amounts which might jeopardize the safety of human beings, and consideration must be given to minimizing the amounts of preservative used. These tests are commonly used to verify that products maintain their preservative effectiveness at the design phase of formulation or in the case of periodic monitoring. Although these tests are not performed for lot release testing, the efficacy of the preservative present in the product packaged in the final containers should be verified throughout the entire dating period.

1. Products and their Categories

The products have been divided into two categories for these tests. Category I products are those made with aqueous bases or vehicles, and Category II products are those made with nonaqueous bases or vehicles. Oil-in-water emulsions are considered Category I products, and water-in-oil emulsions Category II products. Category I is further divided into three subtypes depending on the dosage forms.

Category IA: Injections and other sterile parenterals

Category IB: Nonsterile parenterals

Category IC: Oral products made with aqueous bases (including syrup products to be dissolved or suspended before use)

Category II: All the dosage forms listed under Category I made with nonaqueous bases or vehicles.

2. Test Microorganisms and Culture Media

The following strains or those considered to be equivalent are used as the test microorganisms.

Escherichia coli ATCC 8739, NBRC 3972

Pseudomonas aeruginosa ATCC 9027, NBRC 13275

Staphylococcus aureus ATCC 6538, NBRC 13276

Candida albicans ATCC 10231, NBRC 1594, JCM 2085

Aspergillus niger ATCC 16404, NBRC 9455

These test microorganisms are representative of those that might be found in the environment in which the product is manufactured, used or stored, and they are also recognized

as opportunistic pathogens. In addition to these strains designated as test microorganisms, it is further recommended to use strains that might contaminate the product and grow on or in it, depending on its characteristics. For the test microorganisms received from coordinated collections of microorganisms, one passage is defined as the transfer of microorganisms from an established culture to fresh medium, and microorganisms subjected to not more than five passages should be used for the tests. Single-strain challenges rather than mixed cultures should be used. The test strains can be harvested by growth on solid agar or liquid media.

Cultures on agar plate media: Inoculate each of the five test strains on the surface of agar plates or agar slants. For growth of bacteria, use Soybean-Casein Digest Agar Medium, and for yeasts and moulds, use Sabouraud Agar, Glucose-Peptone Agar or Potato Dextrose Agar Medium. Incubate bacterial cultures at 30°C to 35°C for 18 to 24 hours, the culture of *C. albicans* at 20°C to 25°C for 40 to 48 hours and the culture of *A. niger* at 20°C to 25°C for one week or until good sporulation is obtained. Harvest these cultured cells aseptically using a platinum loop, etc. Suspend the collected cells in sterile physiological saline or in 0.1% peptone water and adjust the viable cell count to about 10⁸ microorganisms per mL. In the case of *A. niger*, suspend the cultured cells in sterile physiological saline or 0.1% peptone water containing 0.05 w/v% of polysorbate 80 and adjust the spore count to about 10⁸ per mL. Use these suspensions as the inocula.

Liquid cultures: After culturing each of the four strains except for *A. niger* in a suitable medium, remove the medium by centrifugation. Wash the cells in sterile physiological saline or 0.1% peptone water and resuspend them in the same solution with the viable cell or spore count of the inoculum adjusted to about 10⁸ per mL.

When strains other than the five listed above are cultured, select a culture medium suitable for growth of the strain concerned. The cell suspension may also be prepared by a method suitable for that strain. Use the inoculum suspensions within 24 hours after they have been prepared from the cultivations on agar plate media or in liquid media. Store the inoculum suspensions in a refrigerator if it is not possible to inoculate them into the test specimens within 2 hours. Titrate the viable cell count of the inocula immediately before use, and then calculate the theoretical viable cell count per mL (g) of the product present just after inoculation.

3. Test Procedure

3.1 Category I products

Inject each of the cell suspensions aseptically into five containers containing the product and mix uniformly. When it is difficult to inject the cell suspension into the container aseptically or the volume of the product in each container is too small to be tested, transfer aseptically a sufficient volume of the product into each of alternative sterile containers, and mix the inoculum. When the product is not sterile, incubate additional containers containing the uninoculated product as controls and calculate their viable cell counts (the viable counts of bacteria and those of yeasts and moulds). A sterile syringe, spatula or glass rod may be used to mix the cell suspension uniformly in the product. The volume of the suspension mixed in the product must not exceed 1/100 of the volume of the product. Generally, the cell suspension is inoculated and mixed so that the concentration of viable cells is

10^5 to 10^6 cells per mL or per gram of the product. Incubate these inoculated containers at 20°C to 25°C with protection from light, and calculate the viable cell count of 1 mL or 1 g of the product taken at 0, 14 and 28 days subsequent to inoculation. Record any marked changes (e.g., changes in color or the development of a bad odor) when observed in the mixed samples during this time. Such changes should be considered when assessing the preservative efficacy of the product concerned. Express sequential changes in the viable counts as percentages, with the count at the start of the test taken as 100. Titration of the viable cell counts is based, in principle, on the Pour Plate Methods in "Microbial Limit Tests". In this case, confirm whether any antimicrobial substance is present in the test specimen. If a confirmed antimicrobial substance needs to be eliminated, incorporate an effective inactivator of the substance in the buffer solution or liquid medium to be used for dilution of the test specimen, as well as in the agar plate count medium. However, it is necessary to confirm that the inactivator has no effect on the growth of the microorganisms. When the occurrence of the preservative or the product itself affects titration of the viable cell count and there is no suitable inactivator available, calculate the viable cell counts by the Membrane Filtration Method in "Microbial Limit Tests".

3.2 Category II products

The procedures are the same as those described for Category I products, but special procedures and considerations are required for both uniform dispersion of the test microorganism in the product and titration of viable cell counts in the samples.

For semisolid ointment bases, heat the sample to 45°C to 50°C until it becomes oily, add the cell suspension and disperse the inoculum uniformly with a sterile glass rod or spatula. Surfactants may also be added to achieve uniform dispersion, but it is necessary to confirm that the surfactant added has no effect on survival or growth of the test microorganisms and that it does not potentiate the preservative efficacy of the product. For titration of the viable cell count, a surfactant or emulsifier may be added to disperse the product uniformly in the buffer solution or liquid medium. Sorbitan monooleate, polysorbate 80 or lecithin may be added to improve miscibility between the liquid medium and semisolid ointments or oils in which test microorganisms were inoculated. These agents serve to inactivate or neutralize many of the most commonly used preservatives.

4. Interpretation

Interpret the preservative efficacy of the product according to Table 1. When the results described in Table 1 are obtained, the product examined is considered to be effectively preserved. There is a strong possibility of massive microbial contamination having occurred when microorganisms other than the inoculated ones are found in the sterile product to be examined, and caution is required in the test procedures and/or the control of the manufacturing process of the product. When the contamination level in a nonsterile product to be examined exceeds the microbial enumeration limit specified in "Microbial Attributes of Nonsterile Pharmaceutical Products" in General Information, caution is also required in the test procedures and/or the control of the manufacturing process of the product.

Table 1. Interpretation criteria by product category

Product category	Microorganisms	Interpretation criteria	
		After 14 days	After 28 days
Category IA	Bacteria	0.1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category IB	Bacteria	1% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category IC	Bacteria	10% of inoculum count or less	Same or less than level after 14 days
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count
Category II	Bacteria	Same or less than inoculum count	Same or less than inoculum count
	Yeasts/moulds	Same or less than inoculum count	Same or less than inoculum count

5. Culture Media

Culture media and buffer solution used for Preservatives-Effectiveness Tests are described below. Other media may be used if they have similar nutritive ingredients and selective and growth-promoting properties for the microorganisms to be tested.

Soybean Casein Digest Agar Medium

Casein peptone	15.0 g
Soybean peptone	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 7.1 – 7.3.

Sabouraud Glucose Agar Medium

Peptone (animal tissue and casein)	10.0 g
Glucose	40.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

Glucose Peptone (GP) Agar Medium

Glucose	20.0 g
Yeast extract	2.0 g
Magnesium sulfate heptahydrate	0.5 g

Peptone	5.0 g
Monobasic potassium phosphate	1.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 5.6 – 5.8.

Potato Dextrose Agar Medium

Potato extract	4.0 g
Glucose	20.0 g
Agar	15.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 5.4 – 5.8.

0.1% Peptone Water

Peptone	1.0 g
Sodium chloride	8.0 g
Water	1000 mL

Mix all of the components and sterilize at 121°C for 15 – 20 minutes in an autoclave. pH after sterilization: 7.2 – 7.4.

20. Qualification of Animals as Origin of Animal-derived Medicinal Products provided in the General Notices of Japanese Pharmacopoeia and Other Standards

Introduction

The Official Gazette issued on March 29, 2002 announced that General Notices of the Japanese Pharmacopoeia and other standards were amended to add a provision that “When a drug product or a drug substance which is used to manufacture a drug product, is manufactured from a raw material of animal origin, the animal in question should be in principle a healthy subject, if not otherwise provided.”.

The Notice Iyaku-hatsu No. 0329001, which was issued on the same date, provided that “Healthy subject herein provided is the animal which does not cause any disease or any infection to human being at an appropriate production process and use of the drug product, and as for the oral or external drug for example, the animal, as its raw material of animal origin, should be confirmed at this stage to meet the Food Standard. It has to be noted that this standard of healthy subject has to be revised timely taking into account the up-to-date information with respect to the amphixenosis infections common between human beings and animals.”.

This General Information describes safety assurance against infection of drugs, which are manufactured from raw materials of animal origin, to follow up the Notice as mentioned above.

1. Basic concept

When drugs derived from raw materials of animal origin including human are used, it is important to take into account any possibility that communicable disease agents such as virus may cause infectious disease or any possible hazards to patients. In such case, it goes without saying that the pri-

mary subject that has to be considered is the absence of any infectious agents such as virus in the raw materials of animal origin including human as the source of the drug. More important points are whether the drugs derived from such raw materials are free of such infectious agents and whether there is any possibility of transmission of infectious agents when the drugs are administered to patient. The eligibility of animals including human, as the source of raw materials of drugs, in other words “the subject which is free from any disease or transmission of infectious agents that is infectious to human being at an appropriate production process and use of the drug product” is that “The drug should be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animals including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indications of the final product.”

2. Animals including human as the source of raw materials of drugs

What is the most clear and appropriate preventive measures against infection to human being due to administration of drugs which are derived from animals including human is to assure the absence of any infectious agents such as virus in its raw materials or an appropriate critical raw material by each of the followings: (1) the use of raw materials of healthy animal origin, which are proved to be free from communicable disease agents to human, or (2) the use of appropriate critical raw materials (e.g., cell substrate, blood plasma, pooled urine after some treatments) for drug production, which are proved to be free from communicable disease agents after certain appropriate processing on raw materials of animal origin.

As for raw materials of drugs of human origin, cell, tissue, blood, placenta, urine, etc. are used. Whenever it is sufficient and possible each donor, as the origin of such raw materials, should be asked his (her) health condition and undergoes his (her) medical examination at this stage, so that the appropriateness as a donor can be confirmed from the standpoint of safety concerning communicable disease agents such as virus.

For example, “Basic concept on handling and use of a drug product, etc. which is derived from cell/tissue” (Attachment 1 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000) and “Guidance for quality and safety assurance of a drug product, etc. which is derived from human cell/tissue (Attachment 2 of the Notice Iyaku-Hatsu No. 1314 dated December 26, 2000)” issued by the Director-General of the Medicinal Safety Bureau, Ministry of Health and Welfare, states that since the cell/tissue supplied by a human donor comes to be applied to patients without processing through any sufficient inactivation or removal of communicable disease agents, the selection and qualification criteria on such donor has to be established. These criteria are to be composed with the respect to the check items on the case history and the physical conditions as well as the test items on the various transmission of infectious agents through cell/tissue, and that the appropriateness of these criteria has to be clarified. Hepatitis Type-B (HBV), Hepatitis Type-C (HCV), Human Immune Deficiency Viral infections (HIV), Adult T-Cell Leukemia and Parvovirus B19 Infections should be denied through the interview to the donor and the tests (sero-

logic test, nucleic-acid amplification test, etc.). Further, if necessary, Cytomegalovirus infection and EB Virus infection should be denied by tests. "Infections caused by bacteria such as *Treponema pallidum*, Chlamydia, Gonococci, *Tubercule bacillus*, etc.", "septicemia and its suspicious case", "vicious tumor", "serious metabolic or endocrine-related disorders", "collagenosis and haematological disorder", "hepatic disease" and "dementia (transmissible spongiform encephalopathies and its suspicious case)" should be checked on the case history or by the interview, etc. and the experience of being transfused or/and transplanted should be checked to confirm eligibility as a donor. The most appropriate check items and test methods then available are to be used, which need to be reconsidered at appropriate timing taking into account the updated knowledge and the progress of the science and the technologies. At screening of a donor, reexaminations has to be made at appropriate timing using the eligible check items and the test methods taking into account the window period (Initial period after infection, in which antibody against bacteria, fungi or virus is not detected.)

In the case of plasma derivatives produced from the donated blood in Japan, the donor should be checked by means of self-assessed report about health conditions, and a serologic check and a nucleic acid amplification test (NAT) on mini pooled plasma should be performed at the stage of donated blood. Further, the plasma material (i.e., critical raw material) for fractionation should be stored 4 months in minimum so that the arrangement could be taken based on the information available after collection of the blood and the blood infusion to exclude the possibility of using any critical raw material which might cause infection to patients.

On the other hand, as for the materials such as urine which are taken from the unspecified number of the donors and come to be critical raw materials for drug production after some treatments, it is unrealistic and not practical to conduct the tests of virus infection, etc. on the individual donor. Consequently, appropriate tests such as virus test has to be performed on such critical raw materials for drug production.

In the case of the animals besides human, the wild ones should be excluded. Only the animals, which are raised under well sanitarly controlled conditions taken to prevent bacterial contamination or under the effective bacterial pollution monitoring systems, have to be used, and it is recommended that the animals from a colony appropriately controlled under specific pathogen-free (SPF) environment are to be used as far as possible. Further, for the animals regulated under the Food Standard, only the animals that met this standard should be used. It should be confirmed by appropriate tests that the animals were free from pathogen, if necessary.

The concrete measures to avoid transmittance or spread of infectivity of prion, which is considered to be the pathogen of transmissible spongiform encephalopathies (TSEs), as far as possible are the followings: ① avoidance of use of animals, which are raised in the areas where high incidence or high risk of TSEs (Scrapie in sheep and goat, bovine spongiform encephalopathies (BSE) in cattle, chronic wasting disease (CWD) in deer, new type of Creutzfeldt-Jacob-Disease (CJD) in human, etc.) is reported, and humans, who have stayed long time (more than 6 months) in such areas, as raw materials or related substances of drugs; ② avoidance of use of any substances that are derived from the individual infected with scrapie, BSE, CJD, etc.; ③ avoidance of using a material

derived from organ, tissue and cell, etc. of high risk of TSEs; and ④ taking appropriate measures basing on the information collected, which includes incidence of TSEs, the results of epidemiological investigation and the experimental research on prion, and incidence of tardive infection on donors after collecting raw materials, etc.

3. Human or animal cells which are used as critical raw materials for drug production

Cell substrates derived from humans or animals are used for drug production. In such case, it is desirable that the humans or the animals, which are the origins of the cell substrates, are healthy subjects. However, it is considered practical that viral safety of the drugs derived from the cell substrates are evaluated on the cells, which are so called critical raw materials for production of such drugs. In such case, the safety should be confirmed through the test and analysis on established cell bank thoroughly with respect to virus etc., as far as possible. The items and the methods of the tests that have been followed in this case are described in detail in the Notice of Japanese version on the internationally accepted ICH Guideline entitled "Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin" (Iyakushin No. 329 issued on February 22, 2000 by Director, Evaluation and Licensing Division, Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare). In the meantime, it is important how to handle the cell in case that any virus has been detected under the cell level tests. This Notice describes how to cope with this situation as follows: "It is recognised that some cell lines used for the manufacture of product will contain endogenous retroviruses, other viruses or viral sequences. In such circumstances, the action plan recommended for manufacturer is described in Section V (Rationale and action plan for viral clearance studies and virus tests on purified bulk) of the Notice. The acceptability of cell lines containing viruses other than endogenous retroviruses will be considered on an individual basis by the regulatory authorities, by taking into account a risk/benefit analysis based on the benefit of the product and its intended clinical use, the nature of the contaminating viruses, their potential for infecting humans or for causing disease in humans, the purification process for the product (e.g., viral clearance evaluation data), and the extent of the virus tests conducted on the purified bulk." For example, it is well known that Type A-, R- and C-endogenous particles like retrovirus are observed in the cells of the rodents used most often for drug production. It is also known that they are not infectious to human and is not dangerous, and CHO cells are generally used for drug production. The established cell lines (e.g., NAMALWA Cell, BALL-1 Cell, etc.) derived from cancer patients are sometimes used, but through the thorough virus tests, etc., their safety are confirmed. The established cell lines are assumed to be safer than the primary cultured cells which are hard to conduct the thorough virus test.

4. Establishment and control of appropriate production process and adherence to the clinical indication of final product for safety assurance

Safety assurance against potential infections at only the level of animals that are source of raw materials of drugs is limited. Further, "health of animal" can not be defined univocally, and the various factors have to be taken into account. The final goal of this subject is to protect human from

any infectious disease caused by drugs. Achieving this goal, the establishment and control of appropriate production processes of each drug and the adherence to the clinical indications of the final product are important.

As mentioned above, the rodent cells used most often for the production of the drugs are known to have endogenous retrovirus sometimes. The reason why such cells can be used for the production of the drugs is that multiple measures are applied for safety in the purification stages which include appropriate inactivation or removal processes. There are cases in which the production procedure involves intentional use of a virus or a microorganism. In this case, relevant measures capable of removing or inactivating of such virus or microorganism are appropriately incorporated in the purification process, so that the risk of infection to human can be fully denied and its safety can be assured when it is used as a drug. Further, even in the case that it is difficult to clarify the risk of contamination of the infectious agents or that the raw material are contaminated by viruses etc., the raw material in question may be used for the production of drugs so long as appropriate inactivation or removal processes are introduced, their effectiveness can be confirmed and the safety can be assured by appropriate control of the manufacturing processes under GMP, etc.

5. Conclusion

The qualification of animals including human, as the source of raw materials of drugs, in other words “the subject which does not cause any infectious diseases to human being at an appropriate production process and use of the drug product” is that “the drug has to be entirely free from any risk of infections by means of whole procedures which include evaluation of appropriateness of the animal including human as the source of their raw materials, establishment of appropriate production processes and their appropriate control, and strict adherence to the clinical indication of the final product.”

To cope with this subject, the advanced scientific measures, which actually reflect the updated knowledge and progress of the science and the technology about infectious diseases in human and infection of animal origin, have to be taken into account timely.

21. Quality Control of Water for Pharmaceutical Use

Water used for producing pharmaceutical products, cleaning pharmaceutical containers and equipment, and the like is referred to as “Pharmaceutical Water.” To consistently assure the quality of pharmaceutical water, it is important to verify through appropriate validation that water of required quality is supplied, and to maintain that quality by routine control.

Types of Pharmaceutical Water

① Water

The specifications for *Water* are specified in the corresponding monograph of the Japanese Pharmacopoeia. It is required for *Water* to meet the quality standards for drinking water provided by the Japanese Water Supply Law and an additional requirement for ammonium of “no greater than

0.05 mg/L.” In preparing *Water* at various facilities from source water such as well water or industrial water, it is needed to assure compliance with specifications set forth for *Water* in the Japanese Pharmacopoeia. Furthermore, when *Water* is used after storage for a long period of time, microbial proliferation should be prevented.

Water is used as source water for “*Purified Water*” and “*Water for Injection*.” It also is used in the production of intermediates of active pharmaceutical ingredients (APIs) and in pre-washing the equipments used for pharmaceutical purposes.

② Purified Water

The specifications of *Purified Water* are included in “Official Monographs” of the Japanese Pharmacopoeia. *Purified Water* is prepared from *Water* by distillation, ion-exchange treatment, reverse osmosis (RO), ultrafiltration (UF) capable of removing substances having molecular weights of 6,000 and above, or a combination of these methods. Because *Purified Water* contains no components capable of inhibiting microbial growth, appropriate microbiological control is needed. Particularly in the case of ion-exchange treatment, reverse osmosis or ultrafiltration, the appropriate treatment for microbial growth inhibition or periodical sterilization should be conducted.

Purified Water which has been sterilized or treated with chemical agents for the purpose of microbial growth inhibition or maintaining the endotoxin level within an appropriate control range should be appropriately controlled in order to maintain the quality in compliance with standards set forth for the individual purposes of use. *Purified Water* that has been sterilized is referred to as “*Sterilized Purified Water*.”

③ Water for Injection

The specifications of “*Water for Injection*” are described in “Official Monographs” of the Japanese Pharmacopoeia. *Water for Injection* is prepared from *Water* or *Purified Water* by distillation, or from *Purified Water* by reverse osmosis (RO), ultrafiltration (UF) capable of removing substances having molecular weights of 6,000 and above, or a combination of RO and UF. The quality of processing water by RO and/or UF should be maintained consistently at the same level as that prepared by distillation, by the validation through long-term operation of the process and elaborate routine control. Because *Water for Injection* contains no components that inhibit microbial growth, stringent control is needed for microorganisms and endotoxins. The standard for endotoxins requires a level lower than 0.25 EU/mL.

Selection of Pharmaceutical Water

Depending on the purpose of use, pharmaceutical water can be selected from the above-described group of pharmaceutical waters that allow us to assure the quality of the final product without causing any trouble during the production process. Table 1 shows some example criteria for such selection (in the case of charge-in water for the preparation of drug products).

For the production of sterile drug products, for which contamination by microorganisms or endotoxins is not permitted, *Water for Injection* should be used. For ophthalmics and eye ointments, *Water for Injection* or *Purified Water* should be used. For the production of non-sterile drug products, water of a quality higher than that of *Purified Water* should be used, however for a non-sterile drug product that requires

Table 1. Criteria for Selecting Pharmaceutical Water (Charge-In Water)

Classification	Class of Pharmaceutical Water	Application	Remarks
Drug Product	<i>Water for Injection</i>	Injections, Ophthalmics, Eye Ointments	
	<i>Purified Water</i>	Ophthalmics, Eye Ointment	For ophthalmics and eye ointments for which precautions should be taken against microbial contamination, purified water that is subjected to microbiological control by treatment with sterilization, ultrafiltration, or the like, should be used.
		Aerosols, Liquids and Solutions, Extracts, Elixirs, Capsules, Granules, Pills, Suspensions and Emulsions, Suppositories, Powders, Spirits, Tablets, Syrups, Infusions and Decoctions, Plasters, Tinctures, Troches, Ointments, Cataplasms, Aromatic Waters, Liniments, Lemonades, Fluidextracts, Lotions, and Pharmaceutical preparations of percutaneous absorption type	For liquids, solutions, ointments, suspensions, emulsions, aerosols, and the like for which precautions should be taken against microbial contamination, select and use purified water subjected to appropriate microbiological control.
Active Pharmaceutical Ingredient (API)	<i>Water for Injection</i>	Sterile APIs, and APIs rendered sterile in the formulation process	
	<i>Purified Water</i>	APIs, APIs rendered sterile in the formulation process, and API intermediates	In the production of APIs that are rendered sterile in the formulation process and have no subsequent processes capable of removing endotoxins, Purified Water that is controlled to maintain endotoxins at a low level should be used.
	<i>Water</i>	API Intermediates	

care against microbiological contamination, such as liquids, ointments, suspensions, emulsions, suppositories, and aerosols, water should have a quality appropriately controlled from microbiological viewpoints, considering the possible impacts of preservatives formulated in the dosage form. Water for pre-washing containers or equipment surfaces that come in direct contact with the drug should be of a quality higher than that of *Water*. Water for final rinsing should have the same quality as that of charge-in water.

For the production of non-sterile drug products, the quality of water should be higher than that of *Purified Water*; however for the production of liquids, ointments, suspensions, emulsions, suppositories, and aerosols for which care should be taken against microbial contamination, the quality of water should be controlled appropriately from microbiological viewpoints in consideration of the possible effects of formulated preservatives. Water for pre-washing containers or equipment surfaces that come in direct contact with the product should be of a quality not less than that of *Water*. The water for final rinsing should be the same quality as that of charge-in water.

In selecting pharmaceutical water for Active Pharmaceutical Ingredient (API), the characteristics and formulation

process of the drug products for which the API is used should be considered, so that the quality of the final drug products is maintained at an appropriate level. Water used in the production of sterile API or to clean pharmaceutical containers or equipment surfaces that come in direct contact with the product should have the quality controlled chemically and microbiologically at the level of *Water* or higher, even if the water is to be used at an earlier stage of a synthetic or extraction process. In the final purification process, water should have a quality equal to *Purified Water* or higher. Water used for the final rinsing of containers or equipment surfaces that come in direct contact with the product should have the same quality as that of the charge-in water. Pharmaceutical water used in sterile API should be *Water for Injection*. Similarly, in the case of APIs for drug products, where endotoxin control is required and there are no subsequent processes capable of removing endotoxins, *Water for Injection* or *Purified Water*, for which endotoxins are controlled to an appropriate level, should be used.

Quality Control of Pharmaceutical Water

3.1 Outline

Routine control of pharmaceutical water is conducted on

the premise that the production of water of a required quality has been thoroughly verified by validation studies at an earlier stage of the system that produces the pharmaceutical water (pharmaceutical water system). If these requirements are met, the following control methods are applicable.

For routine control, the control of electrical conductivity and Total Organic Carbon (TOC) is markedly useful. Items to be controlled regularly should be determined according to the intended use of the pharmaceutical water. In addition to the above, control should be made for several chemical substances, viable counts, endotoxins, insoluble foreign particles, and the like. The measurement frequency should be determined in consideration of the stability of the water quality in question.

The following shows microbiological control items and control items for electrical conductivity and Total Organic Carbon (TOC), for which special attention should be paid. Similar considerations should be made of the other control items so that the water quality meets the specifications of pharmaceutical water.

3.2 Sampling

Monitoring should be conducted at a sufficient enough frequency to ensure that the pharmaceutical water system is in control and that water of acceptable quality is continuously produced. Sampling should be made at representative locations in the production and supply systems of the pharmaceutical water, with particular care that the collected sample reflects the pharmaceutical water system. Normally, point-of-use sites are appropriate for the sampling locations. Sampling frequency is established on the basis of data from validation studies of the pharmaceutical water system. In making a sampling plan for pharmaceutical water system, it is important to cover all important points of the system by thoroughly considering the quality characteristics required for the water to be sampled. Particularly in microbiological control, attention should be paid to the difference in conditions among sampling locations.

3.3 Alert and Action Levels

In a pharmaceutical water system, monitoring is done for microorganisms and other quality attributes that assure water of acceptable quality is produced during operation within the designed specifications. Monitoring data thus obtained is compared against an alert level, action level, other control levels, and allowable limits for pharmaceutical waters. This implies the alert level and action level are used for process control, rather than for judging the acceptance.

Definition of Alert Level

An alert level indicates that, when exceeded, a process may have drifted from its normal operating condition. Alert levels constitute a warning and exceeding them does not necessarily require a corrective action. Alert levels are generally established either at a mean + 2 σ on the basis of past trend analyses or at a level of 70% (50% for viable counts) of the action criteria, whichever is smaller.

Definition of Action Level

An action level indicates that, when exceeded, a process has drifted from its normal operating range. Exceeding an action level indicates that corrective action must be taken to bring the process back within its normal operating range.

Alert and action levels should be established within process and product specification tolerances and based on integrated

considerations for technology and product quality. Consequently, exceeding an alert or action level does not necessarily indicate that the product quality has been compromised.

3.4 Microbiological Monitoring

The main purpose of a microbiological monitoring program for pharmaceutical water system is to predict any microbiological quality deterioration of the produced water and to prevent any adverse effects it may have on product quality. Consequently, detecting all of the microorganisms present may not be necessary; however it is required to adopt a monitoring technique capable of detecting a wide range of microorganisms, including slow growing microorganisms.

The following indicates culture-based microbiological monitoring methods for pharmaceutical water systems. In adopting a rapid detection system for microorganisms, it is needed to confirm in advance that the obtained microbial counts are equivalent to or above the data obtained through incubation.

3.4.1 Media and Incubation Conditions

There are many mesophilic bacteria of heterotrophic type that are adaptable to poor nutrient water environments. In many pharmaceutical water systems, heterotrophic bacteria may form bio-films and cause water quality deterioration. It, therefore, is useful to monitor the water quality by use of R2A Agar Medium, which is excellent for growing bacteria of oligotrophic type. On the other hand, in routine microbial monitoring, an approach that identifies the trend in microbiological quality change is widely employed; a standard agar plate is used for counting the total number of viable microorganisms capable of proliferating at 30 – 35°C in a comparatively short period of time.

Table 2 shows examples of measurement methods, minimum sample sizes, media, and incubation periods for estimating viable counts.

3.4.2 Media Growth Promotion Test

In the media growth promotion test with R2A Agar Medium, use the strains listed below or other strains considered to be equivalent to these strains. Prior to the media growth promotion test, inoculate these strains into sterile purified water and incubate at 20 – 25°C for 3 days.

Methylobacterium extorquens: NBRC 15911

Pseudomonas fluorescens: NBRC 15842, ATCC 17386, or the like

Dilute the fluid containing the starved strain in purified water with sterile purified water to prepare a fluid containing about 50 – 200 cfu per mL. When pipeting 1 ml of the diluted solution onto the R2A agar medium to be used for incubating at 20 – 25°C or 30 – 35°C for 4 – 7 days, a sufficient number of recovered colonies of the microorganism must be observed in comparison with the inoculated cfus.

In the media growth promotion test with standard agar medium, use the strains listed below or other strains considered to be equivalent to these strains. When pipeting 1 mL of the solution containing the microorganism for incubating at 30 – 35°C for 48 hours, a sufficient number of recovered colonies of the microorganism must be observed in comparison with the inoculated counts.

Staphylococcus aureus: ATCC 6538, NCIMB 9518, CIP 4.83 or NBRC 13276

Pseudomonas aeruginosa: ATCC 9027, NCIMB 8626, CIP 82.118 or NBRC 13275

Table 2. Methods for Assessment of Viable Counts in pharmaceutical Water

Method	Pharmaceutical Water		
	Water	Purified Water in Bulk	Water for Injection in Bulk
Measurement Method	Pour Plate Method or Membrane Filtration	Pour Plate Method or Membrane Filtration	Membrane Filtration
Minimum Sample Size	1.0 mL	1.0 mL	100 mL
Media	Standard Agar Medium	R2A Agar Medium, Standard Agar Medium	R2A Agar Medium, Standard Agar Medium
Incubation Period	Standard Agar Medium: 48 – 72 hours (or longer)	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)	R2A Agar Medium: 4 – 7 days (or longer) Standard Agar Medium: 48 – 72 hours (or longer)
Incubation Temperature	Standard Agar Medium: 30 – 35°C	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C	R2A Agar Medium: 20 – 25°C or 30 – 35°C Standard Agar Medium: 30 – 35°C

Colon bacillus (*Escherichia coli*): ATCC 8739, NCIMB 8545, CIP 53.126 or NBRC 3972

3.4.3 Action Levels for Microorganisms in Pharmaceutical Water System

The following action levels are considered appropriate and generally applicable to pharmaceutical water systems. Action levels for *Purified Water* and *Water for Injection* should be those obtained using R2A Agar Medium.

Action Levels for viable counts in various pharmaceutical waters

Water: 100 cfu/mL (according to the criteria in the Water Supply Law)

Purified Water: 100 cfu/mL

Water for Injection: 10 cfu/100 mL

(cfu: colony-forming units)

When actual counts exceed such action levels in validation studies or routine control studies, it is necessary to characterize the isolated microorganisms and to sterilize or disinfect the affected system.

3.5 Physicochemical Monitoring

Physicochemical monitoring of a pharmaceutical water system is generally performed using electrical conductivity and Total Organic Carbon (TOC) as indicators. Monitoring by electrical conductivity can predict probable quantities of the total inorganic salts present. Monitoring by TOC can estimate the total quantity of the organic compounds present. Basically, the tests for electrical conductivity and total organic carbon specified in “General Tests” of the Japanese Pharmacopoeia are applied as these physicochemical monitoring methods. However, tests for monitoring are different in profile from those described in “Monographs” of the Japanese Pharmacopoeia. The following describes the particulars for completing the part that cannot be worked out with the individual descriptions in “General Tests” of the Pharmacopoeia alone. When monitoring is done at a production

facility using electrical conductivity or TOC as an indicator, appropriate alert and action levels, as well as countermeasures against contingency should be established for each indicator.

3.5.1 Monitoring with an Indicator of Electrical Conductivity

Measurement of electrical conductivity for monitoring is usually conducted continuously with an in-line meter having a flow-through type or pipe-insertion type cell. Alternatively, batch testing may be done using a dip type cell with samples taken at point-of-use sites or other appropriate locations of the pharmaceutical water system. The following guidelines for the operation control of a pharmaceutical water system address how to interpret the results of electrical conductivity tests, and how to approve the continuation of operation, depending on whether measurements are made at the standard temperature (20°C) or at temperatures outside the standard.

(1) When monitoring is done at the standard temperature (20°C)

Tests for electrical conductivity of the Japanese Pharmacopoeia commonly require the tester to measure at the standard temperature (20°C), however measurement at a temperature within a range of $20 \pm 5^\circ\text{C}$ may be acceptable on condition that the correct equation is used. When monitoring electrical conductivity at the standard temperature of *Purified Water* and *Water for Injection*, the recommended allowable (action level) conductivity is as follows.

· Action Level 1.0 $\mu\text{S}/\text{cm}$ (20°C)

The above allowable conductivity is established on the supposition of in-line monitoring, so this standard level may be changed in batch testing.

(2) When monitoring is done at temperatures other than the standard temperature

When the quality of water is monitored by the measurement of electrical conductivity at a temperature other than

the standard temperature, the following three-stage approach is applied. The method described below complies with the description in “General Chapter <645> WATER CONDUCTIVITY” of the United States Pharmacopoeia (USP28, 2005), which specifies the establishment of allowable levels of electrical conductivity individually corresponding to different measurement temperatures and pH levels of specimens measured by the three-stage approach (Stage 1 to Stage 3).

Stage 1

1. When a non-temperature-compensated conductivity instrument is used, measure the temperature of the water specimen, in addition to the conductivity.

2. Using Table 3, find the conductivity value corresponding to the measured temperature or a temperature listed in Table 3 just below the measured temperature that can be used to calculate the allowable electrical conductivity at the measured temperature.

3. If the measured conductivity is not greater than the allowable conductivity value, the water specimen is judged as passing the electrical conductivity test. If the conductivity value exceeds the allowable conductivity value, proceed with Stage 2.

Table 3 Stage 1 Allowable Electrical Conductivity Measured at Different Temperatures*

Temperature (°C)	Allowable Conductivity (μS/cm)	Temperature (°C)	Allowable Conductivity (μS/cm)
0	0.6		
5	0.8	55	2.1
10	0.9	60	2.2
15	1.0	65	2.4
20	1.1	70	2.5
25	1.3	75	2.7
30	1.4	80	2.7
35	1.5	85	2.7
40	1.7	90	2.7
45	1.8	95	2.9
50	1.9	100	3.1

*Applied to the conductivity value measured with a non-temperature-compensated conductivity instrument.

Stage 2

1. Transfer a sufficient amount of water specimen to a suitable container, and stir the specimen. Adjust the temperature ($25 \pm 1^\circ\text{C}$), and while vigorously agitating the specimen, measure the conductivity periodically. If the change in conductivity is not greater than $0.1 \mu\text{S}/\text{cm}$, due to the uptake of atmospheric carbon dioxide, interpret the observed value as the conductivity of the specimen (in equilibrium with the atmosphere).

2. If the observed conductivity value at 25°C (with the specimen in equilibrium with the atmosphere) is not greater than $2.1 \mu\text{S}/\text{cm}$, the specimen is judged as passing the conductivity test. If the observed value is greater than the level, proceed with Stage 3.

Stage 3

1. While maintaining the sample at $25 \pm 1^\circ\text{C}$, perform the conductivity measurement as explained in Step 1 of Stage 2. Add 0.3 mL of saturated potassium chloride solution to 100

mL of the water specimen, and determine the pH to the nearest 0.1 pH.

2. Use Table 4 to find the allowable conductivity value corresponding to the observed pH. If the observed conductivity is lower than the allowable level, the water specimen is judged as passing the electrical conductivity test. If the observed value is greater than the allowable level, or if the pH of the specimen is outside of a range of pH 5.0 – 7.0, the water specimen is judged as unacceptable.

Table 4 Third Step Allowable Electrical Conductivity at Different pHs*

pH	Allowable Conductivity (μS/cm)	pH	Allowable Conductivity (μS/cm)
5.0	4.7		
5.1	4.1	6.1	2.4
5.2	3.6	6.2	2.5
5.3	3.3	6.3	2.4
5.4	3.0	6.4	2.3
5.5	2.8	6.5	2.2
5.6	2.6	6.6	2.1
5.7	2.5	6.7	2.6
5.8	2.4	6.8	3.1
5.9	2.4	6.9	3.8
6.0	2.4	7.0	4.6

*Applied only to a sample in equilibrium with the air at 25°C .

3.5.2 Monitoring with an Indicator of Total Organic Carbon (TOC)

Drinking Water Standards (Prescribed under the Article 4 of the Japanese Water Supply Law) require that TOC should be “not greater than 5 ppm”. However it is preferable for individual facilities to conduct TOC monitoring on *Water* with alert and action levels separately determined for water-quality control by TOC monitoring.

The Japanese Pharmacopoeia specifies the Test for Total Organic Carbon, and usually, TOC measurement is conducted using an apparatus in compliance with the specifications, however if the apparatus conforms to the apparatus suitability test requirements described in “General Chapter <643> TOTAL ORGANIC CARBON” of the United States Pharmacopoeia (USP28, 2005), otherwise described in “Methods of Analysis 2.2.44. TOTAL ORGANIC CARBON IN WATER FOR PHARMACEUTICAL USE” of the European Pharmacopoeia (EP 5.0, 2005), the apparatus may be used for monitoring a pharmaceutical water supplying system, provided that sufficiently pure water not contaminated with ionic organic substances or organic substances containing nitrogen, sulfur or chlorine atoms is used as source water for the system.

A TOC apparatus, characterized by calculating the amount of organic carbon from the difference in conductivity before and after the decomposition of organic substances without separating carbon dioxide from the sample solution, may be influenced negatively or positively, when applied to a sample solution containing ionic organic substances or organic substances comprised of nitrogen, sulfur, or halogens such as chlorine and the like; therefore, the apparatus should be selected appropriately depending on the purity of phar-

maceutical water to be measured and on the contamination risk in the case of apparatus failure.

3.6 Storage of Water for Injection

In storing *Water for Injection*, the water should be heated during circulation or other action to inhibit microbial proliferation. The maximum storage time allowed should also be appropriately established on the basis of validation studies, with considerations given to risks of contamination and quality deterioration.

22. Rapid Identification of Microorganisms Based on Molecular Biological Method

This chapter describes the methods for the identification or estimation of microorganisms (bacteria and fungi), found in in-process control tests or lot release tests of pharmaceutical products, at the species or genus level based on their DNA sequence homology. The identification of isolates found in the sterility test or aseptic processing can be helpful for investigating the causes of contamination. Furthermore, information on microorganisms found in raw materials used for pharmaceutical products, processing areas of pharmaceutical products, and so on is useful in designing measures to control the microbiological quality of drugs. For the identification of microorganisms, phenotypic analysis is widely used, based on morphological, physiological, and biochemical features and analysis of components. Commercial kits based on differences in phenotype patterns have been used for the identification of microorganisms, but are not always applicable to microorganisms found in raw materials used for pharmaceutical products and in processing areas of pharmaceutical products. In general, the identification of microorganisms based on phenotypic analysis needs special knowledge and judgment is often subjective. It is considered that the evolutionary history of microorganisms (bacteria and fungi) is memorized in their ribosomal RNAs (rRNAs), so that systematic classification and identification of microorganisms in recent years have been based on the analysis of these sequences. This chapter presents a rapid method to identify or estimate microorganisms based on partial sequences of divergent regions of the 16S rRNA gene for bacteria and of the internal transcribed spacer 1 (ITS1) region located between 18S rRNA and 5.8S rRNA for fungi, followed by comparison of the sequences with those in the database. Methods described in this chapter do not take the place of usual other methods for the identification, and can be modified based on the examiner's experience, and on the available equipment or materials. Other gene regions besides those mentioned in this chapter can be used if appropriate.

Apparatuses

(1) DNA sequencer

Various types of sequencers used a gel board or capillary can be used.

(2) DNA amplifier

To amplify target DNA and label amplified (PCR) products with sequencing reagents.

Procedures

The following procedures are described as an example.

1. Preparation of template DNA

It is important to use a pure cultivated bacterium or fungus for identification. In the case of colony samples, colonies are picked up with a sterilized toothpick (in the case of fungi, a small fragment of colony sample is picked up), and suspended in 0.3 mL of DNA releasing solution in a 1.5 mL centrifuge tube. In the case of culture fluid, a 0.5 mL portion of fluid is put in a 1.5 mL centrifuge tube and centrifuged at 10,000 rpm for 10 min. After removal of the supernatant, the pellet is suspended in 0.3 mL of DNA releasing solution, and then heated at 100°C for 10 min. In general, PCR can be run for bacteria and yeasts heated in DNA releasing solution. For fungi, DNA extraction after treatment with a mixer or ultrasonic generator may be necessary before PCR.

2. PCR

Add 2 μ L of template DNA in PCR reaction solution. Use 10F/800R primers for bacteria and ITS1F/ITS1R primers for fungi, and then perform 30 amplification cycles at 94°C for 30 sec, 55°C for 60 sec, and 72°C for 60 sec. DNA fragments are amplified about 800 bp in the case of bacteria and about 150 – 470 bp depending on the strain in the case of fungi. Include a negative control (water instead of the test solution) in the PCR.

3. Confirmation of PCR products

Mix 5 μ L of PCR product with 1 μ L of loading buffer solution, place it in a 1.5 w/v% agarose gel well, and carry out electrophoresis with TAE buffer solution (1-fold concentration). Carry out the electrophoresis together with appropriate DNA size markers. After the electrophoresis, observe PCR products on a trans-illuminator (312 nm) and confirm the presence of a single band of the targeted size. If multiple bands are observed, cut the targeted band out of the gel, and extract DNA by using appropriate commercial DNA extraction kit.

4. Purification of PCR products

Remove unincorporated PCR primers and deoxynucleoside triphosphates (dNTP) from PCR products by using appropriate purification methods.

5. Quantification of purified DNA

When purified DNA is measured by spectrophotometer, calculate 1 OD_{260 nm} as 50 μ g/mL.

6. Labeling of PCR products with sequencing reagents

Use an appropriate fluorescence-labeled sequencing reagent suitable for the available DNA sequencer or its program and label the PCR products according to the instructions provided with the reagent.

7. Purification of sequencing reagent-labeled PCR products

Transfer the product in 75 μ L of diluted ethanol (7 in 10) into a 1.5 mL centrifuge tube, keep in an ice bath for 20 min, and centrifuge at 15,000 rpm for 20 min. After removal of supernatant, add 250 μ L of diluted ethanol (7 in 10) to the precipitate and centrifuge at 15,000 rpm for 5 min. Remove the supernatant and dry the precipitate.

8. DNA homology analysis

Place sequencing reagent-labeled PCR products in the DNA sequencer and read the nucleotide sequences of the PCR products. Compare the partial nucleotide sequence with those in the BLAST database.

Judgment

If sequencing data show over 90% identity with a sequence in the database, in general, judgment may be made as follows.

1. In the case of bacteria, compare about 300 nucleotides between positions 50 to 350 in the product obtained with the 10F primer, with the BLAST database. Higher ranked species are judged as identified species or closely related species.
2. In the case of fungi, compare sequencing data for the product obtained with the ITS1F primer, with the BLAST database. Higher ranked species are judged as identified species or closely related species.

Reagents, Test Solutions

(1) 0.5 mol/L Disodium dihydrogen ethylenediamine tetraacetate TS

Dissolve 18.6 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in water to make 100 mL.

(2) 1 mol/L Tris buffer solution, pH 8.0

Dissolve 24.2 g of 2-amino-2-hydroxymethyl-1,3-propanediol in a suitable amount of water, adjust the pH to 8.0 with 0.2 mol/L hydrochloric acid TS, and add water to make 200 mL.

(3) TE buffer solution

Mix 1.0 mL of 1 mol/L tris buffer solution, pH 8.0 and 0.2 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 100 mL.

(4) DNA releasing solution

Divide TE buffer solution containing Triton X-100 (1 in 100) into small amounts and store frozen until use.

(5) PCR reaction solution

10-fold buffer solution*	5 μ L
dNTP mixture**	4 μ L
10 μ mol/L Sense primer	1 μ L
10 μ mol/L Anti-sense primer	1 μ L
Heat-resistant DNA polymerase (1 U/ μ L)	1 μ L
Water	36 μ L

* Being composed of 100 mmol/L 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloride, pH 8.4, 500 mmol/L potassium chloride, 20 mmol/L magnesium chloride and 0.1 g/L gelatin.

** A solution containing 2.5 mmol/L each of dGTP (sodium 2'-deoxyguanosine 5'-triphosphate), dATP (sodium 2'-deoxyadenosine 5'-triphosphate), dCTP (sodium 2'-deoxycytidine 5'-triphosphate) and dTTP (sodium 2'-deoxythymidine 5'-triphosphate). Adequate products containing these components as described above may be used.

(6) Sequencing reagent

There are many kinds of sequencing methods, such as the dye-primer method for labeling of primer, the dye-terminator method for labeling of dNTP terminator and so on. Use an appropriate sequencing reagent kit for the apparatus and program to be used.

(7) 50-Fold concentrated TAE buffer solution

Dissolve 242 g of 2-amino-2-hydroxymethyl-1,3-propanediol in 57.1 mL of acetic acid (100) and 100 mL of 0.5 mol/L disodium dihydrogen ethylenediamine tetraacetate TS, and add water to make 1000 mL.

(8) 1-Fold concentrated TAE buffer solution

Diluted 50-fold concentrated TAE buffer solution (1 in 50) prepared before use is referred to as 1-fold concentrated TAE buffer solution.

(9) Agarose gel

Mix 1.5 g of agarose, 2.0 mL of 50-fold concentrated TAE buffer solution, 10 μ L of a solution of ethidium bromide (3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide) (1 in

100) and 100 mL of water. After dissolving the materials by heating, cool the solution to about 60°C, and prepare gels.

(10) Loading buffer solution (6-fold concentrated)

Dissolve 0.25 g of bromophenol blue, 0.25 g of xylene cyanol FF and 1.63 g of disodium dihydrogen ethylenediamine tetraacetate dihydrate in 50 mL of water, and add 30 mL of glycerol and water to make 100 mL.

(11) PCR primers

For	Primer
Bacteria	10F 5'-GTTTGATCCTGGCTCA-3' 800R 5'-TACCAGGGTATCTAATCC-3'
Fungi	ITS1F 5'-GTAACAAGGT(T/C)TCCGT-3' ITS1R 5'-CGTTCTTCATCGATG-3'

23. SDS-Polyacrylamide Gel Electrophoresis

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

The SDS-Polyacrylamide Gel Electrophoresis is used for the characterization of proteins in biotechnological and biological products and for control of purity and quantitative determinations.

This technique is a suitable analytical method with which to identify and to assess the homogeneity of proteins in biotechnological and biological products. The method is also routinely used for the estimation of protein subunit molecular masses and for determining the subunit compositions of purified proteins.

Ready-to-use gels and reagents are widely available on the market and can be used instead of those described in this text, provided that they give equivalent results and that they meet the validity requirements given below under Validation of the Test.

1. Characteristics of Polyacrylamide Gels

The sieving properties of polyacrylamide gels are afforded by the three-dimensional network of fibers and pores which is formed as the bifunctional bisacrylamide cross-links adjacent polyacrylamide chains. Polymerization is catalyzed by a free radical-generating system composed of ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine (TEMED).

As the acrylamide concentration of a gel increases, its effective pore size decreases. The effective pore size of a gel is operationally defined by its sieving properties; that is, by the resistance it imparts to the migration of macromolecules. There are limits on the acrylamide concentration that can be used. At high acrylamide concentrations, gels break much more easily and are difficult to handle. As the pore size of a gel decreases, the migration rate of a protein through the gel decreases. By adjusting the pore size of a gel, through manipulating the acrylamide concentration, the resolution of the method can be optimized for a given protein product. Thus, the physical characteristics of a given gel are determined by the relative concentrations of acrylamide and bisacrylamide, used in its preparation.

In addition to the composition of the gel, the state of the

protein is an important determinant of the electrophoretic mobility. In the case of proteins, the electrophoretic mobility is dependent on the pK values of the charged groups and the size of the molecule. It is also influenced by the type, concentration and pH of the buffer, the temperature and the field strength, as well as by the nature of the support material.

2. Polyacrylamide Gel Electrophoresis under Denaturing Conditions

The method cited as an example is limited to the analysis of monomeric polypeptides with a mass range of 14,000 to 100,000 daltons. It is possible to extend this mass range by various techniques (e.g., by using gradient gels, particular buffer systems, etc.), but those techniques are not discussed in this chapter.

Analysis by electrophoresis on sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-Polyacrylamide Gel Electrophoresis) under denaturing conditions is the most common mode of electrophoresis used in assessing the quality of proteins in biotechnological and biological products, and will be the focus of the example described here. Typically, analytical electrophoresis of proteins is carried out in polyacrylamide gels under conditions that ensure dissociation of the proteins into their individual polypeptide subunits and that minimize aggregation. Most commonly, the strongly anionic detergent SDS is used in combination with heat to dissociate the proteins before they are loaded on the gel. The denatured polypeptides bind to SDS, become negatively charged and exhibit a consistent charge-to-mass ratio regardless of protein type. Because the amount of SDS bound is almost always proportional to the molecular mass of the polypeptide and is independent of its amino acid sequence, SDS-polypeptide complexes migrate through polyacrylamide gels with mobilities that are dependent on the size of the polypeptides.

The electrophoretic mobilities of the resultant SDS-polypeptide complexes all assume the same functional relationship to their molecular masses. Migration of SDS-complexes occurs toward the anode in a predictable manner, with low-molecular-mass complexes migrating faster than larger ones. The molecular mass of a protein can therefore be estimated from its relative mobility calibrated in SDS-Polyacrylamide Gel Electrophoresis and the occurrence of a single band in such a gel is a criterion of purity.

However, modifications to the polypeptide backbone, such as N- or O-linked glycosylation, have a significant impact on the apparent molecular mass of a protein, since SDS does not bind to a carbohydrate moiety in a manner similar to a polypeptide. Thus, a consistent charge-to-mass ratio is not maintained. The apparent molecular masses of proteins that have undergone post-translational modifications do not truly reflect the masses of the polypeptides.

1) Reducing conditions

Polypeptide subunits and three-dimensional structure of proteins are often fixed, at least in part, by the presence of disulfide bonds. A goal of SDS-Polyacrylamide Gel Electrophoresis under reducing conditions is to disrupt this structure by reducing the disulfide bonds. Complete denaturation and dissociation of proteins by treatment with 2-mercaptoethanol or dithiothreitol (DTT) will result in unfolding of the polypeptide backbone and subsequent complexation with SDS. Under these conditions, the molecular masses of the polypeptide subunits can be calculated by interpolation in the

presence of suitable molecular-mass standards.

2) Non-reducing conditions

For some analyses, complete dissociation of the protein of interest into subunit peptides is not desirable. In the absence of treatment with reducing agents such as 2-mercaptoethanol or DTT, disulfide covalent bonds remain intact, preserving the oligomeric form of the protein. Oligomeric SDS-protein complexes migrate more slowly than their SDS-polypeptide subunits. In addition, non-reduced proteins may not be completely saturated with SDS and, hence, may not bind the detergent in the expected mass ratio. This makes molecular-mass determinations of these molecules by SDS-Polyacrylamide Gel Electrophoresis less straightforward than analyses of fully denatured polypeptides, since it is necessary that both standards and unknown proteins be in similar configurations for valid comparisons. However, the staining of a single band in such a gel is a criterion of purity.

3. Characteristics of Discontinuous Buffer System Gel Electrophoresis

The most widely used electrophoretic method for the analysis of complex mixtures of proteins involves the use of a discontinuous buffer system consisting of two contiguous, but distinct gels: a resolving or separating (lower) gel and a stacking (upper) gel. The two gels are cast with different porosities, pH, and ionic strengths. In addition, different mobile ions are used in the gel and electrode buffers. The buffer discontinuity acts to concentrate large-volume samples in the stacking gel, resulting in improved resolution. When power is applied, a voltage drop develops across the sample solution which drives the proteins into the stacking gel. Glycinate ions from the electrode buffer follow the proteins into the stacking gel. A moving boundary region is rapidly formed with the highly mobile chloride ions in the front and the relatively slow glycinate ions in the rear. A localized high-voltage gradient forms between the leading and trailing ion fronts, causing the SDS-protein complex to form into a very thin zone (called the stack) and to migrate between the chloride and glycinate phases. Regardless of the height of the applied sample solution in the wells, all SDS-protein complexes condense within broad limits and enter the resolving gel as a well-defined, thin zone of high protein density. The large-pore stacking gel does not retard the migration of most proteins and serves mainly as an anticonvective medium. At the interface of the stacking and resolving gels, the proteins experience a sharp increase in retardation due to the smaller pore size of the resolving gel. Once the proteins are in the resolving gel, their mobility continues to be slowed down by the molecular sieving effect of the matrix. The glycinate ions overtake the proteins, which then move in a space of uniform pH formed by the tris(hydroxymethyl)aminomethane and glycine. Molecular sieving causes the SDS-polypeptide complexes to separate on the basis of their molecular masses.

4. Preparing Vertical Discontinuous Buffer SDS-Polyacrylamide Gels

1) Assembling of the gel moulding cassette

Clean the two glass plates (size: e.g. 10 cm × 8 cm), the sample comb made of polytetrafluoroethylene, the two spacers and the silicone rubber tubing (diameter, e.g. 0.6 mm × 35 cm) with mild detergent and rinse extensively with water. Dry all the items with a paper towel or tissue. Lubricate the spacers and the silicone rubber tubing with non-silicone grease. Apply the spacers along each of the two short

sides of the glass plate 2 mm away from the edges and 2 mm away from the long side corresponding to the bottom of the gel. Begin to lay the silicone rubber tubing on the glass plate by using one spacer as a guide. Carefully twist the silicone rubber tubing at the bottom of the spacer and follow the long side of the glass plate. While holding the silicone rubber tubing with one finger along the long side again twist the tubing and lay it on the second short side of the glass plate, using the spacer as a guide. Place the second glass plate in perfect alignment and hold the mould together by hand pressure. Apply two clamps on each of the two short sides of the mould. Carefully apply four clamps on the longer side of the gel mould, thus forming the bottom of the gel mould. Verify that the silicone rubber tubing is running along the edge of the glass plates and has not been extruded while placing the clamps.

2) Preparation of the gel

In a discontinuous buffer SDS polyacrylamide gel, it is recommended to pour the resolving gel, let the gel set, and then pour the stacking gel, since the compositions of the two gels in acrylamide-bisacrylamide, buffer and pH are different. *Preparation of the resolving gel:* In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide for the resolving gel, using the values given in Table 1. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the tetramethylethylenediamine (TEMED), filter the solution if necessary under vacuum through a cellulose acetate membrane (pore size: 0.45 μm); keep the solution under vacuum by swirling the filtration unit until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 1, swirl and pour immediately into the gap between the two glass plates of the mould. Leave sufficient space for the stacking gel (the length of the teeth of the sample comb plus 1 cm). Using a pipette, carefully overlay the solution with water-saturated isobutanol. Leave the gel in a vertical position at room temperature to allow polymerization to occur.

Preparation of the stacking gel: After polymerization is complete (about 30 minutes), pour off the isobutanol and wash the top of the gel several times with water to remove the isobutanol overlay and any unpolymerized acrylamide. Drain as much fluid as possible from the top of the gel, and then remove any remaining water with the edge of a paper towel.

In a conical flask, prepare the appropriate volume of solution containing the desired concentration of acrylamide, using the values given in Table 2. Mix the components in the order shown. Where appropriate, before adding the ammonium persulfate solution and the TEMED, filter the solution if necessary under vacuum through a cellulose acetate membrane (pore size: 0.45 μm); keep the solution under vacuum by swirling the filtration unit until no more bubbles are formed in the solution. Add appropriate amounts of ammonium persulfate solution and TEMED as indicated in Table 2, swirl and pour immediately into the gap between the two glass plates of the mould directly onto the surface of the polymerized resolving gel. Immediately insert a clean sample comb into the stacking gel solution, taking care to avoid trapping air bubbles. Add more stacking gel solution to fill completely the spaces of the sample comb. Leave the gel in a vertical position and allow to polymerize at room temperature.

3) Mounting the gel in the electrophoresis apparatus and

electrophoretic separation

After polymerization is complete (about 30 minutes), remove the sample comb carefully. Rinse the wells immediately with water or with the running buffer for SDS-Polyacrylamide Gel Electrophoresis to remove any unpolymerized acrylamide. If necessary, straighten the teeth of the sample comb of the stacking gel with a blunt hypodermic needle attached to a syringe. Remove the clamps on one short side, carefully pull out the silicone rubber tubing and replace the clamps. Proceed similarly on the other short side. Remove the silicone rubber tubing from the bottom part of the gel. Mount the gel in the electrophoresis apparatus. Add the electrophoresis buffers to the top and bottom reservoirs. Remove any bubbles that become trapped at the bottom of the gel between the glass plates. This is best done with a bent hypodermic needle attached to a syringe. Never pre-run the gel before loading solutions, such as samples, since this will destroy the discontinuity of the buffer systems. Before loading solutions, such as samples, carefully rinse the stacking gel wells with the running buffer for SDS-Polyacrylamide Gel Electrophoresis. Prepare the test and reference solutions in the recommended sample buffer and treat as specified in the individual monograph. Apply the appropriate volume of each solution to the stacking gel wells. Start the electrophoresis using suitable operating conditions for the electrophoresis equipment to be used. There are commercially available gels of different surface area and thickness that are appropriate for various types of electrophoresis equipment. Electrophoresis running time and current/voltage may need to be altered depending on the type of apparatus used, in order to achieve optimum separation. Check that the dye front is moving into the resolving gel. When the dye is reaching the bottom of the gel, stop the electrophoresis. Remove the gel assembly from the apparatus and separate the glass plates. Remove the spacers, cut off and discard the stacking gel and immediately proceed with staining.

5. Detection of Proteins in Gels

Coomassie staining is the most common protein staining method, with a detection level of the order of 1 μg to 10 μg of protein per band. Silver staining is the most sensitive method for staining proteins in gels and a band containing 10 ng to 100 ng can be detected. All of the steps in gel staining are done at room temperature with gentle shaking in any convenient container. Gloves must be worn when staining gels, since fingerprints will stain.

1) Coomassie staining

Immerse the gel in a large excess of Coomassie staining TS and allow to stand for at least 1 hour. Remove the staining solution.

Destain the gel with a large excess of destaining TS. Change the destaining solution several times, until the stained protein bands are clearly distinguishable on a clear background. The more thoroughly the gel is destained, the smaller is the amount of protein that can be detected by the method. Destaining can be speeded up by including 2 to 3 g of anion-exchange resin or a small sponge in the destaining TS.

NOTE: the acid-alcohol solutions used in this procedure do not completely fix proteins in the gel. This can lead to losses of some low-molecular-mass proteins during the staining and destaining of the gel. Permanent fixation is obtainable by allowing the gel to stand in trichloroacetic acid TS for fixing for 1 hour before it is immersed in Coomassie staining TS.

Table 1. Preparation of resolving gel

Solution components	Component volumes (mL) per gel mould volume of							
	5 mL	10 mL	15 mL	20 mL	25 mL	30 mL	40 mL	50 mL
6% Acrylamide								
Water	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
Acrylamide solution ⁽¹⁾	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Acrylamide								
Water	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
Acrylamide solution ⁽¹⁾	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Acrylamide								
Water	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
Acrylamide solution ⁽¹⁾	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Acrylamide								
Water	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
Acrylamide solution ⁽¹⁾	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
14% Acrylamide								
Water	1.4	2.7	3.9	5.3	6.6	8.0	10.6	13.8
Acrylamide solution ⁽¹⁾	2.3	4.6	7.0	9.3	11.6	13.9	18.6	23.2
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.2	2.5	3.6	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Acrylamide								
Water	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
Acrylamide solution ⁽¹⁾	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 mol/L Tris solution (pH 8.8) ⁽²⁾	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
100 g/L SDS ⁽³⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
100 g/L APS ⁽⁴⁾	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED ⁽⁵⁾	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

(1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution

(2) 1.5 mol/L Tris solution (pH 8.8): 1.5 mol/L tris-hydrochloride buffer solution, pH 8.8

(3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate

(4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.

(5) TEMED: *N,N,N',N'*-tetramethylethylenediamine

Table 2. Preparation of stacking gel

Solution components	Component volumes (mL) per gel mould volume of							
	1 mL	2 mL	3 mL	4 mL	5 mL	6 mL	8 mL	10 mL
Water	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
Acrylamide solution ⁽¹⁾	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 mol/L Tris solution (pH 6.8) ⁽²⁾	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
100 g/L SDS ⁽³⁾	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
100 g/L APS ⁽⁴⁾	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED ⁽⁵⁾	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

- (1) Acrylamide solution: 30% acrylamide/bisacrylamide (29:1) solution
- (2) 1.0 mol/L Tris solution (pH 6.8): 1 mol/L tris-hydrochloride buffer solution, pH 6.8
- (3) 100 g/L SDS: 100 g/L solution of sodium dodecyl sulfate
- (4) 100 g/L APS: 100 g/L solution of ammonium persulfate. Ammonium persulfate provides the free radicals that drive polymerization of acrylamide and bisacrylamide. Since ammonium persulfate solution decomposes slowly, fresh solutions must be prepared before use.
- (5) TEMED: *N,N,N',N'*-tetramethylethylenediamine

2) Silver staining

Immerse the gel in a large excess of fixing TS and allow to stand for 1 hour. Remove the fixing solution, add fresh fixing solution and incubate either for at least 1 hour or overnight, if convenient. Discard the fixing solution and wash the gel in water for 1 hour. Soak the gel for 15 minutes in a 1 vol% glutaraldehyde solution. Wash the gel twice for 15 minutes in water. Soak the gel in fresh silver nitrate TS for silver staining for 15 minutes, in darkness. Wash the gel three times for 5 minutes in water. Immerse the gel for about 1 minute in developer TS until satisfactory staining has been obtained. Stop the development by incubation in blocking TS for 15 minutes. Rinse the gel with water.

6. Drying of Stained SDS-Polyacrylamide Gels

Depending on the staining method used, gels are pretreated in a slightly different way. For Coomassie staining, after the destaining step, allow the gel to stand in a diluted solution of concentrated glycerin (1 in 10) for at least 2 hours (overnight incubation is possible). For silver staining, add to the final rinsing a step of 5 minutes in a diluted solution of concentrated glycerin (1 in 50).

Immerse two sheets of porous cellulose film in water and incubate for 5 to 10 minutes. Place one of the sheets on a drying frame. Carefully lift the gel and place it on the cellulose film. Remove any trapped air bubbles and pour 2 to 3 mL of water around the edges of the gel. Place the second sheet on top and remove any trapped air bubbles. Complete the assembly of the drying frame. Place in an oven or leave at room temperature until dry.

7. Molecular-Mass Determination

Molecular masses of proteins are determined by comparison of their mobilities with those of several marker proteins of known molecular mass. Mixtures of proteins with precisely known molecular masses blended for uniform staining are commercially available for calibrating gels. They are obtainable in various molecular mass ranges. Concentrated stock solutions of proteins of known molecular mass are diluted in the appropriate sample buffer and loaded on the same gel as the protein sample to be studied.

Immediately after the gel has been run, the position of the bromophenol blue tracking dye is marked to identify the

leading edge of the electrophoretic ion front. This can be done by cutting notches in the edges of the gel or by inserting a needle soaked in India ink into the gel at the dye front. After staining, measure the migration distances of each protein band (markers and unknowns) from the top of the resolving gel. Divide the migration distance of each protein by the distance traveled by the tracking dye. The normalized migration distances so obtained are called the relative mobilities of the proteins (relative to the dye front) and conventionally denoted as R_f . Construct a plot of the logarithm of the relative molecular masses (M_r) of the protein standards as a function of the R_f values. Note that the graphs are slightly sigmoid. Unknown molecular masses can be estimated by linear regression analysis or interpolation from the curves of $\log M_r$ against R_f as long as the values obtained for the unknown samples are positioned along the linear part of the graph.

8. Suitability of the Test (Validation)

The test is not valid unless the front end of the molecular mass marker migrates 80% of the migrating distance of the dye, and over the required separation range (e.g., the range covering the product and its dimer or the product and its related impurities) the separation obtained for the relevant protein bands shows a linear relationship between the logarithm of the molecular mass and the R_f as described in 7. Additional requirements with respect to the solution under test may be specified in individual monographs.

9. Quantification of Impurities

Where the impurity limit is specified in the individual monograph, a reference solution corresponding to that level of impurity should be prepared by diluting the test solution. For example, where the limit is 5%, a reference solution would be a 1:20 dilution of the test solution. No impurity (any band other than the main band) in the electrophoretogram obtained with the test solution may be more intense than the main band obtained with the reference solution.

Under validated conditions, impurities may be quantified by normalization to the main band, using an integrating densitometer. In this case, the responses must be validated for linearity.

Test solutions:

Coomassie staining TS Dissolve 125 mg of coomassie brilliant blue R-250 in 100 mL of a mixture of water, methanol and acetic acid (100) (5:4:1), and filter.

Developer TS Dissolve 2 g of citric acid monohydrate in water to make 100 mL. To 2.5 mL of this solution add 0.27 mL of formaldehyde solution and water to make 500 mL.

Fixing TS To 250 mL of methanol add 0.27 mL of formaldehyde solution and water to make 500 mL.

Silver nitrate TS for silver staining To 40 mL of sodium hydroxide TS add 3 mL of ammonia solution (28), then add dropwise 8 mL of a solution of silver nitrate (1 in 5) while stirring, and add water to make 200 mL.

Destaining TS A mixture of water, methanol and acetic acid (100) (5:4:1).

Blocking TS To 10 mL of acetic acid (100) add water to make 100 mL.

Trichloroacetic acid TS for fixing Dissolve 10 g of trichloroacetic acid in a mixture of water and methanol (5:4) to make 100 mL.

24. Solid and Particle Densities

Density of a solid or a powder as a state of aggregation has different definitions depending on the way of including of the interparticulate and intraparticulate voids that exist between the particles or inside the powder. Different figures are obtained in each case, and there are different practical meanings. Generally, there are three levels of definitions of the solid or powder density.

(1) **Crystal density:** It is assumed that the system is homogeneous with no intraparticulate void. Crystal density is also called true density.

(2) **Particle density:** The sealed pores or the experimentally non-accessible open pores is also included as a part of the volumes of the solid or the powder.

(3) **Bulk density:** The interparticulate void formed in the powder bed is also included as a part of the volumes of the solid or the powder. Bulk density is also called apparent density. Generally, the powder densities at loose packing and at tapping are defined as the bulk density and the tapped density, respectively.

Generally, the densities of liquid or gas are affected only by temperature and pressure, but the solid or powder density is affected by the state of aggregation of the molecules or the particles. Therefore, the solid or powder densities naturally vary depending on crystal structure or crystallinity of the substance concerned, and also varies depending on the method of preparation or handling if the sample is amorphous form or partially amorphous. Consequently, even in a case that two solids or powders are chemically identical, it may be possible that the different figures of density are obtained if their crystal structures are different. As the solid or powder particle densities are important physical properties for the powdered pharmaceutical drugs or the powdered raw materials of drugs, the Japanese Pharmacopoeia specifies each density determination as "Powder Particle Density Determination" for the particle density and as "Determination of Bulk and Tapped Densities" for the bulk density.

The solid or powder densities are expressed in mass per unit volume (kg/m^3), and generally expressed in g/cm^3 (1

$\text{g/cm}^3 = 1000 \text{ kg/m}^3$).

Crystal Density

The crystal density of a substance is the average mass per unit volume, exclusive of all voids that are not a fundamental part of the molecular packing arrangement. It is an intrinsic property concerning the specific crystal structure of the substance, and is not affected by the method of determination. The crystal density can be determined either by calculation or by simple measurement.

A. The calculated crystal density is obtained using:

- 1) For example, the crystallographic data (volume and composition of the unit cell) obtained by indexing the perfect crystal X-ray diffraction data from single crystal or the powder X-ray diffraction data.
- 2) Molecular mass of the substance.

B. The measured crystal density is obtained as the mass to volume ratio after measuring the single crystal mass and volume.

Particle Density

The particle density takes account both the crystal density and the intraparticulate porosity (sealed and/or experimentally non-accessible open pores) as a part of the particle volume. The particle density depends on the value of the volume determined, and the volume in turn depends on the method of measurement. Particle density can be determined either by gas displacement pycnometry or mercury porosimetry, but the Japanese Pharmacopoeia specifies the pycnometry as the "Powder Particle Density Determination".

A. The pycnometric density is obtained by assuming that the volume of the gas displaced, which is measured with the gas displacement pycnometer, is equivalent to that of a known mass of the powder. In pycnometric density measurements, any volume with the open pores accessible to the gas is not included as a part of volume of the powder, but the sealed pores or pores inaccessible to the gas is included as a part of the volume of the powder. Due to the high diffusivity of helium which can penetrate to most open pores, it is recommendable as the measurement gas of particle density. Therefore, the pycnometric particle density of a finely milled powder is generally not very different from the crystal density. Hence, the particle density by this method is the best estimate of the true density of an amorphous or partially crystalline sample, and can be widely used for manufacturing control of the processed pharmaceutical powder samples.

B. The mercury porosimetric density is also called granular density. This method also includes the sealed pores as a part of the volumes of the solid or the powder, but excludes the volume only from the open pores larger than some size limit. This pore size limit or minimal access diameter depends on the maximal mercury intrusion pressure applied during the measurement and under normal operating pressure, the mercury does not penetrate the finest pores accessible to helium. Since this method is capable of measuring the density which corresponds to the pore size limit at each mercury intrusion pressure, the various granular densities can be obtained from one sample.

Bulk Density and Tapped Density

The bulk density of a powder includes the contribution of

interparticulate void volume as a part of the volume of the powder. Therefore, the bulk density depends on both the powder particle density and the space arrangement of particles in the powder bed. Further, since the slightest disturbance of the bed may result in variation of the space arrangement, it is often very difficult to determine the bulk density with good reproducibility. Therefore, it is essential to specify how the determination was made upon reporting the bulk density.

The Japanese Pharmacopoeia specifies "Determination of Bulk and Tapped Densities".

- A. The bulk density is determined by measuring the apparent volume of a known mass of powder sample that has been passed through a screen in a graduated cylinder (constant mass method). Separately, the Pharmacopoeia specifies the method of determining bulk density by measuring the mass of powder in a vessel having a known volume (constant volume method).
- B. The tapped density is obtained by mechanically tapping a measuring cylinder containing a powder sample. After determining the initial bulk volume, carry out tapping under a fixed measurement condition (tapping rate and drop height), and the measurement is carried out repeatedly until the bulk volume variation obtained at consecutive two measurements is within an acceptable range (constant mass method). Separately, the Pharmacopoeia specifies the method of determining the tapped density by measuring the mass of a fixed volume of the tapped powder (constant volume method).

25. Sterility Assurance for Terminally Sterilized Pharmaceutical Products

As indicated in the "Terminal Sterilization and Sterilization Indicators", the pharmaceuticals to which terminal sterilization can be applied, generally must be sterilized so that a sterility assurance level of 10^{-6} or less is obtained. The sterility assurance level of 10^{-6} or less can be proven by using a sterilization process validation based on physical and microbiological methods, but cannot be proven by sterility tests of the sterilized products. This chapter deals with the necessary requirements for the appropriate management of the important control points of the sterilization process for the parametric release of products, without performing sterility tests on products which have been subjected to terminal sterilization (in the case of radiation sterilization, called dosimetric release). Parametric release is a method that can be applied in cases where the sterilization system is clearly defined, important control points are clearly specified, and the sterilization system process can be validated by microbiological methods using appropriate biological indicators.

1. Definitions

The definitions of the terminology used in this chapter are provided below.

1.1 Terminal sterilization

A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

1.2 Validation

A documented procedure for obtaining, recording and interpreting the results needed to show that a process will consistently yield a product complying with predetermined specifications.

1.3 Periodic re-validation

Validation that is regularly performed to reconfirm that a process is consistently yielding a product complying with predetermined specifications. It should confirm that variables and the acceptable ranges are permissible to yield a product consistently of the required quality.

1.4 Facility/equipment qualification

This is to provide evidence that the manufacturing facilities/equipment, measuring equipment, and manufacturing environment control facilities, etc. have been properly selected, correctly installed, and are operated in conformity with the specifications at the time of installation and during operation.

1.5 Operation qualification

This is to provide evidence to confirm physically, chemically and microbiologically that equipment, operated in accordance with its operational instructions, operates as specified and affords a product meeting the specifications.

1.6 Support system for sterilization process

This refers to the facility/equipment that is associated with the sterilization devices, such as the preconditioning and aeration for ethylene oxide sterilization, the steam supply equipment for moist heat sterilization, and the loading devices for radiation sterilization.

1.7 Quality system

The procedures, resources and organizational structure of a manufacturer (responsibilities, authorities and relationships between these) required to implement quality management.

1.8 Change control system

A system designed to evaluate all of the changes that may affect the quality of the pharmaceutical product, in order to ensure that the process is continuously controlled.

1.9 F_0 value

Assume a value of 10°C for the Z value defined as the number of degrees of temperature required for a 10-fold change in the D value. The F_0 value indicates the time (minutes) required to give the equivalent lethality at T_b of the sterilization heat obtained by integrating the lethality rate (L) over an entire heating cycle.

$$L = \log^{-1} \frac{T_0 - T_b}{Z} = 10^{\frac{T_0 - T_b}{Z}}$$

T_0 = Temperature inside the chamber or inside the product to be sterilized

T_b = Reference temperature (121°C)

$$F_0 = \int_{t_0}^{t_1} L dt$$

$t_1 - t_0$ = Processing time (minutes)

1.10 Control device

A general term for the devices and measurement equipment, including the equipment for controlling, measuring and recording the physical parameters that can be measured (temperature, humidity, pressure, time, radiation dose, etc.).

1.11 Parametric release

A release procedure based on an evaluation of the produc-

tion records and critical parameters of the sterilization process (temperature, humidity, pressure, time, radiation dose, etc.) based on the results of validation, in lieu of release based on testing results of the final product.

2. Sterilization Validation

2.1 Subject of the Implementation

A manufacturer of sterile pharmaceuticals (hereafter, "manufacturer") must establish a quality system, implement product sterilization validation for the categories below as a general rule, and continuously control the sterilization process based on the results of the sterilization validation.

- a. Sterilization process
- b. Sterilization process support system

2.2 Documenting Sterilization Validation Procedure

2.2.1 The manufacturer must prepare a "Sterilization Validation Procedure" defining the items listed below regarding the procedures for managing the sterilization process.

- a. Details related to the range of duties of the persons responsible for the validation, as well as the extent of their authority
- b. Details related to the implementation period for the sterilization validation
- c. Details related to the creation, modification, and approval of the sterilization validation plan documents
- d. Details related to the reporting, evaluation, and approval of the sterilization validation implementation results
- e. Details related to the storage of documentation concerning the sterilization validation
- f. Other required matters

2.2.2 The sterilization validation procedure must list the names of the enactors, the date of enactment, and when there are revisions, must also list the revisers, date of revisions, revised sections and reasons for the revisions.

2.2.3 The manufacturer must properly store and maintain the sterilization validation procedure after clarifying the procedures related to alterations and deletions of the contents of the sterilization validation procedure.

2.3 Persons Responsible for the Validation

The manufacturer must assign persons to be responsible for the sterilization validation. The responsible parties must perform each of the duties listed below according to the sterilization validation procedure.

2.3.1 For products that are to be produced according to the sterilization validation procedure, a written sterilization validation implementation plan must be prepared. The implementation plan will specify the following points based on a consideration of the implementation details of the sterilization validation.

- a. Subject pharmaceutical name (product name)
- b. Purpose of the applicable sterilization validation
- c. Expected results
- d. Verification methods (including inspection results and evaluation methods)
- e. Period of verification implementation
- f. Names of persons performing the sterilization validation (persons-in-charge)
- g. Names of the persons who created the plan, creation date, and in the event of revisions, the names of the revisers, date of the revisions, revised sections, and reasons for revision.
- h. Technical requirements for the applicable sterilization validation

i. Other required matters for the implementation of the applicable sterilization validation

2.3.2 The following sterilization validation is implemented according to the plan defining the items above.

a. When the manufacturing license and additional (modification) licenses for product production are obtained, implementation items for the sterilization validation to be executed

- 1 Product qualification
- 2 Facility/equipment qualification
 - 1) Installation qualification
 - 2) Operation qualification
- 3 Performance qualification
 - 1) Physical performance qualification
 - 2) Microbiological performance qualification

b. Sterilization validation to be executed until it is time to renew the manufacturing license

- 1 Re-validation when there are changes
- 2 Periodic re-validation (The items implemented, etc. must be determined based on a consideration of relevant factors such as the sterilization method.)

2.3.3 Evaluate the results of the sterilization validation and verify that sterility is assured.

2.3.4 Make a written report of the results of the sterilization validation to the manufacturer's authorized person.

2.3.5 Perform the day-to-day management of the sterilization process.

3. Microorganism Control Program

When parametric release is adopted, it is important to control the bioburden in the raw materials of the product, the containers and stoppers, and in the product before sterilization. The bioburden is measured with a previously specified method and frequency, and when required, surveys of the characteristics of the isolated microorganisms are made to investigate their resistance to the applicable sterilization method. Refer to the "Microbiological Evaluation of Process Areas for Sterile Pharmaceutical Products" regarding the method for evaluating the environmental microorganisms in the processing areas of pharmaceutical products.

4. Sterilization Indicators

Biological indicators (BI), chemical indicators (CI), and dosimeters are among the means used to monitor a sterilization process and as indices of sterility (refer to Terminal Sterilization and Sterilization Indicators). When using sterilization indicators it is important to consider environmental and human safety, and to take all necessary precautions. The BI used for sterilization validation and daily process control must be defined in the specification, and recorded in writing. When BI are used for daily process control it must be verified that the loading pattern on the form, product, or simulated product has a resistance equal to or greater than that used for the microbiological performance qualification.

5. Establishment of a Change Control System

Changes which have a large effect on the sterile quality, such as changes in sterilization equipment, loading pattern, and sterilization conditions, correspond to changes of the parametric release conditions for the relevant pharmaceutical product. A change control system must be defined in the sterilization validation procedure; and when there are changes in the causes of variation that have been previously specified, there must be an investigation of the causes of vari-

ation and of acceptable conditions to verify that the pharmaceutical product is guaranteed always to conform to the quality standards. Furthermore, before modifications are made to a sterilization process that has been validated, it is mandatory to obtain approval for the implementation of the modifications in question from the appropriate authorized person.

6. Release Procedure

A release procedure must be created to clarify the conditions required for shipment based on parametric release of terminally sterilized products. The following points must be evaluated and recorded when a product is released.

Depending on the sterilization method, some of these items may be omitted or modified.

- a) Batch record
- b) Microorganism evaluation data of production environment
- c) Bioburden data for the raw materials and product before sterilization
- d) Data related to the sterilization indicators
- e) Data on the maintenance management of the sterilization process and sterilization process support systems
- f) Data on the management of sterilization parameters
- g) Data on the calibrations of measurement equipment
- h) Re-validation data
- i) Other

7. Critical Control Points

The important control points for each sterilization method are presented.

7.1 Moist heat sterilization

Moist heat sterilization is a method for killing microorganisms in which saturated water vapor is generated or introduced into a sterilization chamber at the appropriate temperature and pressure, and the chamber is then heated for a certain period of time. It is roughly classified into saturated vapor sterilization, in which the target microorganisms are directly exposed to the saturated vapor, and unsaturated vapor sterilization, in which the fluid inside a container, such as an ampule, is subjected to moist heat energy or high-frequency energy from the outside.

7.1.1 Important control points

A process control procedure must be created, specifying the process parameters that affect the sterile quality of the pharmaceutical product, and the permissible range of variation for each parameter. The important control points for the moist heat sterilization are indicated below.

- a) Heating history (usually indicated by F_0 value)
- b) Temperature
- c) Pressure
- d) Time
- e) Product loading format/loading density
- f) Other necessary matters

7.1.2 Utilities

The utilities and control devices required for moist heat sterilization determine the quality and precision.

- a) Quality of the vapor used
- b) Quality of the air introduced into the sterilization chamber to restore pressure, etc.
- c) Quality of the water used for cooling
- d) Precision of the temperature control devices
- e) Precision of the pressure control devices
- f) Precision of the time control devices

g) Other

7.2 Ethylene oxide gas sterilization

Ethylene oxide gas allows sterilization at low temperatures, so there is typically little injury to the substance being sterilized; however, since the gas is toxic it must be handled with extreme caution. The sterilization process consists of preconditioning, a sterilization cycle, and aeration. The preconditioning is performed before the sterilization cycle to process the product so that temperature and relative humidity in the room or container are within the range in the specifications. The sterilization cycle indicates the stage at which the actual sterilization is performed, and consists of removal of the air, conditioning (when used), injection of the sterilization gas, maintenance of the sterilization conditions, removal of the sterilization gas, and replacement of the air. The aeration is the process of eliminating the residual ethylene oxide gas from the product, either inside the sterilization chamber or in a separate location.

7.2.1 Important control points

The important control points for the ethylene oxide gas sterilization are indicated below.

7.2.1.1 Preconditioning (when performed)

- a) Time, temperature, humidity
- b) Product loading pattern/loading density
- c) Sterilization loading temperature and/or humidity
- d) Time from the end of preconditioning until the start of the sterilization
- e) Other necessary matters

7.2.1.2 Conditioning

- a) If pressure reduction is performed, the pressure achieved and required time
- b) Reduced pressure maintenance period
- c) Time, temperature, pressure, humidity
- d) Sterilization loading temperature and humidity
- e) Other necessary matters

7.2.1.3 Sterilization cycle

- a) Pressure increase, injection time, and final pressure for the injection of the sterilization gas
- b) Concentration of the ethylene oxide gas (it is desirable to analyze directly the gas concentration inside the sterilization chamber, but the following alternatives are acceptable if direct analysis is difficult)

- i) Mass of gas used
- ii) Volume of gas used
- iii) Conversion calculation using the initial low pressure level and the gas injection pressure

- c) Temperature within the sterilization chamber
- d) Temperature of the loaded products to be sterilized
- e) Effect time (exposure time)
- f) Product loading pattern/loading density
- g) BI placement points and cultivation results
- h) Other necessary matters

7.2.1.4 Aeration

- a) Time, temperature
- b) Loaded sterilized substance temperature
- c) Pressure variation in the sterilization chamber and/or the aeration room
- d) Rate of change of the air or other gases in the aeration room
- e) Other necessary matters

7.2.2 Utilities

The utilities and control devices required for ethylene oxide sterilization determine the quality and precision.

- a) Quality of the ethylene oxide gas
- b) Quality of the injected vapor or water
- c) Quality of the replacement air after the completion of sterilization
- d) Quality of the BI
- e) Precision of the temperature control devices
- f) Precision of the pressure control devices
- g) Precision of the humidity control devices
- h) Precision of the time control devices
- i) Other

7.3 Irradiation Sterilization

Irradiation sterilization refers to methods of killing microorganisms through exposure to ionizing radiation. The types of ionizing radiation used are gamma-rays (γ -rays) emitted from a radioisotope such as ^{60}Co or ^{137}Cs , or electron beams and bremsstrahlung (X-ray) generated from an electron accelerator. In the case of γ -rays, the cells are killed by secondarily generated electrons, while in the case of the electron beam, the cells are killed by the electrons generated directly from the electron accelerator. For this reason, the processing time for electron beam sterilization is generally shorter than that for γ -ray sterilization; but, since the penetration of the γ -rays is better than that of the electron beam, there must be appropriate consideration of the density and thickness of the substance being sterilized when choosing between these methods. For an irradiation sterilization process, the control procedures primarily make use of dosimeters and measure the absorbed dose in the substance being sterilized. This is called dosimetric release.

7.3.1 Important control points

The important control points for the irradiation sterilization are indicated below.

7.3.1.1 γ -ray radiation

- a) Irradiation time (timer setting or conveyor speed)
- b) Absorbed dose
- c) Product loading pattern
- d) Other necessary matters

7.3.1.2 Electron beam and X-ray radiation

- a) Electron beam characteristics (average electron beam current, electron energy, scan width)
- b) Conveyor speed
- c) Absorbed dose
- d) Product loading pattern
- e) Other necessary matters

7.3.2 Utilities

A traceable calibration, performed according to national standards, must be performed for the radiation devices and dose measurement systems. This calibration must be performed as specified in a written plan in order to verify that the equipment is kept within the required range of accuracy.

7.3.2.1 Required calibration items for gamma-radiation equipment

- a) Cycle time or conveyor speed
- b) Weighing device
- c) Dose measurement system
- d) Other

7.3.2.2 Required calibration items for electron-beam and X-ray radiation equipment

- a) Electron beam characteristics
- b) Conveyor speed
- c) Weighing device
- d) Dose measurement system
- e) Other

References

- 1) Validation Standards, PAB Notification No.158, Ministry of Health and Welfare 1995
- 2) Sterilization Validation Standards, PMSB/IGD Notification No.1, Ministry of Health and Welfare 1997
- 3) Quality Assurance Standards for Medical Devices, PAB Notification No.1128, Ministry of Health and Welfare 1994
- 4) ISO 9000 series, International Standards for Quality Assurance
- 5) ISO 11134 Industrial moist heat sterilization
- 6) ISO 11135 Ethylene oxide sterilization
- 7) ISO 11137 Radiation sterilization
- 8) ISO 11138 Biological indicators
- 9) ISO 11140 Chemical indicators
- 10) ISO 11737-1 Microbiological Methods Part 1: Estimation of population of microorganisms on products
- 11) USP <1222> Terminally Sterilized Pharmaceutical Products - Parametric Release

26. Tablet Friability Test

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia.

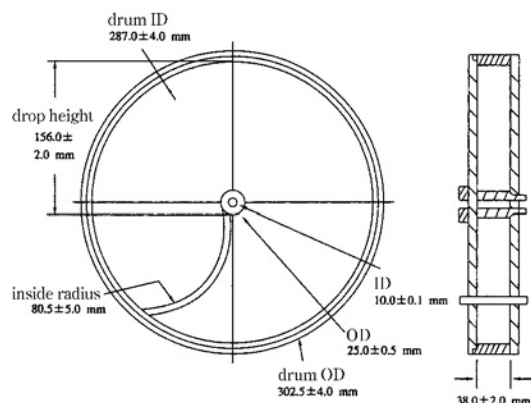
The Tablet Friability Test is a method to determine the friability of compressed uncoated tablets. The test procedure presented in this chapter is generally applicable to most compressed tablets. Measurement of tablets friability supplements other physical strength measurement, such as tablet crushing strength.

Use a drum, with an internal diameter between 283 and 291 mm and a depth between 36 and 40 mm, of transparent synthetic polymer with polished internal surface, and not subject to minimum static build-up (see figure for a typical apparatus). One side of the drum is removable. The tablets are tumbled at each turn of the drum by a curved projection with an inside radius between 75.5 and 85.5 mm that extends from the middle of the drum to the outer wall. The outside diameter of the central shaft ring is between 24.5 and 25.5 mm. The drum is attached to the horizontal axis of a device that rotates at 25 ± 1 rpm. Thus, at each turn the tablets roll or slide and fall onto the drum wall or onto each other.

For tablets with a unit mass equal to or less than 650 mg, take a sample of whole tablets n corresponding as near as possible to 6.5 g. For tablets with a unit mass of more than 650 mg, take a sample of 10 whole tablets. The tablets should be carefully dedusted prior to testing. Accurately weigh the tablet sample, and place the tablets in the drum. Rotate the drum 100 times, and remove the tablets. Remove any loose dust from the tablets as before, and accurately weigh.

Generally, the test is run once. If obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample fails the test. If the results are difficult to interpret or if the weight loss is greater than the targeted value, the test should be repeated twice and the mean of the three tests determined. A maximum mean weight loss from the three samples of not more than 1% is considered acceptable for most products.

If tablet size or shape causes irregular tumbling, adjust the drum base so that the base forms an angle of about 10° with



the bench top and the tablets no longer bind together when lying next to each other, which prevents them from falling freely.

Effervescent tablets and chewable tablets may have different specifications as far as friability is concerned. In the case of hygroscopic tablets, an appropriate humidity-controlled environment is required for testing.

Drums with dual scooping projections, or apparatus with more than one drum, for the running of multiple samples at one time, are also permitted.

27. Terminal Sterilization and Sterilization Indicators

Sterilization is a process whereby the killing or removal of all forms of viable microorganisms in substances is accomplished. It is achieved by terminal sterilization or a filtration method. For substances to which terminal sterilization can be applied, an appropriate sterilization method should be selected in accordance with the properties of the product, including the packaging, after full consideration of the advantages and disadvantages of each sterilization method, from among the heat method, irradiation method and gas method. After installation of the sterilizer (including design and development of the sterilization process), validation is required to confirm that the sterilization process is properly performing its designed function, under conditions of loading and unloading of the product, on the basis of sufficient scientific evidence. After the process has been validated and the sterilization of the product commenced, the process must be controlled correctly, and qualification tests of the equipment and procedures must be performed regularly. The bioburden per product, prior to terminal sterilization, must be evaluated periodically or on the basis of batches. Refer to the ISO standard (ISO 11737-1) relevant to bioburden estimation. For a substance to which terminal sterilization can be applied, generally use sterilization conditions such that a sterility assurance level of less than 10^{-6} can be obtained. The propriety of the sterilization should be judged by employing an appropriate sterilization process control, with the use of a suitable sterilization indicator, and if necessary, based on the result of the sterility test. The filtration procedure is used for the sterilization of a liquid product, to which terminal sterilization can not be applied. Concerning the disinfection and/or sterilization necessary for processing equipment and areas

of pharmaceutical products, and performing microbiological tests specified in the monographs, see Disinfection and Sterilization Methods.

1. Definitions

The definitions of the terms used in this text are as follows.

Terminal sterilization: A process whereby a product is sterilized in its final container or packaging, and which permits the measurement and evaluation of quantifiable microbial lethality.

Product: A generic term used to describe raw materials, intermediate products, and finished products, to be sterilized.

Bioburden: Numbers and types of viable microorganisms in a product to be sterilized.

Sterility assurance level (SAL): Probability of a viable microorganism being present in a product unit after exposure to the proper sterilization process, expressed as 10^{-n} .

Integrity test: A non-destructive test which is used to predict the functional performance of a filter instead of the microorganism challenge test.

D value: The value which shows the exposure time (decimal reduction time) or absorbed dose (decimal reduction dose) required to cause a 1-logarithm or 90% reduction in the population of test microorganisms under stated exposure conditions.

Sterilization indicator: Indicators used to monitor the sterilization process, or as an index of sterility, including biological indicators (BI), chemical indicators (CI), dosimeters and the like.

2. Sterilization

2-1. Heat Method

In the heat method, microorganisms are killed by heating.

(i) Moist heat method

Microorganisms are killed in saturated steam under pressure. In this method, factors which may affect the sterilization include temperature, steam pressure and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, steam pressure and exposure time, and they should be included in the specifications of the sterilizer.

(ii) Dry-heat method

Microorganisms are killed in dry heated air. This method is usually conducted in a batch-type dry heat sterilizer or a tunnel-type dry heat sterilizer. In this method, factors which may affect the sterilization include temperature and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature and exposure time, and they should be included in the specifications of the sterilizer.

2-2. Irradiation method

Microorganisms are directly killed by ionizing radiation, or by the heat generated by microwave radiation.

(i) Radiation method

Ionizing radiations which may be used are gamma (γ) rays emitted from a radioisotope such as cobalt 60, an electron beam and bremsstrahlung (X rays) generated from an electron accelerator. Although any procedure can be applied to thermally unstable products with no radioactivity residue, it is necessary to consider the possibility of material degradation. Although a 25 kGy dose is traditionally used as a sterilization dose, there are some ways to calculate the dose as follows: the bioburden of the substance to be sterilized is measured and the sterilization dose is calculated based on the

mean bioburden and the standard resistance distribution (Method 1 in ISO 11137), the dose is calculated from the fraction positive information from a sterility test in which representative product samples are exposed to a substerilizing dose (Method 2 in ISO 11137), or the dose is calculated based on the bioburden and D value of the most resistant microorganisms (Log method) (see 5-3). In the case of the radiation sterilization procedure, factors which may affect the sterilization include dose (absorbed dose) and exposure time. Therefore, in γ ray sterilization process control, it is required to determine the dose (the absorbed dose) at appropriate intervals and to monitor continuously the exposure time in terms of the operating parameters (the conveyer speed, the cycle time). The dose control mechanism should be included in the specifications of the sterilizer. In the case of electron beam or bremsstrahlung irradiation, it is required to monitor the acceleration voltage, the beam current and beam scanning width besides the above-mentioned items.

(ii) Microwave method

Microorganisms are killed by the heat generated by microwave radiation, usually at the frequency of 2450 ± 50 MHz. This method is applied to liquids or water-rich products in sealed containers. Since a glass or plastic container may be destroyed or deformed due to the rise of the inner pressure, the containers must be certified to be able to withstand the heat and the inner pressure generated during microwave sterilization. Leakage of electromagnetic radiation must be at a sufficiently low level to cause no harm to humans and no interference with radio communications and the like. In this method, factors which may affect the sterilization include temperature, processing time and microwave output power. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, time and the microwave output power, and they should be included in the specifications of the sterilizer.

2-3. Gas method

Ethylene oxide (EO) is widely used as a sterilization gas. Since EO gas has an explosive nature, a 10–30% mixture with carbon dioxide is commonly used. Also, as EO gas is a strong alkylating agent, it can not be applied to the products which are likely to react with or absorb it. Furthermore, because EO gas is toxic, the residual concentration of EO gas and other secondarily generated toxic gases in products sterilized with EO gas must be reduced to less than the safe levels thereof by means of aeration and the like before the product is shipped. In this method, factors which may affect the sterilization include temperature, gas concentration (pressure), humidity and exposure time. Therefore, in routine sterilization process control, it is required to monitor continuously the temperature, gas concentration (pressure), humidity and exposure time, and they should be included in the specifications of the sterilizer.

3. Filtration method

Microorganisms are removed by using a sterilizing filter made of an appropriate material. However, this method is not intended for microorganisms smaller than bacteria. Generally, a sterilizing filter challenged with more than 10^7 microorganisms of a strain of *Brevundimonas diminuta* (ATCC 19146, NBRC 14213, JCM 2428), cultured under the appropriate conditions, per square centimeter of effective filter area should provide a sterile effluent. In this method, factors which may affect the sterilization include pressure,

flow rate, filter unit characteristics and the like. In routine filtration process control, it is required to perform integrity tests of the sterilizing filter after each filtration process (also prior to the filtration process, if necessary).

4. Sterilization Indicators

4-1. Biological indicator (BI)

A BI is prepared from specific microorganisms resistant to the specified sterilization process and is used to develop and/or validate a sterilization process. The dry type BI is classified into two kinds. In one, bacterial spores are added to a carrier such as filter paper, glass or plastic and then the carriers are dried and packaged. In the other, bacterial spores are added to representative units of the product to be sterilized or to simulated products. Packaging materials of the BI should show good heat penetration in dry heat sterilization and good gas or steam penetration in ethylene oxide and moist heat sterilizations. It should be confirmed that any carrier does not affect the D value of the spores. In the case of a liquid product, the spores may be suspended in the same solution as the product or in a solution showing an equivalent effect in the sterilization of biological indicator. However, when the spores are suspended in liquid, it is necessary to ensure that the resistance characteristics of the spores are not affected due to germination.

Typical examples of biological indicator

Sterilization	Representative microorganisms*	Strain name
Moist heat method	<i>Geobacillus</i>	ATCC 7953, NBRC 13737, JCM 9488
	<i>stearo-thermophilus</i>	ATCC 12980, NBRC 12550, JCM 2501
	<i>Bacillus atrophaeus</i>	ATCC 9372, NBRC 13721
Dry heat method	<i>Bacillus atrophaeus</i>	ATCC 9372, NBRC 13721
Gas method	<i>Bacillus atrophaeus</i>	ATCC 9372, NBRC 13721

* In addition to these microorganisms, other microorganisms with the greatest resistance to the sterilization procedure concerned, found in the bioburden, can be used as the biological indicator.

4-1-1. D value of BI

Methods for determination of the D value include the survival curve method and the fraction negative method (Stumbo, Murphy & Cochran procedure, Limited Spearman-Kärber procedure and the like). In using marketed BIs, it is usually unnecessary to determine the D value before use if the D value indicated on the label has been determined by a standardized biological indicator evaluation resistometer (BIER) under strictly prescribed conditions in accordance with ISO 11138-1. It is acceptable that the D value indicated on the label shows a scattering of not more than ± 30 seconds.

4-1-2. Setting up procedure of BI

(i) In the case of dry materials

A Dry type BI is placed at predetermined cold spots in the product to be sterilized or a suitable product showing an equivalent effect in the sterilization. The BIs are usually primary packaged in the same way as the product, including a secondary packaging, if applicable.

(ii) In the case of wet materials

Spores are suspended as the BI in the same solution as the

product or in an appropriate similar solution, and should be placed at cold spots in the sterilizer.

4-1-3. Culture conditions of BI

Soybean casein digest medium is generally used. General culture conditions are at 55 – 60°C for 7 days in the case of *G. stearothermophilus* and at 30 – 35°C for 7 days in the case of *B. atrophaeus*.

4-2. Chemical indicator (CI)

CI is an indicator which shows a color change of a substance applied to a paper slip, etc. as a result of physical and/or chemical change due to exposure to heat, gas or radiation. The CI can be classified into three types. The first is employed to identify whether or not sterilization has already been implemented, the second is employed to control the sterilization process (for example, its color changes after sterilization for a sufficient time), and the third is the Bowie & Dick type used to evaluate the effectiveness of air removal during the pre-vacuum phase of the pre-vacuum sterilization cycle.

4-3. Dosimeter

In the radiation (γ -ray) method, the sterilization effect depends on the absorbed radiation dose, so the sterilization process control is mainly performed by measuring the dose. A dosimeter is installed at a position corresponding to the minimum dose region of an exposed container or a position where the dose is in a known relation to that in the above region. Measurement should be done for each radiation batch. If there are many containers in the same batch, dosimeters should be employed so that more than one dosimeter is always installed at the effective radiation section of the irradiation chamber. It should be noted that dosimeters may be affected by environmental conditions (temperature, humidity, ultraviolet light, time until reading, etc.) before and during irradiation. Practical dosimeters for γ -ray and bremsstrahlung sterilization include the dyed polymethylmethacrylate dosimeter, clear polymethylmethacrylate dosimeter, ceric-cerous sulfate dosimeter, alanine-EPR dosimeter and the like. A dosimeter for gamma radiation can not generally be used for sterilization process control with an electron beam of less than 3 MeV energy. Dosimeters for electron beam sterilization include the cellulose acetate dosimeter, radiochromic film dosimeter and the like. A practical dosimeter must be calibrated against an appropriate national or international standard dosimetry system.

5. Determination of sterilization conditions using microorganism as an indicator

Taking account of the characteristics upon the sterilization concerned, bioburden, etc. of a product to be sterilized, chose a suitable method from the followings and determine the conditions.

5-1. Half-cycle method

In this method, a sterilization time of twice as long as that required to inactivate all of 10^6 counts of BI placed in the product is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization.

5-2. Overkill method

In this method, a sterilization condition giving a sterility assurance level of not more than 10^{-6} counts is used, regardless of bioburden count in the product being sterilized or the resistance of the objective microorganisms to the sterilization. Generally, a sterilization condition providing 12

logarithmic reduction (12D) of a known count of BI of more than 1.0 D value is used.

5-3. Combination of BI and bioburden

Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is considered as the maximum bioburden count, and the sterilization time (or radiation dose) is calculated with the bioburden count based on an objective sterility assurance level. When this procedure is used, it is required to determine the resistance of the bioburden to the sterilization as well as the bioburden count in the product being sterilized. If a more resistant microorganism than the BI spore is found in the bioburden estimation, it should be used as the BI.

$$\text{Sterilization time (or radiation dose)} = D \times \log \frac{N_0}{N}$$

D: D value of the BI

N: Sterility assurance level

*N*₀: Maximum bioburden count in the product

5-4. Absolute bioburden method

The sterilization conditions are determined by employing the D value of the most resistant microorganism found in the product or environment by the resistant estimation and being based on the bioburden count in the product. Generally, a count of mean bioburden added three times of its standard deviation obtained by an extensive bioburden estimation is employed as the bioburden count. When this procedure is used, it is required to make frequent counting and resistance determination of microorganisms in daily bioburden estimation.

References

- 1) ISO 11134 Industrial moist heat sterilization
- 2) ISO 11135 Ethylene oxide sterilization
- 3) ISO 11137 Radiation sterilization
- 4) ISO 11138 Biological indicators
- 5) ISO 11140 Chemical indicators
- 6) ISO 11737 Microbiological methods
Part 1: Estimation of population of microorganisms on products

28. Test for Trace Amounts of Aluminum in Trans Parenteral Nutrition (TPN) Solutions

Trans parenteral nutrition solutions (TPNs) are nutrient preparations for intravenous injection packed in a large volume. Since toxic effects to the central nervous system, bone, etc. due to trace amounts of aluminum have recently been reported in several countries, testing methods for trace amounts of aluminum contaminating TPNs are required for the official standard. The following three analytical methods are available: (1) High-Performance Liquid Chromatography using a fluorescence photometric detector (HPLC with fluorescence detection), (2) Inductivity Coupled Plasma-Atomic Emission Spectrometry (ICP-AES method), (3) Inductivity Coupled Plasma-Mass Spectrometry (ICP-MS method). Detection sensitivity by HPLC with fluorescence detection is about 1 $\mu\text{g/L}$ (ppb), while ICP-AES fitted with special apparatus and ICP-MS have higher sensitivity.

Since TPNs are nutrient preparations, they contain many nutrients such as sugars, amino acids, electrolytes, etc., in various compositions. Thus, care is needed in the selection of a suitable analytical method, because these coexisting components may affect the measurement of trace amounts of aluminum.

In view of the general availability of HPLC apparatus, the present general information describes procedures for the determination of trace levels of aluminum in TPNs by means of HPLC with a fluorescence photometric detector, using two kinds of fluorescent chelating agents, i.e., (1) Quinolinol complexing method, (2) Lumogallion complexing method.

(1) Quinolinol complexing method

After forming a complex of aluminum ion in the sample solution with quinolinol, the assay for aluminum by HPLC fitted with a fluorescence photometer is performed.

Preparation of sample solution

Pipet 1 mL of the sample (TPNs) exactly, and after adding 10 μ L of water for aluminum test, make up the sample solution to 10 mL exactly by adding the mobile phase.

Preparation of a series of standard solutions for calibration curve

Pipet 1 mL of water for aluminum test exactly, and after adding 10 μ L each of standard solutions of aluminum (1)?? (5), make up the standard solutions for calibration curve to 10 mL (Aluminum concentration: 0, 1.25, 2.5, 5.0, and 10.0 ppb).

Standard testing method

Pipet 0.1 mL each of the sample solution and standard solutions, and perform the test by HPLC under the following conditions. Calculate the aluminum content in the sample solution using a calibration curve method.

Operating conditions—

Detector: Fluorescence photometer (excitation wavelength: 380 nm, emission wavelength: 520 nm)

Column: A stainless steel column 4.6 mm in inside diameter and 15 cm in length, packed with phenylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: A mixture of 8-quinolinol in acetonitrile (3 in 100) and diluted 0.5 mol/L ammonium acetate TS (2 in 5) (1:1).

Flow rate: Adjust the flow rate so that the retention time of aluminum/8-quinolinol complex is about 9 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

Furthermore there is an alternative method, in which the chelating agent 8-quinolinol is not included in the mobile phase. In this method also, aluminum is detected as a complex with 8-quinolinol in the sample solution by using HPLC fitted with fluorescence photometer. But it is necessary to form a more stable aluminum/8-quinolinol complex in the sample solution, because the chelating agent is not included in the mobile phase. Further, since the analytical wavelength for the fluorescence detection is different from that in the standard method, excitation WL: 370 nm, emission WL: 504 nm, the detection sensitivity is different. Thus, it is appropriate to obtain the calibration curve between 0 – 25 ppb of alu-

minum. Other than the above-mentioned differences, the size of column, column temperature, and the mobile phase are also different from those used in the standard method, so suitable analytical conditions should be established for performing precise and reproducible examinations of trace amounts of aluminum in the sample specimen.

(2) Lumogallion complexing method

After forming a complex of aluminum ion in the sample specimen with the fluorescent reagent of lumogallion, the solution is examined by HPLC fitted with a fluorescence photometer.

Preparation of sample solution

Pipet 70 μ L of the sample specimen (TPN) exactly, add 0.15 mL of lumogallion hydrochloric acid TS and 0.6 mL of buffer solution for aluminum test, pH 7.2 exactly, then mix the solution. After this solution has been allowed to stand for 4 hours at 40°C, it can be used for the measurement as a sample solution.

Preparation of a series of standard solutions for calibration curve

Pipet 1 mL each of standard aluminum solutions (1) – (5) exactly, and add diluted nitric acid for aluminum test (1 in 100) to make exactly 100 mL. Pipet 70 μ L each of these solutions exactly, and add exactly 0.15 mL of lumogallion hydrochloric acid TS and exactly 0.6 mL of buffer solution for aluminum test, pH 7.2 then allow to stand for 4 hours at 40°C to make a series of standard solutions for obtaining the calibration curve (Aluminum: 0, 1.07, 2.13, 4.27, and 8.54 ppb).

Standard examination method

Take 0.1 mL each of the sample solution and standard aluminum solutions for the calibration curve, and perform HPLC analysis under the following conditions. Calculate the aluminum content in the sample solution by using a calibration curve method.

Operating conditions—

Detector: Fluorescence photometer (excitation wavelength 505 nm, emission wavelength 574 nm)

Column: A stainless steel column 6.0 mm in inside diameter and 10 cm in length, packed with octylsilylated silica gel for liquid chromatography (5 μ m in particle diameter).

Column temperature: A constant temperature of about 40°C.

Mobile phase: Take 100 mL of 2-propanol, and add a diluted 1 mol/L acetic acid-sodium acetate buffer solution of pH 5.0 (1 in 10) to make 1000 mL.

Flow rate: Adjust the flow rate so that the retention time of aluminum/lumogallion complex is about 5 minutes.

System suitability—

The correlation coefficient of the calibration curve, which is prepared using a series of standard solutions, is not less than 0.99.

The following points should be noted in connection with measuring trace amounts of aluminum in TPN preparations.

- 1) Regarding water, solvents, reagents, vessels and other tools used for the examination, select those not contaminated with aluminum. Further, keep the testing environment clean and free from dust in the testing room.
- 2) Before the measurement, it is necessary to confirm that the characteristic properties of the sample do not affect the formation of the complex.

- 3) Reference substances of river water for analysis of trace elements, distributed by the Japan Society for Analytical Chemistry, contain certified amounts of aluminum: JSAC 0301-1 and JSAC 0302 (a known amount of aluminum is artificially added to JSAC 0301-1).

Standard Solutions, Reagents and Test Solutions

Other than the standard solutions, reagents and test solutions specified in the Japanese Pharmacopoeia, those described below can be used in this test.

***N,N*-Bis(2-hydroxyethyl)-2-aminoethane sulfonic acid** $C_6H_{15}NO_5S$ White crystals or powder.

Hydrochloric acid for aluminum test Same as the reagent *Hydrochloric acid*. Further, it contains not more than 1 ppb of aluminum.

Lumogallion [5-Chloro-2-hydroxy-3(2,4-dihydroxyphenylazo)benzenesulfonic acid] $C_{12}H_9ClN_2O_6S$ Red-brown to dark brown powder. Further, it contains not more than 1 ppm of aluminum.

Lumogallion hydrochloric acid TS Dissolve 0.86 g of lumogallion in 300 mL of 2-propanol, and add 350 mL of diluted *Hydrochloric acid for aluminum test* (9 in 50) and *Water for aluminum test* to make 1000 mL exactly.

Nitric acid for aluminum test Same as the reagent *Nitric acid*. Further, it contains not more than 1 ppb of aluminum.

pH buffer solution for aluminum test, pH 7.2 Dissolve 106.6 g of *N,N*-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid in 800 mL of *Water for aluminum test*, adjust the pH 7.2 by using *Tetramethylammonium hydroxide aqueous solution*, and add *Water for aluminum test* to make 1000 mL.

Standard aluminum solution Pipet a constant volume each of *Water for aluminum test* or the *Standard aluminum stock solution*, dilute and adjust the aluminum concentration to 0, 1.25, 2.5, 5.0, and 10 ppm by using diluted *Nitric acid for aluminum test* (1 in 100), to make Standard aluminum solutions (1) – (5).

Tetramethylammonium hydroxide TS $(CH_3)_4NOH$

It is a 25% aqueous solution, prepared for aluminum test. Further, it contains not more than 1 ppb of aluminum.

Water for aluminum test Same as the monograph *Purified Water*. Further, it contains not more than 1 ppb of aluminum.

29. Total Protein Assay

This test is harmonized with the European Pharmacopoeia and the U.S. Pharmacopoeia. The parts of the text that are not harmonized are marked with symbols (♦ ♦).

The following procedures are provided as illustrations of the determination of total protein content in pharmacopoeial preparations. Other techniques, such as HPLC, are also acceptable if total protein recovery is demonstrated. Many of the total protein assay methods described below can be performed successfully using kits from commercial sources.

Note: Where water is required, use distilled water.

Method 1

Protein in solution absorbs UV light at a wavelength of 280 nm, due to the presence of aromatic amino acids, mainly tyrosine and tryptophan. This property is the basis of this method. Protein determination at 280 nm is mainly a function of the tyrosine and tryptophan content of the protein. If the buffer used to dissolve the protein has a high absorbance relative to that of water, there is an interfering substance in the buffer. This interference can be compensated for when the spectrophotometer is adjusted to zero buffer absorbance. If the interference results in a large absorbance that challenges the limit of sensitivity of the spectrophotometer, the results may be compromised. Furthermore, at low concentrations protein can be absorbed onto the cuvette, thereby reducing the content in solution. This can be prevented by preparing samples at higher concentrations or by using a non-ionic detergent in the preparation.

Note: Keep the Test Solution, the Standard Solution, and the buffer at the same temperature during testing.

Standard Solution Unless otherwise specified in the individual monograph, prepare a solution of the reference standard or reference material for the protein under test in the same buffer and at the same concentration as the Test Solution.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration of 0.2 to 2 mg per mL.

Procedure Concomitantly determine the absorbances of the Standard Solution and the Test Solution in quartz cells at a wavelength of 280 nm, with a suitable spectrophotometer, using the buffer as the blank. To obtain accurate results, the response should be linear in the range of protein concentrations to be assayed.

Light-Scattering The accuracy of the UV spectroscopic determination of protein can be decreased by the scattering of light by the test specimen. If the proteins in solution exist as particles comparable in size to the wavelength of the measuring light (250 to 300 nm), scattering of the light beam results in an apparent increase in absorbance of the test specimen. To calculate the absorbance at 280 nm due to light-scattering, determine the absorbances of the Test Solution at wavelengths of 320, 325, 330, 335, 340, 345, and 350 nm. Using the linear regression method, plot the log of the observed absorbance versus the log of the wavelength, and determine the standard curve best fitting the plotted points. From the graph so obtained, extrapolate the absorbance value due to light-scattering at 280 nm. Subtract the absorbance from light-scattering from the total absorbance at 280 nm to obtain the absorbance value of the protein in solution. Filtration with a filter having a 0.2- μ m porosity or clarification by centrifugation may be performed to reduce the effect of light-scattering, especially if the solution is noticeably turbid.

Calculations Calculate the concentration, C_U , of protein in the test specimen by the formula:

$$C_U = C_S (A_U/A_S),$$

in which C_S is the concentration of the Standard Solution; and A_U and A_S are the corrected absorbances of the Test Solution and the Standard Solution, respectively.

Method 2

This method, commonly referred to as the Lowry assay, is based on the reduction by protein of the phosphomolybdic-

tungstic mixed acid chromogen in the Folin-Ciocalteu's phenol reagent, resulting in an absorbance maximum at 750 nm. The Folin-Ciocalteu's phenol reagent (Folin's TS) reacts primarily with tyrosine residues in the protein, which can lead to variation in the response of the assay to different proteins. Because the method is sensitive to interfering substances, a procedure for precipitation of the protein from the test specimen may be used. Where separation of interfering substances from the protein in the test specimen is necessary, proceed as directed below for Interfering Substances prior to preparation of the Test Solution. The effect of interfering substances can be minimized by dilution provided the concentration of the protein under test remains sufficient for accurate measurement. Variations of the Lowry test that are indicated in national regulatory documents¹⁾ can be substituted for the method described below.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 5 and 100 μg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions. An appropriate buffer will produce a pH in the range of 10 to 10.5.

Blank Use the buffer used for the Test Solution and the Standard Solutions.

Reagents and Solutions—

Copper Sulfate Reagent Dissolve 100 mg of copper (II) sulfate pentahydrate and 200 mg of sodium tartrate dihydrate in water, dilute with water to 50 mL, and mix. Dissolve 10 g of anhydrous sodium carbonate in water to a final volume of 50 mL, and mix. Slowly pour the sodium carbonate solution into the copper sulfate solution with mixing. Prepare this solution fresh daily.

5% SDS TS Dissolve 5 g of sodium dodecyl sulfate in water, and dilute with water to 100 mL.

Alkaline Copper Reagent Prepare a mixture of 5% SDS TS, Copper Sulfate Reagent, and Sodium Hydroxide Solution (4 in 125) (2:1:1). This reagent may be stored at room temperature for up to 2 weeks.

Diluted Folin's TS Mix 10 mL of Folin's TS with 50 mL of water. Store in an amber bottle, at room temperature.

Procedure To 1 mL of each Standard Solution, the Test Solution, and the Blank, add 1 mL of Alkaline Copper Reagent, and mix. Allow to stand at room temperature for 10 minutes. Add 0.5 mL of the Diluted Folin's TS to each solution, and mix each tube immediately after the addition, and allow to stand at room temperature for 30 minutes. Determine the absorbances of the solutions from the Standard Solutions and the Test Solution at the wavelength of maximum absorbance at 750 nm, with a suitable spectrophotometer, using the solution from the Blank to set the instrument to zero.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot

the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Interfering Substances In the following procedure, deoxycholate-trichloroacetic acid is added to a test specimen to remove interfering substances by precipitation of proteins before testing. This technique also can be used to concentrate proteins from a dilute solution.

Sodium Deoxycholate Reagent Prepare a solution of sodium deoxycholate in water having a concentration of 150 mg in 100 mL.

Trichloroacetic Acid Reagent Prepare a solution of trichloroacetic acid in water having a concentration of 72 g in 100 mL.

Procedure Add 0.1 mL of Sodium Deoxycholate Reagent to 1 mL of a solution of the protein under test. Mix on a vortex mixer, and allow to stand at room temperature for 10 minutes. Add 0.1 mL of Trichloroacetic Acid Reagent, and mix on a vortex mixer. Centrifuge at $3000 \times g$ for 30 minutes, decant the liquid, and remove any residual liquid with a pipet. Redissolve the protein pellet in 1 mL of Alkaline Copper Reagent. Proceed as directed for Test Solution. [Note: Color development reaches a maximum in 20 to 30 minutes during incubation at room temperature, after which there is a gradual loss of color. Most interfering substances cause a lower color yield; however, some detergents cause a slight increase in color. A high salt concentration may cause a precipitate to form. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.]

Method 3

This method, commonly referred to as the Bradford assay, is based on the absorption shift from 470 nm to 595 nm observed when the brilliant blue G-250 dye binds to protein. The Coomassie Brilliant Blue G-250 dye binds most readily to arginyl and lysyl residues in the protein, which can lead to variation in the response of the assay to different proteins.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 100 μg and 1 mg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Coomassie Reagent Dissolve 100 mg of brilliant blue G-250²⁾ in 50 mL of ethanol (95). [Note: Not all dyes have the same brilliant blue G content, and different products may give different results.] Add 100 mL of phosphoric acid, dilute with water to 1000 mL, and mix. Filter the solution through filter paper (Whatman No.1 or equivalent), and store the filtered reagent in an amber bottle at room temperature. [Note: Slow precipitation of the dye will occur during storage of the rea-

¹⁾ Example: the Minimum Requirements for Biological Products and individual monograph of JP.♦

²⁾ Dye purity is important in the reagent preparation.

gent. Filter the reagent before use.]

Procedure Add 5 mL of the Coomassie Reagent to 100 μ L of each Standard Solution, the Test Solution, and the Blank, and mix by inversion. Avoid foaming, which will lead to poor reproducibility. Determine the absorbances of the solutions from the Standard Solutions and the Test Solution at 595 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero.

[Note: Do not use quartz (silica) spectrophotometer cells: the dye binds to this material. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.] There are relatively few interfering substances, but detergents and ampholytes in the test specimen should be avoided. Highly alkaline specimens may interfere with the acidic reagent.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 4

This method, commonly referred to as the bicinchoninic acid or BCA assay, is based on reduction of the cupric (Cu^{2+}) ion to cuprous (Cu^+) ion by protein. The bicinchoninic acid reagent is used to detect the cuprous ion. The method has few interfering substances. When interfering substances are present, their effect may be minimized by dilution, provided that the concentration of the protein under test remains sufficient for accurate measurement.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 10 and 1200 μ g of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

BCA Reagent Dissolve about 10 g of bicinchoninic acid, 20 g of sodium carbonate monohydrate, 1.6 g of sodium tartrate dihydrate, 4 g of sodium hydroxide, and 9.5 g of sodium hydrogen carbonate in water. Adjust, if necessary, with sodium hydroxide or sodium hydrogen carbonate to a pH of 11.25. Dilute with water to 1000 mL, and mix.

Copper Sulfate Reagent Dissolve about 2 g of copper (II) sulfate pentahydrate in water to a final volume of 50 mL.

Copper-BCA Reagent Mix 1 mL of Copper Sulfate Reagent and 50 mL of BCA Reagent.

Procedure Mix 0.1 mL of each Standard Solution, the Test Solution, and the Blank with 2 mL of the Copper-BCA Reagent. Incubate the solutions at 37°C for 30 minutes, note the time, and allow to come to room temperature. Within 60 minutes following the incubation time, determine the absor-

bances of the solutions from the Standard Solutions and the Test Solution in quartz cells at 562 nm, with a suitable spectrophotometer, using the Blank to set the instrument to zero. After the solutions are cooled to room temperature, the color intensity continues to increase gradually. If substances that will cause interference in the test are present, proceed as directed for Interfering Substances under Method 2. Because different protein species may give different color response intensities, the standard protein and test protein should be the same.

Calculations [Note: The relationship of absorbance to protein concentration is nonlinear; however, if the standard curve concentration range is sufficiently small, it will approach linearity.] Using the linear regression method, plot the absorbances of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the absorbance of the Test Solution, determine the concentration of protein in the Test Solution.

Method 5

This method, commonly referred to as the Biuret assay, is based on the interaction of cupric (Cu^{2+}) ion with protein in an alkaline solution and the resultant development of absorbance at 545 nm.

Standard Solutions Unless otherwise specified in the individual monograph, prepare a solution of Albumin Human for which the protein content has been previously determined by nitrogen analysis (using the nitrogen-to-protein conversion factor of 6.25) or of the reference standard or reference material for the protein under test in sodium chloride solution (9 in 1000). Dilute portions of this solution with sodium chloride solution (9 in 1000) to obtain not fewer than three Standard Solutions having concentrations between 0.5 and 10 mg per mL, the concentrations being evenly spaced. [Note: Low responses may be observed if the sample under test has significantly different level of proline than that of Albumin Human. A different standard protein may be employed in such cases.]

Test Solution Prepare a solution of the test protein in sodium chloride solution (9 in 1000) having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use sodium chloride solution (9 in 1000).

Biuret Reagent Dissolve about 3.46 g of copper (II) sulfate pentahydrate in 10 mL of water, with heating if necessary, and allow to cool (Solution A). Dissolve about 34.6 g of sodium citrate dihydrate and 20.0 g of anhydrous sodium carbonate in 80 mL of water, with heating if necessary, and allow to cool (Solution B). Mix Solutions A and B, and dilute with water to 200 mL. This Biuret Reagent is stable at room temperature for 6 months. Do not use the reagent if it develops turbidity or contains any precipitate.

Procedure To one volume of the Standard Solutions and a solution of the Test Solution add an equal volume of sodium hydroxide solution (6 in 100), and mix. Immediately add a volume of Biuret Reagent equivalent to 0.4 volume of the Test Solution, and mix. Allow to stand at a temperature between 15°C and 25°C for not less than 15 minutes. Within 90 minutes after the addition of the Biuret Reagent, determine the absorbances of the Standard Solutions and the solution from the Test Solution at the wavelength of maximum absorbance at 545 nm, with a suitable spectrophotome-

ter, using the Blank to set the instrument to zero. [Note: Any solution that develops turbidity or a precipitate is not acceptable for calculation of protein concentration.]

Calculations Using the least-squares linear regression method, plot the absorbances of the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points, and calculate the correlation coefficient for the line. [Note: Within the given range of the standards, the relationship of absorbance to protein concentration is approximately linear.] A suitable system is one that yields a line having a correlation coefficient of not less than 0.99. From the standard curve and the absorbance of the Test Solution, determine the concentration of protein in the test specimen, making any necessary correction.

Interfering Substances To minimize the effect of interfering substances, the protein can be precipitated from the initial test specimen as follows. Add 0.1 volume of 50% trichloroacetic acid to 1 volume of a solution of the test specimen, withdraw the supernatant layer, and dissolve the precipitate in a small volume of 0.5 mol/L sodium hydroxide TS. Use the solution so obtained to prepare the Test Solution.

Comments This test shows minimal difference between equivalent IgG and albumin samples. Addition of the sodium hydroxide and the Biuret Reagent as a combined reagent, insufficient mixing after the addition of the sodium hydroxide, or an extended time between the addition of the sodium hydroxide solution and the addition of the Biuret Reagent will give IgG samples a higher response than albumin samples. The trichloroacetic acid method used to minimize the effects of interfering substances also can be used to determine the protein content in test specimens at concentrations below 500 μg per mL.

Method 6

This fluorometric method is based on the derivatization of the protein with *o*-phthalaldehyde (OPA), which reacts with the primary amines of the protein (i.e., NH_2 -terminal amino acid and the ϵ -amino group of the lysine residues). The sensitivity of the test can be increased by hydrolyzing the protein before testing. Hydrolysis makes the α -amino group of the constituent amino acids of the protein available for reaction with the OPA reagent. The method requires very small quantities of the protein.

Primary amines, such as tris(hydroxymethyl)amino-methane and amino acid buffers, react with OPA and must be avoided or removed. Ammonia at high concentrations will react with OPA as well. The fluorescence obtained when an amine reacts with OPA can be unstable. The use of automated procedures to standardize this procedure may improve the accuracy and precision of the test.

Standard Solutions Unless otherwise specified in the individual monograph, dissolve the reference standard or the reference material for the protein under test in the buffer used to prepare the Test Solution. Dilute portions of this solution with the same buffer to obtain not fewer than five Standard Solutions having concentrations between 10 and 200 μg of protein per mL, the concentrations being evenly spaced.

Test Solution Dissolve a suitable quantity of the protein under test in the appropriate buffer to obtain a solution having a concentration within the range of the concentrations of the Standard Solutions.

Blank Use the buffer used to prepare the Test Solution and the Standard Solutions.

Reagents and Solutions—

Borate Buffer Dissolve about 61.83 g of boric acid in water, and adjust with potassium hydroxide to a pH of 10.4. Dilute with water to 1000 mL, and mix.

Stock OPA Reagent Dissolve about 120 mg of *o*-phthalaldehyde in 1.5 mL of methanol, add 100 mL of Borate Buffer, and mix. Add 0.6 mL of polyoxyethylene (23) lauryl ether, and mix. This solution is stable at room temperature for at least 3 weeks.

OPA Reagent To 5 mL of Stock OPA Reagent add 15 μL of 2-mercaptoethanol. Prepare at least 30 minutes prior to use. This reagent is stable for one day.

Procedure Adjust each of the Standard Solutions and the Test Solution to a pH between 8.0 and 10.5. Mix 10 μL of the Test Solution and each of the Standard Solutions with 100 μL of OPA Reagent, and allow to stand at room temperature for 15 minutes. Add 3 mL of 0.5 mol/L sodium hydroxide TS, and mix. Using a suitable fluorometer, determine the fluorescent intensities of solutions from the Standard Solutions and the Test Solution at an excitation wavelength of 340 nm and an emission wavelength between 440 and 455 nm. [Note: The fluorescence of an individual specimen is read only once because irradiation decreases the fluorescent intensity.]

Calculations The relationship of fluorescence to protein concentration is linear. Using the linear regression method, plot the fluorescent intensities of the solutions from the Standard Solutions versus the protein concentrations, and determine the standard curve best fitting the plotted points. From the standard curve so obtained and the fluorescent intensity of the Test Solution, determine the concentration of protein in the test specimen.

Method 7

This method is based on nitrogen analysis as a means of protein determination. Interference caused by the presence of other nitrogen-containing substances in the test protein can affect the determination of protein by this method. Nitrogen analysis techniques destroy the protein under test but are not limited to protein presentation in an aqueous environment.

Procedure A Determine the nitrogen content of the protein under test as directed elsewhere in the Pharmacopoeia. Commercial instrumentation is available for the Kjeldahl nitrogen assay.

Procedure B Commercial instrumentation is available for nitrogen analysis. Most nitrogen analysis instruments use pyrolysis (i.e., combustion of the sample in oxygen at temperatures approaching 1000°C), which produces nitric oxide (NO) and other oxides of nitrogen (NO_x) from the nitrogen present in the test protein. Some instruments convert the nitric oxides to nitrogen gas, which is quantified with a thermal conductivity detector. Other instruments mix nitric oxide (NO) with ozone (O_3) to produce excited nitrogen dioxide (NO_2^*), which emits light when it decays and can be quantified with a chemiluminescence detector. A protein reference standard or reference material that is relatively pure and is similar in composition to the test proteins is used to optimize the injection and pyrolysis parameters and to evaluate consistency in the analysis.

Calculations The protein concentration is calculated by dividing the nitrogen content of the sample by the known nitrogen content of the protein. The known nitrogen content of the protein can be determined from the chemical composition of the protein or by comparison with the nitrogen

content of the appropriate reference standard or reference material.

30. Validation of Analytical Procedures

The validation of an analytical procedure is the process of confirming that the analytical procedure employed for a test of pharmaceuticals is suitable for its intended use. In other words, the validation of an analytical procedure requires us to demonstrate scientifically that risks in decision by testing caused by errors from analytical steps are acceptably small. The performance of an analytical procedure is established by various kinds of validation characteristics. The validity of a proposed analytical procedure can be shown by demonstrating experimentally that the validation characteristics of the analytical procedure satisfy the standards set up according to the acceptable limits of testing.

When an analytical procedure is to be newly carried in the Japanese Pharmacopoeia, when a test carried in the Japanese Pharmacopoeia is to be revised, and when the test carried in the Japanese Pharmacopoeia is to be replaced with a new test according to regulations in general notices, analytical procedures employed for these tests should be validated according to this document.

Required data for analytical procedures to be carried in the Japanese Pharmacopoeia

(1) Outline

This section should provide a brief explanation of the principle of a proposed analytical procedure, identify the necessity of the analytical procedure and its advantage compared with other procedures, and summarize the validation. When an analytical procedure is revised, the limitation of the current analytical procedure and the advantage offered by the new analytical procedure should be described.

(2) Analytical procedure

This section should contain a complete description of the analytical procedure to enable skilled persons to evaluate correctly the analytical procedure and replicate it if necessary. Analytical procedures include all important operating procedures for performing analyses, the preparation of standard samples, reagents and test solutions, precautions, procedures to verify system suitability (e.g. the verification of the separating performance of a chromatographic system), formulas to obtain results, the number of replications and so forth. Any instruments and apparatus that are not stated in the Japanese Pharmacopoeia should be described in detail. The physical, chemical or biological characteristics of any new reference standards should be clarified and their testing methods should be established.

(3) Data showing the validity of analytical procedures

This section should provide complete data showing the validity of the analytical procedures. This includes the experimental designs to determine the validation characteristics, experimental data, calculation results and results of hypothesis tests.

Validation characteristics

The definition of typical validation characteristics to be assessed in validation of analytical procedures and examples of assessing procedures are given below.

The terminology and definitions of the validation characteristics may possibly vary depending upon the fields to which analytical procedures are applied. The terminology and definitions shown in this document are established for the purpose of the Japanese Pharmacopoeia. Typical methods for assessing the validation characteristics are shown in the item of assessment. Various kinds of methods to determine the validation characteristics have been proposed and any methods that are widely accepted will be accepted for the present purpose. However, since values of the validation characteristics may possibly depend upon methods of determination, it is required to present the methods of determining the validation characteristics, the data and calculation methods in sufficient detail.

Although robustness is not listed as a validation characteristic, it should be considered during the development of analytical procedures. Studying the robustness may help to improve analytical procedures and to establish appropriate analytical conditions including precautions.

(1) Accuracy/Trueness

Definition: The accuracy is a measure of the bias of observed values obtained by an analytical procedure. The accuracy is expressed as the difference between the average value obtained from a large series of observed values and the true value.

Assessment: The estimate of accuracy of an analytical method is expressed as the difference between the total mean of observed values obtained during investigation of the reproducibility and the true value. The theoretical value is used as the true value (e.g., in the case of titration methods, etc.). When there is no theoretical value or it is difficult to obtain a theoretical value even though it exists, a certified value or a consensus value may be used as the true value. When an analytical procedure for a drug product is considered, the observed value of the standard solution of the drug substance may be used as the consensus value.

It may be inferred from specificity data that an analytical procedure is unbiased.

The estimate of accuracy and a 95% confidence interval of the accuracy should be calculated using the standard error based on the reproducibility (intermediate precision). It should be confirmed that the confidence interval includes zero or that the upper or lower confidence limits are within the range of the accuracy required of the analytical procedure.

(2) Precision

Definition: The precision is a measure of the closeness of agreement between observed values obtained independently from multiple samplings of a homogenous sample and is expressed as the variance, standard deviation or relative standard deviation (coefficient of variation) of observed values.

The precision should be considered at three levels with different repetition conditions; repeatability, intermediate precision and reproducibility.

(i) Repeatability/Intra-assay precision

The repeatability expresses the precision of observed values obtained from multiple samplings of a homogenous sample over a short time interval within a laboratory, by the same analyst, using the same apparatus and instruments, lots of reagents and so forth (repeatability conditions).

(ii) Intermediate precision

The intermediate precision expresses the precision of observed values obtained from multiple samplings of a homogenous sample by changing a part of or all of the oper-

ating conditions including analysts, experimental dates, apparatus and instruments and lots of reagents within a laboratory (intermediate precision condition).

(iii) Reproducibility

The reproducibility expresses the precision of observed values obtained from multiple samplings of a homogenous sample in different laboratories (reproducibility condition).

Assessment: A sufficient volume of a homogenous sample should be prepared before studying the precision. The solution is assumed to be homogenous. When it is difficult to obtain a homogenous sample, the following samples may be used as homogenous samples; e.g., a large amount of drug products or mixture of drug substance and vehicles that are crushed and mixed well until they can be assumed to be homogenous.

Suitable experimental designs such as one-way layout may be employed when more than one level of precision is to be investigated simultaneously. A sufficient number of repetitions, levels of operating conditions and laboratories should be employed. Sources of variations affecting analytical results should be evaluated as thoroughly as possible through the validation.

It is required to show the variance, standard deviation and relative standard deviation (coefficient of variation) of each level of precision. The 90% confidence interval of the variance and corresponding intervals of the standard deviation and relative standard deviation should also be established. The validity of the proposed analytical procedure for its intended use may be confirmed by comparing obtained values with the required values of the analytical procedure. Whether the proposed analytical procedure is acceptable may normally be decided based on the reproducibility.

(3) Specificity

Definition: The specificity is the ability of an analytical procedure to measure accurately an analyte in the presence of components that may be expected to be present in the sample matrix. The specificity is a measure of discriminating ability. Lack of specificity of an analytical procedure may be compensated by other supporting analytical procedures.

Assessment: It should be confirmed that the proposed analytical procedure can identify an analyte or that it can accurately measure the amount or concentration of an analyte in a sample. The method to confirm the specificity depends very much upon the purpose of the analytical procedure. For example, the specificity may be assessed by comparing analytical results obtained from a sample containing the analyte only with results obtained from samples containing excipients, related substances or degradation products, and including or excluding the analyte. If reference standards of impurities are unavailable, samples that are expected to contain impurities or degradation products may be used (e.g. samples after accelerated or stress tests).

(4) Detection limit

Definition: The detection limit is the lowest amount or concentration of the analyte in a sample that is detectable, but not necessarily quantifiable.

Assessment: The detection limit should be normally determined so that producer's and consumer's risks are less than 5%. The detection limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the detection limit and the slope of the calibration curve close to the detection limit. The following equation is an example to determine the detection limit using

the standard deviation of responses of blank samples and the slope of the calibration curve.

$$DL = 3.3\sigma/slope$$

DL: detection limit

σ : the standard deviation of responses of blank samples

slope: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the detection limit of the analytical procedure is lower than the specified limit for testing.

(5) Quantitation limit

Definition: The quantitation limit is the lowest amount or concentration of the analyte in a sample that can be determined. The precision expressed as the relative standard deviation of samples containing an analyte at the quantitation limit is usually 10%.

Assessment: The quantitation limit may be calculated using the standard deviation of responses of blank samples or samples containing an analyte close to the quantitation limit and the slope of the calibration curve close to the quantitation limit. The following equation is an example to determine the quantitation limit using the standard deviation of responses of blank samples and the slope of the calibration curve.

$$QL = 10\sigma/slope$$

QL: quantitation limit

σ : the standard deviation of responses of blank samples

slope: slope of the calibration curve

The noise level may be used as the standard deviation of responses of blank samples in chromatographic methods. It should be ensured that the quantitation limit of the analytical procedure is lower than the specified limit for testing.

(6) Linearity

Definition: The linearity is the ability of an analytical procedure to elicit responses linearly related to the amount or concentration of an analyte in samples. A well-defined mathematical transformation may sometimes be necessary to obtain a linear relationship.

Assessment: Responses are obtained after analyzing samples with various amounts or concentrations of an analyte according to described operating procedures. The linearity may be evaluated in terms of the correlation coefficient, and the slope and y-intercept of the regression line. It may be also helpful for evaluating the linearity to plot residual errors from the regression line against the amount or concentration and to confirm that there is no particular tendency in the graph. Samples with five different amounts or concentrations of an analyte should be usually investigated.

(7) Range

Definition: The range for the validation of analytical procedures is the interval between the lower and upper limits of the amount or concentration of an analyte providing sufficient accuracy and precision. The range for the validation of analytical procedures for an analytical procedure with linearity is the interval between the lower and upper limits providing sufficient accuracy, precision and linearity.

Assessment: When the range for the validation of analytical procedures is investigated, 80 to 120% of specified limits

of testing should be usually considered. The accuracy, precision and linearity should be evaluated using samples containing the lower and upper limits and in the middle of the range.

Categories of tests employing analytical procedures

Tests covered with this document are roughly classified into three categories shown below according to their purposes. The table lists the normally required validation characteristics to be evaluated in the validation of analytical procedures used in these tests. This list should be considered to represent typical validation characteristics. A different approach to validating analytical procedures should be considered depending upon the characteristics of analytical procedures and their intended use.

Type I Identification. Tests for identifying major components in pharmaceuticals according to their characteristics.

Type II Impurity tests. Tests for determination of impurities in pharmaceuticals.

Type III Tests for assaying drug substances, active ingredients, and major components in pharmaceuticals.

(Additives such as stabilizing agents and preservatives are included in major components.) Tests for determining performance of pharmaceuticals, such as dissolution testing.

Table Lists of validation characteristics required to be evaluated in tests of each type

Validation characteristics \ Type of test	Type I	Type II		Type III
		Quantitation test	Limit test	
Accuracy/Trueness	–	+	–	+
Precision				
Repeatability	–	+	–	+
Intermediate precision	–	–*	–	–*
Reproducibility	–	+*	–	+*
Specificity**	+	+	+	+
Detection limit	–	–	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

– Usually need not to be evaluated.

+ Usually need to be evaluated.

* Either intermediate precision or reproducibility should be evaluated depending upon circumstances in which analytical procedures or tests are performed. The latter should be normally evaluated in the validation of analytical procedures proposed to be included in the Japanese Pharmacopoeia.

** The lack of the specificity of an analytical procedure may be compensated by other relevant analytical procedures

Terminology used in the validation of analytical procedures

Analytical procedure: This document covers analytical procedures applied to identification, and ones that provides responses depending upon the amount or concentration of analytes in samples.

Laboratory: The laboratory means an experimental room or facility where tests are performed. In this document different laboratories are expected to perform an analytical procedure using different analysts, different experimental apparatus and instruments, different lots of reagents and so forth.

Number of replications: The number of replications is one that is described in analytical procedures. An observed value is often obtained by more than one measurement in order to achieve good precision of analytical procedures. Analytical

procedures including the number of replications should be validated. This is different from repetition in the validation of analytical procedures to obtain accuracy or precision.

Observed value: The value of a characteristic obtained as the result of performing an analytical procedure.

Consumer's risk: This is the probability that products out of the specification of tests are decided to be accepted after testing. It is usually expressed as β , and is called the probability of type II error or the probability of false negative in impurity tests.

Producer's risk: This is the probability that products satisfying the specification of tests are decided to be rejected after testing. It is usually expressed as α , and is called the probability of type I error or the probability of false positive in impurity tests.

Robustness: The robustness is a measure of the capacity to remain unaffected by small but deliberate variations in analytical conditions. The stability of observed values may be studied by changing various analytical conditions within suitable ranges including pH values of solutions, reaction temperature, reaction time or amount of reagents added. When observed values are unstable, the analytical procedure should be improved. Results of studying robustness may be reflected in the developed analytical procedure as precautions or significant digits describing analytical conditions.

Test: Tests mean various tests described in general tests and official monographs in the Japanese Pharmacopoeia such as impurity tests and assay. They includes sampling methods, specification limits and analytical procedures.

Atomic Weight Table (2004)

In 1961 it was decided that the atomic weights of the elements would be based on values relative to the mass number of 12 (no fractions) for carbon (^{12}C). Ever since, there has been a marked improvement in the quality and quantity of data on the nuclide masses and isotope ratios of the elements using physical methods such as mass spectrometry. The Commission on Atomic Weights and Isotope Abundances (CAWIA) of the International Union of Pure and Applied Chemistry (IUPAC) collected and examined newly measured data and then published an atomic weight table. Based on this table, in April of each year the Atomic Weight Subcommittee of the Chemical Society of Japan also publishes an atomic weight table. A simple explanation is provided below so that the table can be used correctly and effectively. For a more detailed explanation, the user is referred to a report¹⁾ and a review²⁾ published by the CAWIA.

The atomic weight values of each of the elements shown in the atomic weight tables are, as stated in the preface to the table, for elements that originate on Earth and are present in substances that exist naturally. Atomic weights are, with the exception of single nuclide elements (elements consisting of one stable nuclide), not natural constants like the speed of light, but rather change depending on a variety of factors, such as the method of treatment or the origin of the substance containing that element. This is because the atomic weight is dependent on the relative frequency (isotope ratio) of the stable nuclides comprising each of the respective elements. Due to advancements in measurement techniques, the isotopic frequencies of each of the elements are not necessarily constant, and fluctuate due to a variety of processes that occur on the Earth. We have come to learn that this is reflected in the atomic weights. The result of this is that differences have arisen in the accuracy of the atomic weights between elements. The figures in parentheses that follow the atomic weight values in the atomic weight tables represent the uncertainty with respect to the last digit in the atomic weight. For example, in the case of hydrogen, 1.00794(7) means 1.00794 ± 0.00007 .

The atomic weight of a single nuclide element is the most accurate and the precision is also high. This is because it is not necessary to consider the isotope ratio since single nuclide elements do not possess a multiple number of stable isotopes. The atomic weights of such elements are determined based on the mass³⁾ of each nuclide determined by physical techniques, taking into consideration the uncertainty with constant criteria.

Among the elements, the majority of samples gathered on Earth exhibit a constant isotope composition, however, some specific samples have isotope compositions that are different to these. These kinds of elements are indicated by a “g”, which means the value in the atomic weight table cannot be used as is, depending on the sample, as the atomic weight of these elements. In relation to this, oxygen for example exists in a number of forms on Earth, such as in air, salt water, fresh water, and in rocks, and because the isotope compositions fluctuate among these substances, oxygen is not an element for which only one value can be used. Thus, an “r” is attached to an element for which a precise atomic weight cannot be given, no matter how much progress is made in techniques for measuring the isotope composition. On the other hand, it is also possible, depending on the element, to use an isotope that has undergone artificial fractionation as a reagent. Typical elements that are representative include hydrogen, lithium, boron, and uranium. This type of element is identified by an “m”, and particularly in cases where the atomic weight is a problem, it is necessary to be careful by referring to the label of the reagent.

- 1) IUPAC Inorganic Chemistry Division, CAWIA: Atomic Weights of the Elements 2001. *Pure Appl. Chem.*, **75**, 1107 (2003).
- 2) J. R. De Laeter et al.: Atomic Weights of the Elements: Review 2000. *Pure Appl. Chem.*, **75**, 683 (2003).
- 3) G. Audi and A. H. Wapstra: The 1993 Atomic Mass Evaluation (I). Atomic Mass Table. *Nucl. Phys.*, **A565**. 1 (1993).

Standard Atomic Weights 2004

(scaled to $A_r(^{12}\text{C})=12$, where ^{12}C is a neutral atom in its nuclear and electronic ground state)

The atomic weights of many elements are not invariant but depend on the origin and treatment of the material. The standard values of $A_r(E)$ and the uncertainties (in parentheses, following the last significant figure to which they are attributed) apply to elements of natural terrestrial origin. The footnotes to this Table elaborate the types of variation which may occur for individual elements and which may be larger than the listed uncertainties of values of $A_r(E)$. Names of elements with atomic number 112 to 116 are provisional.

Name	Symbol	Atomic Number	Atomic Weight	Footnotes	Name	Symbol	Atomic Number	Atomic Weight	Footnotes
Hydrogen	H	1	1.00794(7)	gmr	Praseodymium	Pr	59	140.90765(2)	
Helium	He	2	4.002602(2)	g r	Neodymium	Nd	60	144.24(3)	g
Lithium	Li	3	(6.941(2)) †	gmr	Promethium*	Pm	61		
Beryllium	Be	4	9.012182(3)		Samarium	Sm	62	150.36(3)	g
Boron	B	5	10.811(7)	gmr	Europium	Eu	63	151.964(1)	g
Carbon	C	6	12.0107(8)	g r	Gadolinium	Gd	64	157.25(3)	g
Nitrogen	N	7	14.0067(2)	g r	Terbium	Tb	65	158.92534(2)	
Oxygen	O	8	15.9994(3)	g r	Dysprosium	Dy	66	162.500(1)	g
Fluorine	F	9	18.9984032(5)		Holmium	Ho	67	164.93032(2)	
Neon	Ne	10	20.1797(6)	gm	Erbium	Er	68	167.259(3)	g
Sodium	Na	11	22.989770(2)		Thulium	Tm	69	168.93421(2)	
Magnesium	Mg	12	24.3050(6)		Ytterbium	Yb	70	173.04(3)	g
Aluminium	Al	13	26.981538(2)		Lutetium	Lu	71	174.967(1)	g
Silicon	Si	14	28.0855(3)	r	Hafnium	Hf	72	178.49(2)	
Phosphorus	P	15	30.973761(2)		Tantalum	Ta	73	180.9479(1)	
Sulfur	S	16	32.065(5)	g r	Tungsten	W	74	183.84(1)	
Chlorine	Cl	17	35.453(2)	gm	(Wolfram)				
Argon	Ar	18	39.948(1)	g r	Rhenium	Re	75	186.207(1)	
Potassium	K	19	39.0983(1)		Osmium	Os	76	190.23(3)	g
Calcium	Ca	20	40.078(4)	g	Iridium	Ir	77	192.217(3)	
Scandium	Sc	21	44.955910(8)		Platinum	Pt	78	195.078(2)	
Titanium	Ti	22	47.867(1)		Gold	Au	79	196.96655(2)	
Vanadium	V	23	50.9415(1)		Mercury	Hg	80	200.59(2)	
Chromium	Cr	24	51.9961(6)		Thallium	Tl	81	204.3833(2)	
Manganese	Mn	25	54.938049(9)		Lead	Pb	82	207.2(1)	g r
Iron	Fe	26	55.845(2)		Bismuth	Bi	83	208.98038(2)	
Cobalt	Co	27	58.933200(9)		Polonium*	Po	84		
Nickel	Ni	28	58.6934(2)		Astatine*	At	85		
Copper	Cu	29	63.546(3)	r	Radon*	Rn	86		
Zinc	Zn	30	65.409(4)		Francium*	Fr	87		
Gallium	Ga	31	69.723(1)		Radium*	Ra	88		
Germanium	Ge	32	72.64(1)		Actinium*	Ac	89		
Arsenic	As	33	74.92160(2)		Thorium*	Th	90	232.0381(1)	g
Selenium	Se	34	78.96(3)	r	Protactinium*	Pa	91	231.03588(2)	
Bromine	Br	35	79.904(1)		Uranium*	U	92	238.02891(3)	gm
Krypton	Kr	36	83.798(2)	gm	Neptunium*	Np	93		
Rubidium	Rb	37	85.4678(3)	g	Plutonium*	Pu	94		
Strontium	Sr	38	87.62(1)	g r	Americium*	Am	95		
Yttrium	Y	39	88.90585(2)		Curium*	Cm	96		
Zirconium	Zr	40	91.224(2)	g	Berkelium*	Bk	97		
Niobium	Nb	41	92.90638(2)		Californium*	Cf	98		
Molybdenum	Mo	42	95.94(2)	g	Einsteinium*	Es	99		
Technetium*	Tc	43			Fermium*	Fm	100		
Ruthenium	Ru	44	101.07(2)	g	Mendelevium*	Md	101		
Rhodium	Rh	45	102.90550(2)		Nobelium*	No	102		
Palladium	Pd	46	106.42(1)	g	Lawrencium*	Lr	103		
Silver	Ag	47	107.8682(2)	g	Rutherfordium*	Rf	104		
Cadmium	Cd	48	112.411(8)	g	Dubnium*	Db	105		
Indium	In	49	114.818(3)		Seaborgium*	Sg	106		
Tin	Sn	50	118.710(7)	g	Bohrium*	Bh	107		
Antimony	Sb	51	121.760(1)	g	Hassium*	Hs	108		
Tellurium	Te	52	127.60(3)	g	Meitnerium*	Mt	109		
Iodine	I	53	126.90447(3)		Darmstadtium*	Ds	110		
Xenon	Xe	54	131.293(6)	gm	Roentgenium*	Rg	111		
Caesium	Cs	55	132.90545(2)		Ununbium*	Uub	112		
Barium	Ba	56	137.327(7)		Ununquadium*	Uuq	114		
Lanthanum	La	57	138.9055(2)	g	Ununhexium*	Uuh	116		
Cerium	Ce	58	140.116(1)	g					

*: Element has no stable isotopes.

†: Commercially available Li materials have atomic weights that range between 6.939 and 6.996; if a more accurate value is required, it must be determined for the specific material.

g: geological specimens are known in which the element has an isotopic composition outside the limits for normal material. The difference between the atomic weight of the element in such specimens and that given in the Table may exceed the stated uncertainty.

m: modified isotopic compositions may be found in commercially available material because it has been subjected to an undisclosed or inadvertent isotopic fractionation. Substantial deviations in atomic weight of the element from that given in the Table can occur.

r: range in isotopic composition of normal terrestrial material prevents a more precise $A_r(E)$ being given: the tabulated $A_r(E)$ value should be applicable to any normal material.

INDEX

A

- Absorptive Ointment, 265
Acacia, 1251
Acebutolol Hydrochloride, 265
Aceglutamide Aluminum, 266
Acetaminophen, 267
Acetazolamide, 268
Acetic Acid, 268
Acetohexamide, 269
Acetylcholine Chloride for Injection, 271
Acetylsalicylic Acid, 318
 Tablets, 319
Achyranthes Root, 1252
Aclarubicin Hydrochloride, 272
Acrinol
 and Zinc Oxide Oil, 273
 and Zinc Oxide Ointment, 274
 Hydrate, 274
Actinomycin D, 275
Adrenaline, 276
 Injection, 276
 Solution, 277
Adsorbed
 Diphtheria Toxoid for Adult Use, 596
 Diphtheria-Purified Pertussis-Tetanus Combined Vaccine, 596
 Diphtheria-Tetanus Combined Toxoid, 596
 Habu-venom Toxoid, 716
 Hepatitis B Vaccine, 716
 Purified Pertussis Vaccine, 980
 Tetanus Toxoid, 1156
Agar, 1252
Ajmaline, 278
 Tablets, 279
Akebia Stem, 1253
Alacepril, 279
 Tablets, 280
Albumin Tannate, 282
Alcohol, 638
 for Disinfection, 640
Aldioxa, 282
Alimemazine Tartrate, 283
Alisma Rhizome, 1253
Allopurinol, 284
Aloe, 1254
Alpinia Officinarum Rhizome, 1256
Alprazolam, 284
Alprenolol Hydrochloride, 285
Alprostadil, 286
 Alfadex, 287
Alum, 292
 Solution, 293
Aluminum
 Acetylsalicylate, 319
 Monostearate, 291
 Potassium Sulfate Hydrate, 292
 Sucrose Sulfate Ester, 1118
Amantadine Hydrochloride, 293
Ambenonium Chloride, 294
Amidotrizoic Acid, 295
Amikacin Sulfate, 296
Aminoacetic Acid, 704
Aminobenzylpenicillin, 307
 Sodium, 308
Aminophylline
 Hydrate, 297
 Injection, 297
Amitriptyline Hydrochloride, 298
 Tablets, 299
Ammonia Water, 299
Amobarbital, 300
 Sodium for Injection, 300
Amomum Seed, 1256
Amorphous Insulin Zinc Injection (Aqueous Suspension), 764
Amoxapine, 301
Amoxicillin Hydrate, 302
Amphotericin B, 303
 for Injection, 304
 Syrup, 305
 Tablets, 305
Ampicillin
 Ethoxycarbonyloxyethyl Hydrochloride, 329
 Hydrate, 307
 Sodium, 308
Ampicillinphthalidyl Hydrochloride, 1138
Amyl Nitrite, 309
Anemarrhena Rhizome, 1256
Anesthamine, 644
Anesthetic Ether, 641
Angelica Dahurica Root, 1257
Anhydrous
 Aminobenzylpenicillin, 306
 Ampicillin, 306
 Caffeine, 386
 Citric Acid, 514
 Dibasic Calcium Phosphate, 396
 Ethanol, 639
 Lactose, 802
Antipyrine, 310
Apricot
 Kernel, 1257
 Kernel Water, 1257
Arbekacin
 Sulfate, 311
 Sulfate Injection, 312
Areca, 1258
Arginine Hydrochloride, 313
 Injection, 314
L-Arginine, 313
 Hydrochloride, 313
 Hydrochloride Injection, 314
Aromatic Castor Oil, 415
Arotinolol Hydrochloride, 314
Arsenical Paste, 315
Arsenic Trioxide, 316
Arsenous Acid, 316
Artemisia Capillaris Flower, 1258
Ascorbic Acid, 316
 Injection, 317
 Powder, 317
Asiasarum Root, 1259
Asparagus Tuber, 1259
L-Aspartic Acid, 318
Aspirin, 318
 Aluminum, 319
 Tablets, 319
Aspoxicillin Hydrate, 320
Astragalus Root, 1260
Astromicin Sulfate, 322
Atenolol, 323
Atractylodes
 Lancea Rhizome, 1261
 Rhizome, 1260
Atropine
 Sulfate Hydrate, 324
 Sulfate Injection, 324
Azathioprine, 325
 Tablets, 326
Azithromycin Hydrate, 327
Aztreonam, 328
Afloqualone, 277

B

- Bacampicillin Hydrochloride, 329
Bacitracin, 330
Baclofen, 331
 Tablets, 332
Bamethan Sulfate, 333
Barbital, 333
Barium Sulfate, 334
Bearberry Leaf, 1262
Bear Bile, 1262
Beclometasone Dipropionate, 335
Beef Tallow, 336
Bekanamycin Sulfate, 337
Belladonna
 Extract, 1263
 Root, 1263
Benidipine Hydrochloride, 338

- Tablets, 339
 Benincasa Seed, 1264
 Benoxinate Hydrochloride, 951
 Benserazide Hydrochloride, 340
 Bentonite, 341
 Benzalkonium Chloride, 342
 Concentrated Solution 50, 343
 Solution, 342
 Benzbromarone, 343
 Benzethonium Chloride, 344
 Solution, 345
 Benzocaine, 644
 Benzoic Acid, 345
 Benzoin, 1265
 Benzyl
 Alcohol, 346
 Benzoate, 347
 Benzylpenicillin
 Benzathine Hydrate, 348
 Potassium, 349
 Berberine
 Chloride Hydrate, 351
 Tannate, 352
 Bethahistine Mesilate, 353
 Tablets, 354
 Betamethasone, 355
 Dipropionate, 358
 Sodium Phosphate, 359
 Tablets, 356
 Valerate, 360
 Valerate and Gentamicin Sulfate
 Cream, 361
 Valerate and Gentamicin Sulfate
 Ointment, 362
 Bethanechol Chloride, 363
 Bezafibrate, 364
 Sustained Release Tablets, 365
 Bifonazole, 366
 Biperiden Hydrochloride, 366
 Bisacodyl, 367
 Suppositories, 368
 Bismuth
 Subgallate, 368
 Subnitrate, 369
 Bitter
 Cardamon, 1265
 Orange Peel, 1265
 Tincture, 1266
 Bleomycin
 Hydrochloride, 370
 Sulfate, 372
 Boric Acid, 374
 Bromazepam, 374
 Bromhexine Hydrochloride, 375
 Bromocriptine Mesilate, 376
 Bromovalerylurea, 377
 Bucillamine, 377
 Bucumolol Hydrochloride, 378
 Bufetolol Hydrochloride, 379
 Bufexamac, 380
 Cream, 380
 Ointment, 381
 Bumetanide, 382
 Bunazosin Hydrochloride, 383
 Bupleurum Root, 1266
 Bupranolol Hydrochloride, 383
 Burdock Fruit, 1267
 Burnt Alum, 292
 Busulfan, 384
 Butropium Bromide, 385
 Butyl Parahydroxybenzoate, 386
- C**
- Cacao Butter, 386
 Calciferol, 624
 Calcium
 Chloride Hydrate, 390
 Chloride Injection, 390
 Folinate, 391
 Gluconate Hydrate, 391
 Hydroxide, 392
 Lactate Hydrate, 393
 Leucovorin, 391
 Oxide, 393
 Pantothenate, 394
 Paraaminosalicylate Granules, 394
 Paraaminosalicylate Hydrate, 395
 Polystyrene Sulfonate, 398
 Stearate, 400
 Calumba, 1267
 Camellia Oil, 400
 Camostat Mesilate, 400
d-Camphor, 401
dl-Camphor, 402
 Capsicum, 1268
 and Salicylic Acid Spirit, 1270
 Tincture, 1269
 Capsules, 403
 Captopril, 403
 Carbamazepine, 404
 Carbazochrome Sodium Sulfonate
 Hydrate, 405
 Carbetapentane Citrate, 974
 Carbetapentene Citrate, 974
 Carbidopa Hydrate, 406
 Carbolic Acid, 985
 for Disinfection, 986
 L-Carbocysteine, 407
 Carbon Dioxide, 407
 Carboxymethylcellulose, 408
 Calcium, 409
 Sodium, 410
 Cardamon, 1271
 Carmellose, 408
 Calcium, 409
 Sodium, 410
 Carmofur, 411
 Carnauba Wax, 411
 Carteolol Hydrochloride, 412
 Carumonam Sodium, 412
 Cassia Seed, 1271
 Castor Oil, 414
 Catalpa Fruit, 1271
 Caffeine
 and Sodium Benzoate, 388
 Hydrate, 387
 Cefaclor, 415
 Capsules, 416
 Compound Granules, 419
 Fine Granules, 417
 Cefadroxil, 421
 Cefalexin, 422
 Cefalotin Sodium, 423
 Cefapirin Sodium, 424
 Cefatrizine Propylene Glycolate, 425
 Cefazolin Sodium, 426
 Hydrate, 428
 Cefbuperazone Sodium, 429
 Cefcapene Pivoxil Hydrochloride
 Fine Granules, 432
 Hydrate, 430
 Tablets, 433
 Cefdinir, 434
 Capsules, 435
 Fine Granules, 436
 Cefditoren Pivoxil, 437
 Fine Granules, 438
 Tablets, 438
 Cefepime Dihydrochloride
 for Injection, 441
 Hydrate, 439
 Cefmenoxime Hydrochloride, 443
 Cefmetazole Sodium, 445
 Cefminox Sodium Hydrate, 446
 Cefodizime Sodium, 447
 Cefoperazone Sodium, 448
 Cefotaxime Sodium, 449
 Cefotetan, 451
 Cefotiam
 Hexetil Hydrochloride, 453
 Hydrochloride, 455
 Hydrochloride for Injection, 456
 Cefozopran Hydrochloride, 457
 for Injection, 458
 Cefpiramide Sodium, 458
 Cefpirome Sulfate, 460
 Cefpodoxime Proxetil, 461
 Cefroxadine Hydrate, 462
 Cefsulodin Sodium, 464
 Ceftazidime Hydrate, 466
 Ceferam Pivoxil, 468
 Fine Granules, 469
 Ceftibuten Hydrate, 470
 Ceftizoxime Sodium, 471
 Ceftriaxone Sodium Hydrate, 472
 Cefuroxime
 Axetil, 474
 Sodium, 476
 Cellacelate, 480
 Celmoleukin (Genetical Recombination), 481
 Cetanol, 484
 Cetraxate Hydrochloride, 485
 Cefixime, 442
 Chenodeoxycholic Acid, 486
 Chloral Hydrate, 487
 Chloramphenicol, 487
 Palmitate, 488

- Sodium Succinate, 489
 Chlordiazepoxide, 490
 Powder, 491
 Tablets, 492
 Chlorhexidine
 Gluconate Solution, 493
 Hydrochloride, 493
 Chlorinated Lime, 494
 Chlorladinone Acetate, 494
 Chlorobutanol, 495
 Chlorphenesin Carbamate, 496
 Chlorpheniramine
 and Calcium Powder, 497
 Maleate, 498
 Maleate Injection, 499
 Maleate Powder, 499
 Maleate Tablets, 500
d-Chlorpheniramine Maleate, 501
 Chlorpromazine Hydrochloride, 502
 Injection, 503
 Tablets, 503
 Chlorpropamide, 504
 Tablets, 505
 Cholecalciferol, 506
 Cholera Vaccine, 506
 Cholesterol, 507
 Chorionic Gonadotrophin, 707
 for Injection, 708
 Chrysanthemum Flower, 1271
 Ciclacillin, 507
 Ciclosporin, 509
 A, 509
 Cilastatin Sodium, 510
 Cilostazol, 511
 Tablets, 512
 Cimetidine, 513
 Cimicifuga Rhizome, 1272
 Cinchocaine Hydrochloride, 569
 Cinnamon
 Bark, 1272
 Oil, 1273
 Cisplatin, 513
 Citric Acid Hydrate, 515
 Citrus Unshiu Peel, 1273
 Clarithromycin, 516
 Tablets, 517
 Clemastine Fumarate, 519
 Clematis Root, 1274
 Clindamycin Hydrochloride, 519
 Capsules, 520
 Clindamycin Phosphate, 521
 Clinofibrate, 522
 Clocapramine Hydrochloride Hydrate, 523
 Clofedanol Hydrochloride, 524
 Clomifene Citrate, 526
 Tablets, 527
 Clomipramine Hydrochloride, 528
 Clonazepam, 528
 Clonidine Hydrochloride, 529
 Cloperastine Hydrochloride, 530
 Clotiazepam, 531
 Clotrimazole, 532
 Clove, 1274
 Oil, 1275
 Cloxacillin Sodium Hydrate, 533
 Cloxazolam, 534
 Clofibrate, 525
 Capsules, 526
 CMC, 408
 Calcium, 409
 Sodium, 410
 Cnidium
 Monnieri Fruit, 1275
 Rhizome, 1275
 Cocaine Hydrochloride, 535
 Coconut Oil, 535
 Codeine Phosphate
 Hydrate, 536
 Tablets, 537
 1% Codeine Phosphate Powder, 536
 10% Codeine Phosphate Powder, 537
 Cod Liver Oil, 538
 Coix Seed, 1276
 Colchicine, 538
 Colistin
 Sodium Methanesulfonate, 540
 Sulfate, 541
 Colophonium, 1347
 Compound
 Acrinol and Zinc Oxide Oil, 273
 Diastase and Sodium Bicarbonate Powder, 567
 Hycodone Injection, 952
 Iodine Glycerin, 770
 Methyl Salicylate Spirit, 879
 Oxycodone and Atropine Injection, 953
 Oxycodone Injection, 952
 Phellodendron Powder for Cataplasm, 1332
 Rhubarb and Senna Powder, 1346
 Salicylic Acid Spirit, 1080
 Scopolia Extract and Diastase Powder, 1355
 Thianthol and Salicylic Acid Solution, 1165
 Vitamin B Powder, 1230
 Concentrated
 Glycerin, 703
 Glycerol, 703
 Condurango, 1276
 Fluidextract, 1277
 Coptis Rhizome, 1277
 Corn
 Oil, 542
 Starch, 542
 Cornus Fruit, 1279
 Cortisone Acetate, 543
 Corydalis Tuber, 1279
 Creosote, 544
 Cresol, 544
 Solution, 545
 Croconazole Hydrochloride, 546
 Croscarmellose Sodium, 546
 Crude Glycyrrhiza Extract, 1297
 Crystalline Insulin Zinc Injection (Aqueous Suspension), 765
 Crystal Violet, 881
 Cyanamide, 548
 Cyanocobalamin, 548
 Injection, 549
 Cyclopentolate Hydrochloride, 549
 Cyclophosphamide Hydrate, 550
 Cycloserine, 551
 Cyperus Rhizome, 1280
 Cyproheptadine Hydrochloride Hydrate, 551
 Cytarabine, 552
- ## D
- Daiokanzoto Extract, 1280
 Dantrolene Sodium Hydrate, 553
 Daunorubicin Hydrochloride, 553
 Deferoxamine Mesilate, 554
 Dehydrated Alcohol, 639
 Dehydrocholate Sodium Injection, 557
 Dehydrocholic Acid, 556
 Injection, 557
 Demethylchlortetracycline Hydrochloride, 558
 Dental
 Antiformin, 310
 Iodine Glycerin, 771
 Paraformaldehyde Paste, 969
 Phenol with Camphor, 987
 Sodium Hypochlorite Solution, 310
 Triozinc Paste, 1210
 Dermatol, 368
 Deslanoside, 559
 Injection, 560
 Dexamethasone, 560
 Dextran
 40, 561
 40 Injection, 562
 70, 562
 Sulfate Sodium Sulfur 18, 565
 Sulfate Sodium Sulfur 5, 564
 Dextrin, 565
 Dextromethorphan Hydrobromide Hydrate, 566
 Diagnostic Sodium Citrate Solution, 1100
 Diastase, 567
 and Sodium Bicarbonate Powder, 567
 Diazepam, 567
 Dibasic
 Calcium Phosphate Hydrate, 396
 Sodium Phosphate Hydrate, 568
 Dibekacin Sulfate, 569
 Dibucaine Hydrochloride, 569
 Dichlorphenamide, 571
 Tablets, 572
 Diclofenac Sodium, 570
 Diclofenamide, 571
 Tablets, 572

- Dicloxacillin Sodium Hydrate, 573
 Diethylcarbamazine Citrate, 574
 Tablets, 574
 Diethylstilbestrol Diphosphate, 683
 Tablets, 684
 Difenidol Hydrochloride, 575
 Digenea, 1280
 Digitoxin, 576
 Tablets, 576
 Digoxin, 577
 Injection, 578
 Tablets, 580
 Dihydrocodeine Phosphate, 581
 1% Dihydrocodeine Phosphate Powder, 582
 10% Dihydrocodeine Phosphate Powder, 582
 Dihydroergotamine Mesilate, 583
 Dihydroergotamine Mesilate, 584
 Dilazep Hydrochloride Hydrate, 586
 Diltiazem Hydrochloride, 586
 Dilute
 Hydrochloric Acid, 726
 Iodine Tincture, 769
 Diluted Opium Powder, 940
 Dimemorfan Phosphate, 588
 Dimenhydrinate, 588
 Tablets, 589
 Dimercaprol, 590
 Injection, 590
 Dimorpholamine, 591
 Injection, 591
 Dinoprost, 592
 Dionin, 648
 Dioscorea Rhizome, 1282
 Diphenhydramine, 593
 and Bromovalerylurea Powder, 593
 Hydrochloride, 594
 Tannate, 595
 Phenol and Zinc Oxide Liniment, 595
 Diphenylhydantoin, 991
 Powder, 992
 Sodium for Injection, 992
 Tablets, 992
 Diphtheria
 Toxoid, 596
 Tetanus Combined Toxoid, 596
 Dipyridamole, 597
 Disodium Edetate Hydrate, 598
 Disopyramide, 598
 Distigmine Bromide, 599
 Tablets, 600
 Disulfiram, 600
 Dobutamine Hydrochloride, 601
 Dolichos Seed, 1282
 Dopamine Hydrochloride, 602
 Injection, 602
 Dover's Powder, 1324
 Doxapram Hydrochloride Hydrate, 603
 Doxifluridine, 604
 Capsules, 604
 Doxorubicin Hydrochloride, 605
 Doxycycline Hydrochloride Hydrate, 606
 Dried
 Aluminum Hydroxide Gel, 288
 Aluminum Hydroxide Gel Fine Granules, 289
 Aluminum Potassium Sulfate, 292
 Sodium Carbonate, 1096
 Sodium Sulfite, 1109
 Thyroid, 1171
 Yeast, 1241
 Droperidol, 608
 Dydrogesterone, 609
 Tablets, 610
- E**
- Ecarazine Hydrochloride, 1185
 Ecothiopate Iodide, 610
 Edrophonium Chloride, 611
 Injection, 612
 EDTA Sodium Hydrate, 598
 Elcatonin, 612
 Eleutherococcus Senticosus Rhizome, 1283
 Enoxacin Hydrate, 616
 Enviomycin Sulfate, 616
 Enflurane, 615
 Eperisone Hydrochloride, 618
 Ephedra Herb, 1283
 Ephedrine Hydrochloride, 619
 Injection, 619
 Powder, 620
 Tablets, 621
 10% Ephedrine Hydrochloride Powder, 620
 Epimedium Herb, 1284
 Epinephrine, 276
 Injection, 276
 Solution, 277
 Epirizole, 621
 Epirubicin Hydrochloride, 622
 Ergocalciferol, 624
 Ergometrine Maleate, 625
 Injection, 625
 Tablets, 626
 Ergotamine Tartrate, 627
 Erythromycin, 627
 Ethylsuccinate, 629
 Lactobionate, 629
 Stearate, 630
 Estazolam, 631
 Estradiol Benzoate, 631
 Injection, 632
 Injection (Aqueous Suspension), 633
 Estriol, 633
 Injection (Aqueous Suspension), 634
 Tablets, 635
 Etacrynic Acid, 636
 Tablets, 636
 Ethacridine Lactate, 274
 Ethambutol Hydrochloride, 637
 Ethanol, 638
 for Disinfection, 640
 Ethenzamide, 640
 Ether, 641
 Ethinylestradiol, 642
 Tablets, 642
 Ethionamide, 643
 Ethosuximide, 644
 Ethoxybenzamide, 640
 Ethyl
 Aminobenzoate, 644
 Cysteine Hydrochloride, 645
 L-Cysteine Hydrochloride, 645
 Icosapentate, 646
 Parahydroxybenzoate, 647
 Ethylenediamine, 648
 Ethylmorphine Hydrochloride Hydrate, 648
 Etidronate Disodium, 649
 Tablets, 650
 Etilefrine Hydrochloride, 650
 Tablets, 651
 Etizolam, 652
 Etodolac, 653
 Etoposide, 653
 Eucalyptus Oil, 654
 Eucommia Bark, 1284
 Evodia Fruit, 1285
 Exsiccated Gypsum, 1298
- F**
- Famotidine, 655
 for Injection, 656
 Powder, 657
 Tablets, 657
 Faropenem Sodium
 for Syrup, 659
 Hydrate, 658
 Tablets, 660
 Fenbufen, 660
 Fennel, 1285
 Oil, 1286
 Fentanyl Citrate, 661
 Ferrous Sulfate Hydrate, 661
 Flavin Adenine Dinucleotide Sodium, 662
 Flavoxate Hydrochloride, 664
 Flomoxef Sodium, 664
 for Injection, 666
 Flopropione, 667
 Capsules, 667
 Flucytosine, 668
 Fludiazepam, 669
 Flunitrazepam, 670
 Fluocinolone Acetonide, 670
 Fluocinonide, 672
 Fluorescein Sodium, 673
 Fluorometholone, 673
 Fluorouracil, 674
 Fluoxymesterone, 675

- Fluphenazine Enanthate, 676
 Flurazepam, 677
 Capsules, 678
 Hydrochloride, 678
 Flurbiprofen, 679
 Foeniculated Ammonia Spirit, 1286
 Folic Acid, 680
 Injection, 681
 Tablets, 681
 Formalin, 681
 Water, 682
 Formoterol Fumarate Hydrate, 682
 Forsythia Fruit, 1286
 Fosfestrol, 683
 Tablets, 684
 Fosfomycin
 Calcium Hydrate, 685
 Sodium, 686
 Sodium for Injection, 687
 Fradiomycin Sulfate, 688
 Freeze-dried
 BCG Vaccine (for Percutaneous Use), 335
 Botulism Antitoxin, Equine, 374
 Diphtheria Antitoxin, Equine, 596
 Habu Antivenom, Equine, 716
 Inactivated Tissue Culture Rabies Vaccine, 1054
 Japanese Encephalitis Vaccine, 786
 Live Attenuated Measles Vaccine, 843
 Live Attenuated Mumps Vaccine, 902
 Live Attenuated Rubella Vaccine, 1074
 Mamushi Antivenom, Equine, 841
 Smallpox Vaccine, 1091
 Smallpox Vaccine Prepared in Cell Culture, 1091
 Tetanus Antitoxin, Equine, 1156
 Fritillaria Bulb, 1287
 Fructose, 689
 Injection, 689
 Furosemide, 690
 Tablets, 691
 Fursultiamine Hydrochloride, 692
- G**
- Gabexate Mesilate, 693
 β -Galactosidase
 (Aspergillus), 694
 (Penicillium), 695
 Gallium (⁶⁷Ga) Citrate Injection, 696
 Gambir, 1287
 Gardenia Fruit, 1288
 Gas Gangrene Antitoxin, Equine, 696
 Gastrodia Tuber, 1289
 Gelatin, 696
 Gentamicin Sulfate, 698
 Gentian, 1290
 and Sodium Bicarbonate Powder, 1290
- Geranium Herb, 1291
 Ginger, 1291
 Ginseng, 1292
 Glacial Acetic Acid, 269
 Glehnia Root, 1294
 Glibenclamide, 699
 Glucose, 700
 Injection, 701
 Glutathione, 701
 Glycerin, 702
 and Potash Solution, 704
 Glycerol, 702
 Glyceryl Monostearate, 704
 Glycine, 704
 Glycyrrhiza, 1295
 Extract, 1296
 Gonadorelin Acetate, 705
 Gramicidin, 712
 Griseofulvin, 713
 Guaiacol Glyceryl Ether, 714
 Guaifenesin, 714
 Guanabenz Acetate, 715
 Guanethidine Sulfate, 716
 Gypsum, 1297
- H**
- Haloperidol, 717
 Tablets, 718
 Halothane, 719
 Haloxazolam, 719
 Hemp Fruit, 1298
 Heparin Sodium, 721
 Injection, 722
 Hochuekkito Extract, 1298
 Homatropine Hydrobromide, 722
 Homochlorcyclizine Hydrochloride, 723
 Honey, 1301
 Houttuynia Herb, 1302
 Human
 Chorionic Gonadotrophin, 707
 Chorionic Gonadotrophin for Injection, 708
 Menopausal Gonadotrophin, 708
 Normal Immunoglobulin, 724
 Hycoato Injection, 953
 Hydralazine Hydrochloride, 724
 for Injection, 724
 Powder, 725
 Tablets, 725
 Hydrochloric Acid, 725
 Lemonade, 727
 Hydrochlorothiazide, 727
 Hydrocortisone, 728
 Acetate, 729
 and Diphenhydramine Ointment, 730
 Butyrate, 730
 Sodium Phosphate, 731
 Sodium Succinate, 732
 Succinate, 733
 Hydrocotarnine Hydrochloride
 Hydrate, 734
 Hydrogenated Oil, 735
 Hydrophilic
 Ointment, 735
 Petrolatum, 981
 Hydrous Lanolin, 807
 Hydroxocobalamin Acetate, 736
 Hydroxypropylcellulose, 736
 Hydroxypropylmethylcellulose, 741
 Hydroxyzine
 Hydrochloride, 739
 Pamoate, 739
 Hymecromone, 740
 Hypromellose, 741
 Phthalate, 743
- I**
- Ibuprofen, 744
 Ichthammol, 744
 Idarubicin Hydrochloride, 745
 for Injection, 746
 Idoxuridine, 747
 Ophthalmic Solution, 747
 Ifenprodil Tartrate, 748
 Imipenem
 and Cilastatin for Injection, 750
 Hydrate, 749
 Hydrochloride, 751
 Imipramine Hydrochloride, 751
 Tablets, 752
 Immature Orange, 1302
 Imperata Rhizome, 1302
 Indenolol Hydrochloride, 753
 Indigocarmine, 754
 Injection, 755
 Indium (¹¹¹In) Chloride Injection, 755
 Indometacin, 755
 Capsules, 756
 Suppositories, 757
 Insulin, 758
 Human (Genetical Recombination), 760
 Injection, 762
 Zinc Injection (Aqueous Suspension), 763
 Zinc Protamine Injection (Aqueous Suspension), 766
 Influenza HA Vaccine, 758
 Iodamide, 767
 Iodinated (¹³¹I) Human Serum Albumin Injection, 768
 Iodine, 768
 Salicylic Acid and Phenol Spirit, 772
 Tincture, 769
 Iodoform, 773
 Iopamidol, 773
 Iotalamic Acid, 774
 Iotroxic Acid, 775
 Ipecac, 1303
 Syrup, 1304
 Ipratropium Bromide Hydrate, 776

Iproveratril Hydrochloride, 1225
 Isepanicin Sulfate, 777
 L-Isoleucine, 780
 Isoniazid, 780
 Injection, 781
 Tablets, 781
 Isophane Insulin Injection (Aqueous Suspension), 762
l-Isoprenaline Hydrochloride, 782
 Isopropanol, 783
 Isopropyl Alcohol, 783
 Isopropylantipyrine, 783
 Isosorbide, 784
 Dinitrate, 785
 Dinitrate Tablets, 786
 Isotonic
 Salt Solution, 1098
 Sodium Chloride Injection, 1098
 Sodium Chloride Solution, 1098
 Isoflurane, 778

J

Japanese
 Angelica Root, 1305
 Encephalitis Vaccine, 786
 Gentian, 1306
 Valerian, 1306
 Josamycin, 786
 Propionate, 788
 Jujube, 1307
 Seed, 1307

K

Kainic Acid
 and Santonin Powder, 790
 Hydrate, 789
 Kakkonto Extract, 1308
 Kallidinogenase, 790
 Kamishoyosan Extract, 1309
 Kanamycin
 Monosulfate, 793
 Sulfate, 794
 Kaolin, 795
 Ketamine Hydrochloride, 795
 Ketoprofen, 796
 Ketotifen Fumarate, 797
 Kitasamycin, 798
 Acetate, 799
 Tartrate, 800

L

Lactic Acid, 801
 Lactose, 803
 Hydrate, 803
 Lactulose, 804
 Lanatoside C, 805
 Tablets, 806
 Lard, 808
 Latamoxef Sodium, 809
 Lauromacrogol, 810

Lenampicillin Hydrochloride, 810
 L-Leucine, 812
 Leucomycin, 798
 Acetate, 799
 Tartrate, 800
 Levallorphan Tartrate, 813
 Injection, 814
 Levodopa, 814
 Levomepromazine Maleate, 815
 Levothyroxine Sodium
 Hydrate, 816
 Tablets, 817
 Lidocaine, 818
 Hydrochloride Injection, 818
 Injection, 818
 Light
 Anhydrous Silicic Acid, 1087
 Liquid Paraffin, 967
 Limaprost Alfadex, 819
 Lincomycin Hydrochloride Hydrate, 820
 Lindera Root, 1313
 Liothyronine Sodium, 821
 Tablets, 822
 Liquefied
 Carbolic Acid, 986
 Phenol, 986
 Liquid Paraffin, 966
 Lisinopril
 Hydrate, 823
 Tablets, 824
 Lithium Carbonate, 826
 Lithospermum Root, 1313
 Live Oral Poliomyelitis Vaccine, 1005
 Longgu, 1313
 Loncera Leaf and Stem, 1314
 Loquat Leaf, 1314
 Lorazepam, 827
 Low Substituted Hydroxypropylcellulose, 738
 Loxoprofen Sodium Hydrate, 828
 Lycium
 Bark, 1315
 Fruit, 1315
 Lysine Hydrochloride, 829
 L-Lysine Hydrochloride, 829
 Lysozyme Hydrochloride, 830

M

Macrogol
 400, 830
 1500, 831
 4000, 832
 6000, 832
 20000, 833
 Ointment, 833
 Magnesium
 Carbonate, 834
 Oxide, 835
 Silicate, 836
 Stearate, 837
 Sulfate Hydrate, 838
 Sulfate Injection, 839
 Sulfate Mixture, 839
 Magnolia
 Bark, 1315
 Flower, 1317
 Mallotus Bark, 1317
 Maltose Hydrate, 840
 D-Mannite Injection, 842
 D-Mannitol, 841
 Injection, 842
 Maprotiline Hydrochloride, 842
 Meclofenoxate Hydrochloride, 843
 Mecobalamin, 844
 Medazepam, 845
 Medicinal
 Carbon, 845
 Soap, 846
 Mefenamic Acid, 847
 Mefruside, 849
 Tablets, 849
 Meglumine, 850
 Amidotrizoate Injection, 851
 Iotalamate Injection, 852
 Sodium Amidotrizoate Injection, 853
 Sodium Iodamide Injection, 854
 Melphalan, 855
 Menatetrenone, 855
 Mentha
 Herb, 1317
 Oil, 1318
 Water, 1318
dl-Menthol, 856
l-Menthol, 857
 Mepenzolate Bromide, 857
 Mepirizole, 621
 Mepitostane, 858
 Mepivacaine Hydrochloride, 859
 Injection, 860
 Mequitazine, 861
 Merbromin, 862
 Solution, 863
 Mercaptopurine Hydrate, 861
 Mercurochrome, 862
 Solution, 863
 Meropenem Hydrate, 863
 Mestranol, 864
 Metenolone
 Acetate, 865
 Enanthate, 866
 Enanthate Injection, 866
 Metformin Hydrochloride, 867
 Tablets, 867
 Methamphetamine Hydrochloride, 868
 L-Methionine, 869
 Methotrexate, 869
 Methoxsalen, 870
 Methylbenactyzium Bromide, 871
 Methylcellulose, 871
 Methylidopa
 Hydrate, 873
 Tablets, 874

dl-Methylephedrine Hydrochloride, 875
 Powder, 876
 10% *dl*-Methylephedrine Hydrochloride Powder, 876
 Methylergometrine Maleate, 876
 Tablets, 877
 Methyl
 Parahydroxybenzoate, 878
 Salicylate, 881
 Methylprednisolone, 879
 Succinate, 880
 Methylrosanilinium Chloride, 881
 Methyltestosterone, 882
 Tablets, 883
 Meticrane, 884
 Metildigoxin, 885
 Metoclopramide, 886
 Tablets, 886
 Metoprolol Tartrate, 887
 Tablets, 888
 Metronidazole, 889
 Tablets, 889
 Metyrapone, 890
 Mexiletine Hydrochloride, 891
 Mefloquine Hydrochloride, 848
 Miconazole, 892
 Nitrate, 893
 Microcrystalline Cellulose, 477
 Micronomicin Sulfate, 893
 Midecamycin, 894
 Acetate, 895
 Migrenin, 896
 Minocycline Hydrochloride, 897
 Mitomycin C, 898
 Monobasic Calcium Phosphate Hydrate, 397
 Monosodium Trichloroethyl Phosphate, 1202
 Syrup, 1203
 Morphine
 and Atropine Injection, 899
 Hydrochloride Hydrate, 900
 Hydrochloride Injection, 901
 Hydrochloride Tablets, 902
 Moutan Bark, 1318
 Mulberry Bark, 1320
 Mupirocin Calcium Hydrate, 902

N

Nadolol, 904
 Nalidixic Acid, 905
 Naloxone Hydrochloride, 906
 Naphazoline
 and Chlorpheniramine Solution, 906
 Hydrochloride, 907
 Nitrate, 908
 Naproxen, 908
 Narcotine, 936
 Hydrochloride, 937
 Natamycin, 994

Natural Aluminum Silicate, 289
 Nelumbo Seed, 1320
 Neomycin Sulfate, 688
 Neostigmine Methylsulfate, 909
 Injection, 910
 Netilmicin Sulfate, 910
 Nicardipine Hydrochloride, 911
 Injection, 912
 Nicergoline, 913
 Powder, 914
 Tablets, 915
 Niceritrol, 916
 Nicomol, 917
 Tablets, 918
 Nicorandil, 919
 Nicotinamide, 919
 Nicotinic Acid, 920
 Injection, 921
 Nifedipine, 922
 Nilvadipine, 923
 Tablets, 924
 Nitrazepam, 925
 Nitrendipine, 926
 Tablets, 927
 Nitrogen, 928
 Nitroglycerin Tablets, 928
 Nitrous Oxide, 929
 Noradrenaline, 930
 Hydrochloride Injection, 931
 Noradrenaline Injection, 931
 Norepinephrine, 930
 Hydrochloride Injection, 931
 Injection, 931
 Norethisterone, 932
 Norgestrel, 933
 and Ethinylestradiol Tablets, 934
 Nortriptyline Hydrochloride, 935
 Norfloxacin, 932
 Noscipine, 936
 Hydrochloride Hydrate, 937
 Notopterygium Rhizome, 1321
 Nuphar Rhizome, 1321
 Nux Vomica, 1321
 Extract, 1322
 Extract Powder, 1323
 Tincture, 1323
 Nystatin, 937

O

Olive Oil, 939
 Operidine, 980
 Injection, 981
 Ophiopogon Tuber, 1324
 Opium
 Ipecac Powder, 1324
 Tincture, 940
 Opium Alkaloids
 and Atropine Injection, 942
 and Scopolamine Injection, 943
 Hydrochlorides, 941
 Hydrochlorides Injection, 942
 Orange

Oil, 946
 Peel Syrup, 1325
 Peel Tincture, 1325
 Orciprenaline Sulfate, 946
 Oriental Bezoar, 1325
 Oxapium Iodide, 947
 Oxaprozin, 948
 Oxazolam, 948
 Oxetacaine, 949
 Oxethazaine, 949
 Oxprenolol Hydrochloride, 950
 Oxybuprocaine Hydrochloride, 951
 Oxycodone Hydrochloride Hydrate, 951
 Oxydol, 954
 Oxygen, 955
 Oxymetholone, 956
 Oxytetracycline Hydrochloride, 956
 Oxytocin, 958
 Injection, 960
 Oyster Shell, 1326
 Ofloxacin, 938

P

Panax Japonicus Rhizome, 1326
 Pancreatin, 961
 Pancuronium Bromide, 962
 Panipenem, 962
 Pantethine, 964
 Papaverine Hydrochloride, 965
 Injection, 965
 Paracetamol, 267
 Paraformaldehyde, 968
 Paraffin, 966
 Parnaparin Sodium, 969
 Pas-calcium
 Granules, 394
 Hydrate, 395
 Peach Kernel, 1327
 Peanut Oil, 971
 Penbutolol Sulfate, 972
 Penicillin G Potassium, 349
 Pentazocine, 972
 Pentobarbital Calcium, 973
 Pentoxyverine Citrate, 974
 Peony Root, 1328
 Peplomycin Sulfate, 975
 Perilla Herb, 1329
 Perphenazine, 977
 Maleate, 978
 Maleate Tablets, 979
 Tablets, 977
 Pethidine Hydrochloride, 980
 Injection, 981
 Petroleum Benzoin, 982
 Pharbitis Seed, 1330
 Phellodendron,
 Albumin Tannate and Bismuth Subnitrate Powder, 1332
 Bark, 1330
 Phenazone, 310
 Phenethicillin Potassium, 983

- Phenobarbital, 984
Powder, 985
10% Phenobarbital Powder, 985
Phenol, 985
and Zinc Oxide Liniment, 987
for Disinfection, 987
Phenolated Water, 987
for Disinfection, 986
Phenolsulfonphthalein, 988
Injection, 988
L-Phenylalanine, 989
Phenylbutazone, 990
Phenylephrine Hydrochloride, 990
Phenytoin, 991
Powder, 992
Sodium for Injection, 992
Tablets, 992
Phytomenadione, 993
Phytonadione, 993
Picrasma Wood, 1333
Pilocarpine Hydrochloride, 994
Pimaricin, 994
Pindolol, 995
Pinellia Tuber, 1333
Pipemidic Acid Hydrate, 996
Piperacillin Sodium, 997
for Injection, 998
Piperazine
Adipate, 999
Phosphate Hydrate, 999
Phosphate Tablets, 1000
Pirarubicin, 1000
Pirenoxine, 1001
Pirenzepine Hydrochloride Hydrate, 1002
Piroxicam, 1003
Pivmecillinam Hydrochloride, 1004
Plantago
Herb, 1334
Seed, 1334
Platycodon
Fluidextract, 1334
Root, 1334
Polyethylene Glycol
400, 830
1500, 831
4000, 832
6000, 832
20000, 833
Ointment, 833
Polygala Root, 1335
Polygonatum Rhizome, 1336
Polygonum Root, 1336
Polymixin B Sulfate, 1006
Polyoxyethylene Lauryl Alcohol Ether, 810
Polyoxyl 40 Stearate, 1007
Polyporus Sclerotium, 1336
Polysorbate 80, 1007
Polyvidone, 1015
Polyvinylpyrrolidone, 1015
Poria Sclerotium, 1337
Potash Soap, 1007
Potassium
Bromide, 1008
Canrenoate, 1008
Carbonate, 1009
Chloride, 1009
Clavulanate, 1010
Guaiacolsulfonate, 1011
Hydroxide, 1012
Iodide, 1013
Permanganate, 1013
Sulfate, 1014
Potato Starch, 1014
Povidone, 1015
Iodine, 1017
Powdered
Acacia, 1251
Agar, 1253
Alisma Rhizome, 1253
Aloe, 1255
Amomum Seed, 1256
Atractylodes Lancea Rhizome, 1261
Atractylodes Rhizome, 1260
Calumba, 1268
Capsicum, 1269
Cellulose, 480
Cinnamon Bark, 1273
Clove, 1274
Cnidium Rhizome, 1276
Coix Seed, 1276
Coptis Rhizome, 1278
Cyperus Rhizome, 1280
Dioscorea Rhizome, 1282
Fennel, 1285
Gambir, 1287
Gardenia Fruit, 1288
Gentian, 1290
Geranium Herb, 1291
Ginger, 1292
Ginseng, 1293
Glycyrrhiza, 1296
Ipecac, 1303
Japanese Angelica Root, 1305
Japanese Gentian, 1306
Japanese Valerian, 1307
Magnolia Bark, 1316
Moutan Bark, 1319
Opium, 939
Oyster Shell, 1326
Panax Japonicus Rhizome, 1327
Peach Kernel, 1327
Peony Root, 1329
Phellodendron Bark, 1331
Picrasma Wood, 1333
Platycodon Root, 1335
Polygala Root, 1335
Polyporus Sclerotium, 1337
Poria Sclerotium, 1337
Processed Aconite Root, 1339
Rhubarb, 1345
Rose Fruit, 1346
Scutellaria Root, 1359
Senega, 1361
Senna Leaf, 1362
Smilax Rhizome, 1364
Sophora Root, 1364
Sweet Hydrangea Leaf, 1365
Swertia Herb, 1366
Tragacanth, 1369
Zanthoxylum Fruit, 1371
Pranoprofen, 1018
Pravastatin Sodium, 1018
Prazepam, 1020
Tablets, 1021
Precipitated Calcium Carbonate, 389
Prednisolone, 1021
Acetate, 1023
Sodium Succinate for Injection, 1025
Succinate, 1024
Tablets, 1022
Primidone, 1026
Probenecid, 1027
Tablets, 1027
Procainamide Hydrochloride, 1028
Injection, 1029
Tablets, 1029
Procaine Hydrochloride, 1030
Injection, 1030
Procarbazine Hydrochloride, 1031
Procaterol Hydrochloride Hydrate, 1032
Prochlorperazine Maleate, 1033
Tablets, 1033
Processed
Aconite Root, 1338
Ginger, 1341
Progesterone, 1034
Injection, 1034
Proglumide, 1035
Promethazine Hydrochloride, 1036
Propantheline Bromide, 1036
Propranolol Hydrochloride, 1037
Tablets, 1038
Propylene Glycol, 1039
Propyl Parahydroxybenzoate, 1039
Propylthiouracil, 1040
Tablets, 1040
Propyphenazone, 783
Prostaglandin
E₁, 286
E₁ α-Cyclodextrin Clathrate Compound, 287
F_{2α}, 592
Protamine Sulfate, 1041
Injection, 1042
Prothionamide, 1042
Protirelin, 1043
Tartrate Hydrate, 1044
Prunella Spike, 1341
Pueraria Root, 1341
Pullulan, 1045
Purified
Dehydrocholic Acid, 556
Gelatin, 697
Lanolin, 808

Shellac, 1085
 Water, 1236
 Pyrantel Pamoate, 1045
 Pyrazinamide, 1046
 Pyridostigmine Bromide, 1047
 Pyridoxine Hydrochloride, 1047
 Injection, 1048
 Pyroxylin, 1049
 Pyrrolnitrin, 1049

Q

Quick Lime, 393
 Quinidine Sulfate Hydrate, 1050
 Quinine
 Ethyl Carbonate, 1051
 Hydrochloride Hydrate, 1052
 Sulfate Hydrate, 1053

R

Ranitidine Hydrochloride, 1054
 Rape Seed Oil, 1055
 Red Ginseng, 1342
 Rehmannia Root, 1343
 Reserpine, 1055
 Injection, 1056
 Powder, 1057
 Tablets, 1057
 0.1% Reserpine Powder, 1057
 Retinol
 Acetate, 1058
 Palmitate, 1059
 Rhubarb, 1344
 Ribostamycin Sulfate, 1063
 Riboflavin, 1059
 Butyrate, 1060
 Phosphate, 1061
 Phosphate Injection, 1062
 Powder, 1060
 Sodium Phosphate, 1061
 Sodium Phosphate Injection, 1062
 Rice Starch, 1346
 Rifampicin, 1064
 Capsules, 1065
 Ringer's Solution, 1066
 Ritodrine Hydrochloride, 1067
 Tablets, 1068
 Rokitamycin, 1069
 Rose Fruit, 1346
 Rosin, 1347
 Roxatidine Acetate Hydrochloride, 1070
 Extended-release Capsules, 1071
 Roxithromycin, 1073
 Ryokeijutsukanto Extract, 1347

S

Saccharated Pepsin, 1074
 Saccharin, 1075
 Sodium, 1076
 Sodium Hydrate, 1076

Saireito Extract, 1349
 Salazosulfapyridine, 1077
 Salbutamol Sulfate, 1078
 Salicylated Alum Powder, 1078
 Salicylic Acid, 1079
 Adhesive Plaster, 1080
 Spirit, 1080
 Santonin, 1081
 Saponated Cresol Solution, 545
 Saposhnikovia Root, 1352
 Sappan Wood, 1352
 Saussurea Root, 1352
 Saffron, 1349
 Safflower, 1348
 Schisandra Fruit, 1352
 Schizonepeta Spike, 1353
 Scopolamine
 Butylbromide, 1082
 Hydrobromide Hydrate, 1083
 Scopolia Extract, 1353
 Papaverine and Ethyl Aminobenzoate Powder, 1356
 and Carbon Powder, 1355
 and Ethyl Aminobenzoate Powder, 1355
 and Tannic Acid Suppositories, 1357
 Powder, 1353
 Rhizome, 1357
 Scutellaria Root, 1358
 Senega, 1360
 Syrup, 1361
 Senna Leaf, 1361
 Serrapeptase, 1083
 Serum Gonadotrophin, 710
 for Injection, 711
 Sesame Oil, 1084
 Siccanin, 1086
 Silver
 Nitrate, 1088
 Nitrate Ophthalmic Solution, 1088
 Protein, 1088
 Protein Solution, 1089
 Simple
 Ointment, 1089
 Syrup, 1089
 Sinomenium Stem, 1363
 Sisomicin Sulfate, 1090
 Slaked Lime, 392
 Smilax Rhizome, 1364
 Sodium
 Acetate Hydrate, 1091
 Aurothiomalate, 1092
 Benzoate, 1093
 Bicarbonate, 1093
 Bicarbonate and Bitter Tincture Mixture, 1364
 Bicarbonate Injection, 1094
 Bisulfite, 1094
 Borate, 1095
 Bromide, 1095
 Carbonate Hydrate, 1096
 Chloride, 1097
 Chromate (⁵¹Cr) Injection, 1099
 Citrate Hydrate, 1099
 Citrate Injection for Transfusion, 1099
 Cromoglicate, 1100
 Fusidate, 1101
 Hydrogen Sulfite, 1094
 Hydroxide, 1101
 Iodide, 1102
 Iodide (¹²³I) Capsules, 1103
 Iodide (¹³¹I) Capsules, 1103
 Iodide (¹³¹I) Solution, 1103
 Iodohippurate (¹³¹I) Injection, 1103
 Iotalamate Injection, 1103
 Lauryl Sulfate, 1104
 Metabisulfite, 1108
 Pertechnetate (^{99m}Tc) Injection, 1105
 Picosulfate Hydrate, 1105
 Polystyrene Sulfonate, 1106
 Prasterone Sulfate Hydrate, 1107
 Pyrosulfite, 1108
 Salicylate, 1108
 Thiosulfate Hydrate, 1109
 Thiosulfate Injection, 1110
 Valproate, 1110
 0.9% Sodium Chloride Injection, 1098
 10% Sodium Chloride Injection, 1098
 Sophora Root, 1364
 Sorbitan Sesquioleate, 1111
 D-Sorbitol, 1112
 Solution, 1113
 Soybean Oil, 1113
 Spectinomycin Hydrochloride Hydrate, 1114
 Spiramycin Acetate, 1114
 Spironolactone, 1116
 Stearic Acid, 1116
 Stearyl Alcohol, 1117
 Sterile Purified Water, 1236
 Streptomycin Sulfate, 1117
 Sucralfate Hydrate, 1118
 Sucrose, 1120
 Sulbactam Sodium, 1122
 Sulbenicillin Sodium, 1123
 Sulfadiazine Silver, 1124
 Sulfafurazole, 1128
 Sulfamethizole, 1125
 Sulfamethoxazole, 1126
 Sulfamonomethoxine Hydrate, 1126
 Sulfasalazine, 1077
 Sulfobromophthalein Sodium, 1129
 Injection, 1130
 Sulfur, 1130
 Salicylic Acid and Thianthol Ointment, 1131
 and Camphor Lotion, 1130
 Sulpiride, 1131
 Capsules, 1132
 Tablets, 1132
 Sulpyrine
 Hydrate, 1133

Injection, 1134
 Sultamicillin Tosilate Hydrate, 1134
 Sultiame, 1136
 Sulfinpyrazone, 1127
 Tablets, 1128
 Sulfisomezole, 1126
 Sulfisoxazole, 1128
 Suxamethonium Chloride
 for Injection, 1137
 Hydrate, 1137
 Injection, 1138
 Sweet Hydrangea Leaf, 1365
 Swertia
 and Sodium Bicarbonate Powder,
 1367
 Herb, 1365
 Synthetic
 Aluminum Silicate, 290
 Camphor, 402

T

Talampicillin Hydrochloride, 1138
 Talc, 1139
 Tamsulosin Hydrochloride, 1140
 Tannic Acid, 1141
 Tartaric Acid, 1141
 Taurine, 1142
 Teceleukin
 (Genetical Recombination), 1143
 for Injection (Genetical Recombina-
 tion), 1148
 Tegafur, 1149
 Teicoplanin, 1150
 Terbutaline Sulfate, 1153
 Termeric, 1367
 Testosterone
 Enanthate, 1154
 Enanthate Injection, 1154
 Propionate, 1155
 Propionate Injection, 1155
 Tetracaine Hydrochloride, 1156
 Tetracycline Hydrochloride, 1157
 Thallium (²⁰¹Tl) Chloride Injection,
 1158
 Theophylline, 1158
 Thiamazole, 1159
 Tablets, 1160
 Thiamine
 Chloride Hydrochloride, 1160
 Chloride Hydrochloride Injection,
 1161
 Chloride Hydrochloride Powder,
 1162
 Nitrate, 1162
 Thiamylal Sodium, 1163
 for Injection, 1164
 Thianthol, 1165
 Thiopental Sodium, 1166
 for Injection, 1167
 Thioridazine Hydrochloride, 1168
 Thiotepa, 1168
 L-Threonine, 1169

Thrombin, 1170
 Thymol, 1170
 Tiaramide Hydrochloride, 1171
 Tablets, 1172
 Ticlopidine Hydrochloride, 1173
 Timepidium Bromide Hydrate, 1174
 Timolol Maleate, 1174
 Tinidazole, 1175
 Tiopepidine Hibenzate, 1176
 Tablets, 1177
 Titanium Oxide, 1178
 Tizanidine Hydrochloride, 1179
 Toad Venom, 1368
 Tobramycin, 1180
 Tocopherol, 1181
 Acetate, 1182
 Calcium Succinate, 1183
 Nicotinate, 1184
dl- α -Tocopherol, 1181
 Acetate, 1182
 Nicotinate, 1184
 Todralazine Hydrochloride Hydrate,
 1185
 Tofisopam, 1186
 Tolazamide, 1187
 Tolbutamide, 1188
 Tablets, 1188
 Tolnaftate, 1189
 Solution, 1189
 Tolperisone Hydrochloride, 1190
 Tragacanth, 1369
 Tranexamic Acid, 1191
 Capsules, 1192
 Injection, 1193
 Tablets, 1193
 Trapidil, 1194
 Trepibutone, 1195
 Tretoquinol Hydrochloride, 1209
 Triamcinolone, 1195
 Acetonide, 1196
 Triamterene, 1197
 Tribulus Fruit, 1369
 Trichlormethiazide, 1198
 Tablets, 1199
 Trichomycin, 1201
 Trichosanthes Root, 1369
 Triclofos Sodium, 1202
 Syrup, 1203
 Trihexyphenidyl Hydrochloride, 1204
 Tablets, 1204
 Trimebutine Maleate, 1206
 Trimetazidine Hydrochloride, 1207
 Tablets, 1207
 Trimethadione, 1208
 Tablets, 1209
 Trimetoquinol Hydrochloride Hydrate,
 1209
 Tropicamide, 1211
 L-Tryptophan, 1211
 Tubocurarine Chloride Hydrochloride
 Hydrate, 1212
 Injection, 1213
 Tubocurarine Hydrochloride, 1212

Injection, 1213
 Tulobuterol Hydrochloride, 1213
 Turpentine Oil, 1214

U

Ubidecarenone, 1214
 Ulinastatin, 1215
 Uncaria Hook, 1370
 Urapidil, 1217
 Urea, 1218
 Urokinase, 1219
 Ursodeoxycholic Acid, 1220
 Uva Ursi Fluidextract, 1371

V

L-Valine, 1221
 Vancomycin Hydrochloride, 1221
 for Injection, 1223
 Vasopressin Injection, 1223
 Verapamil Hydrochloride, 1225
 Tablets, 1226
 Vinblastine Sulfate, 1226
 for Injection, 1227
 Vincristine Sulfate, 1228
 Vitamin A
 Acetate, 1058
 Capsules, 1230
 Oil, 1229
 Oil Capsules, 1230
 Palmitate, 1059
 Vitamin B₁
 Hydrochloride, 1160
 Hydrochloride Injection, 1161
 Hydrochloride Powder, 1162
 Nitrate, 1162
 Vitamin B₂, 1059
 Phosphate Ester, 1061
 Phosphate Ester Injection, 1062
 Powder, 1060
 Vitamin B₆, 1047
 Injection, 1048
 Vitamin B₁₂, 548
 Injection, 549
 Vitamin C, 316
 Injection, 317
 Powder, 317
 Vitamin D₂, 624
 Vitamin D₃, 506
 Vitamin E, 1181
 Acetate, 1182
 Calcium Succinate, 1183
 Nicotinate, 1184
 Vitamin K₁, 993
 Voglibose, 1231
 Tablets, 1232

W

Warfarin Potassium, 1233
 Tablets, 1234
 Water, 1235

for Injection, 1235

Weak Opium Alkaloids and Scopolamine Injection, 945

Weil's Disease and Akiyami Combined Vaccine, 1237

Wheat Starch, 1237

White

Beeswax, 336

Ointment, 1238

Petrolatum, 981

Shellac, 1085

Soft Sugar, 1121

Whole Human Blood, 1238

Wine, 1238

X

Xylitol, 1240

Injection, 1241

Y

Yellow

Beeswax, 336

Petrolatum, 982

Z

Zaltoprofen, 1242

Tablets, 1243

Zanthoxylum Fruit, 1371

Zedoary, 1372

Zinc

Chloride, 1244

Oxide, 1245

Oxide Oil, 1245

Oxide Ointment, 1246

Oxide Starch Powder, 1246

Sulfate Hydrate, 1246

Sulfate Ophthalmic Solution, 1247

Zinostatin Stimalamer, 1247

INDEX IN LATIN NAME

A

Achyranthis Radix, 1252
Adeps
 Lanae Rurificatus, 808
 Suillus, 808
Agar, 1252
 Pulveratum, 1253
Akebiae Caulis, 1253
Alismatis
 Rhizoma, 1253
 Rhizoma Pulveratum, 1253
Aloe, 1254
 Pulverata, 1255
Alpiniae
 Fructus, 1265
 officinari Rhizoma, 1256
Amomi
 Semen, 1256
 Semen Pulveratum, 1256
Amylum
 Maydis, 542
 Oryzae, 1346
 Solani, 1014
 Triticum, 1237
Anemarrhenae Rhizoma, 1256
Angelicae
 Radix, 1305
 Radix Pulverata, 1305
Arctii Fructus, 1267
Arecae Semen, 1258
Armeniacae Semen, 1257
Artemisiae Capillaris Flos, 1258
Asiasari Radix, 1259
Asparagi Tuber, 1259
Astragali Radix, 1260
Atractylodis
 Lanceae Rhizoma, 1261
 Lanceae Rhizoma Pulveratum, 1261
 Rhizoma, 1260
 Rhizoma Pulveratum, 1260
Aurantii
 Bobilis Pericarpium, 1273
 Fructus Immaturus, 1302
 Pericarpium, 1265

B

Belladonnae Radix, 1263
Benincasae Semen, 1264
Benzoinum, 1265
Bezoar Bovis, 1325
Bufois Venenum, 1368
Bupleuri Radix, 1266

C

Calumbae
 Radix, 1267
 Radix Pulverata, 1268
Cannabis Fructus, 1298
Capsici
 Fructus, 1268
 Fructus Pulveratus, 1269
Cardamomi Fructus, 1271
Carthami Flos, 1348
Caryophylli
 Flos Pulveratus, 1274
 Flos, 1274
Cassiae Semen, 1271
Catalpae Fructus, 1271
Cellulose Acetate Phthalate, 480
Cera
 Alba, 336
 Carnauba, 411
 Flava, 336
Chrysanthemi Flos, 1271
Cimicifugae Rhizoma, 1272
Cinnamomi
 Cortex, 1272
 Cortex Pulveratus, 1273
Clematidis Radix, 1274
Cnidii
 Monnieris Fructus, 1275
 Rhizoma, 1275
 Rhizoma Pulveratum, 1276
Coicis
 Semen, 1276
 Semen Pulveratum, 1276
Condurango Cortex, 1276
Coptidis
 Rhizoma, 1277
 Rhizoma Pulveratum, 1278
Corni Fructus, 1279
Corydalis Tuber, 1279
Crocus, 1349
Curcumae Rhizoma, 1367
Cyperii
 Rhizoma, 1280
 Rhizoma Pulveratum, 1280

D

Digenea, 1280
Dioscoreae
 Rhizoma, 1282
 Rhizoma Pulveratum, 1282
Dolichi Semen, 1282

E

Eleutherococci senticosi Rhizoma, 1283
Ephdrae Herba, 1283
Epimedii Herba, 1284
Eriobotryae Folium, 1314
Eucommiae Cortex, 1284
Evodiae Fructus, 1285
Extractum Belladonnae, 1263

F

Fel Ursi, 1262
Foeniculi
 Fructus, 1285
 Fructus Pulveratus, 1285
Forsythiae Fructus, 1286
Fossilia Ossis Mastodi, 1313
Fritillariae Bulbus, 1287

G

Gambir, 1287
 Pulveratum, 1287
Gardeniae
 Fructus, 1288
 Fructus Pulveratus, 1288
Gastrodiae Tuber, 1289
Gentianae
 Radix, 1290
 Radix Pulverata, 1290
 Scabrae Radix, 1306
 Scabrae Radix Pulverata, 1306
Geranii
 Herba, 1291
 Herba Pulverata, 1291
Ginseng
 Radix, 1292
 Radix Pulverata, 1293
 Radix Rubra, 1342
Glehniae Radix cum Rhizoma, 1294
Glycyrrhizae
 Radix, 1295
 Radix Pulverata, 1296
Gummi
 Arabicum, 1251
 Arabicum Pulveratum, 1251
Gypsum
 Exsiccatum, 1298
 Fibrosum, 1297

H

Houttuyniae Herba, 1302

Hydrangeae

- Dulcis Folium*, 1365
Dulcis Folium Pulveratum, 1365

I*Imperatae Rhizoma*, 1302*Ipecacuanhae*

- Radix*, 1303
Radix Pulverata, 1303

L*Linderae Radix*, 1313*Lithospermi Radix*, 1313*Lonicerae Folium Cum Caulis*, 1314*Lycii*

- Cortex*, 1315
Fructus, 1315

M*Magnoliae*

- Cortex Pulveratus*, 1316
Flos, 1317

Malloti Cortex, 1317*Mel*, 1301*Menthae Herba*, 1317*Mori Cortex*, 1320*Moutan*

- Cortex*, 1318
Cortex Pulveratus, 1319

N*Nelumbis Semen*, 1320*Notopterygii Rhizoma*, 1321*Nupharis Rhizoma*, 1321**O***Oleum*

- Arachidis*, 971
Aurantii, 946
Cacao, 386
Camelliae, 400
Caryophylli, 1275
Cinnamomi, 1273
Cocois, 535
Eucalypti, 654
Foeniculi, 1286
Maydis, 542
Menthae Japonicae, 1318
Olivae, 939
Rapae, 1055
Ricini, 414
Sesami, 1084
Sojae, 1113
Terebinthinae, 1214
Ophiopogonis Tuber, 1324
Opium Pulveratum, 939

Ostreae

- Testa*, 1326
Testa Pulverata, 1326

P*Paeoniae*

- Radix*, 1328
Radix Pulverata, 1329

Panacis Japonici

- Rhizoma*, 1326
Rhizoma Pulveratum, 1327

Perillae Herba, 1329*Persicae*

- Semen*, 1327
Semen Pulveratum, 1327

Pharbitidis Semen, 1330*Phellodendri*

- Cortex*, 1330
Cortex Pulveratus, 1331

Picrasmae

- Lignum*, 1333
Lignum Pulveratum, 1333

Pinelliae Tuber, 1333*Plantaginis*

- Herba*, 1334
Semen, 1334

Platycodi

- Radix*, 1334
Radix Pulverata, 1335

Polygalae

- Radix*, 1335
Radix Pulverata, 1335

Polygonati Rhizoma, 1336*Polygoni Multiflori Radix*, 1336*Polyporus*, 1336

- Pulveratus*, 1337

Poria, 1337

- Pulveratum*, 1337

Processi

- Aconiti Radix Pulverata*, 1339
Aconiti Radix, 1338

Prunellae Spica, 1341*Puerariae Radix*, 1341**R***Rehmanniae Radix*, 1343*Resina Pini*, 1347*Rhei*

- Rhizoma*, 1344
Rhizoma Pulveratum, 1345

Rosae

- Fructus*, 1346
Fructus Pulveratus, 1346

S*Saposhnikoviae Radix*, 1352*Sappan Lignum*, 1352*Saussureae Radix*, 1352*Schisandrae Fructus*, 1352*Schizonepetae Spica*, 1353*Scopoliae Rhizoma*, 1357*Scutellariae*

- Radix*, 1358
Radix Pulverata, 1359

Senegae

- Radix*, 1360
Radix Pulverata, 1361

Sennae

- Folium*, 1361
Folium Pulveratum, 1362

Sevum Bovinum, 336*Sinomeni Caulis et Rhizoma*, 1363*Smilacis*

- Rhizoma*, 1364
Rhizoma Pulveratum, 1364

Sophorae

- Radix*, 1364
Radix Pulverata, 1364

Strychni Semen, 1321*Swertiae*

- Herba*, 1365
Herba Pulverata, 1366

Syrupus

- Ipecacuanha*, 1304
Senegae, 1361

T*Tinctura Amara*, 1266*Tragacantha*, 1369

- Pulverata*, 1369

Tribuli Fructus, 1369*Trichosanthis Radix*, 1369**U***Uncariae Uncis Cum Ramulus*, 1370*Uvae Ursi Folium*, 1262**V***Valerianae*

- Radix*, 1306
Radix Pulverata, 1307

Z*Zanthoxyli*

- Fructus*, 1371
Fructus Pulveratus, 1371

Zedoariae Rhizoma, 1372*Zingiberis*

- Processum Rhizoma*, 1341
Rhizoma, 1291

- Rhizoma Pulveratum*, 1292

Zizyphi

- Fructus*, 1307
Semen, 1307

INDEX IN JAPANESE

ア

亜鉛華デンプン 1246
亜鉛華軟膏 1246
亜酸化窒素 929
アカメガシワ 1317
アクチノマイシン D 275
アクリルピシリン塩酸塩 272
アクリノール・チンク油 273
アクリノール・亜鉛華軟膏 274
アクリノール水和物 274
アザチオプリン 325
アザチオプリン錠 326
亜硝酸アミル 309
アジスロマイシン水和物 327
アジピン酸塩ピペラジン 999
アジマリン 278
アジマリン錠 279
アスコルビン酸 316
アスコルビン酸散 317
アスコルビン酸注射液 317
アストロマイシン硫酸塩 322
L-アスパラギン酸 318
アスピリン 318
アスピリンアルミニウム 319
アスピリン錠 319
アスポキシシリン水和物 320
アズトレオナム 328
アセグルタミドアルミニウム 266
アセタゾラミド 268
アセトアミノフェン 267
アセトヘキサミド 269
アセプトロール塩酸塩 265
アセンヤク 1287
アセンヤク末 1287
アテノロール 323
アトロピン硫酸塩水和物 324
アトロピン硫酸塩注射液 324
アドレナリン 276
アドレナリン液 277
アドレナリン注射液 276
亜ヒ酸パスタ 315
アフロクアロン 277
アヘン・トコ散 1324
アヘンアルカロイド・アトロピン注射液 942
アヘンアルカロイド・スコポラミン注射液 943
アヘンアルカロイド塩酸 941
アヘンアルカロイド塩酸注射液 942
アヘン散 940
アヘンチンキ 940
アヘン末 939
アマチャ 1365
アマチャ末 1365

アマンタジン塩酸塩 293
アミカシン硫酸塩 296
アミトリプチリン塩酸塩 298
アミトリプチリン塩酸塩錠 299
アミドトリゾ酸 295
アミドトリゾ酸ナトリウムメグルミン注射液 853
アミドトリゾ酸メグルミン注射液 851
アミノ安息香酸エチル 644
アミノフィリン水和物 297
アミノフィリン注射液 297
アムホテリシン B シロップ 305
アムホテリシン B 錠 305
アムホテリシン B 303
アモキサピン 301
アモキシシリン水和物 302
アモバルビタール 300
アラセプリル 279
アラセプリル錠 280
アラビアゴム 1251
アラビアゴム末 1251
アリメマジン酒石酸塩 283
亜硫酸水素ナトリウム 1094
L-アルギニン 313
L-アルギニン塩酸塩 313
L-アルギニン塩酸塩注射液 314
アルジオキサ 282
アルプラゾラム 284
アルプレノロール塩酸塩 285
アルプロスタジル 286
アルプロスタジルアルファデクス 287
アルベカシン硫酸塩 311
アルベカシン硫酸塩注射液 312
アロエ 1254
アロエ末 1255
アロチノロール塩酸塩 314
アロプリノール 284
安息香酸 345
安息香酸ナトリウム 1093
安息香酸ナトリウムカフェイン 388
安息香酸ベンジル 347
アンソッコウ 1265
アンチピリン 310
アンピシリンナトリウム 308
アンピシリン水和物 307
アンペノニウム塩化物 294
アンモニア・ウイキョウ精 1286
アンモニア水 299

イ

イオウ 1130
イオウ・カンフルローション 1130
イオウ・サリチル酸・チアントール軟膏

1131

イオタラム酸 774
イオタラム酸ナトリウム注射液 1103
イオタラム酸メグルミン注射液 852
イオトロクス酸 775
イオパミドール 773
イクタモール 744
イコサペント酸エチルエステル 646
イセパマイシン硫酸塩 777
イソソルビド 784
イソニアジド 780
イソニアジド錠 781
イソニアジド注射液 781
イソフェンインスリン水性懸濁注射液 762
イソフルラン 778
L-イソプレナリン塩酸塩 782
イソプロパノール 783
イソプロピルアンチピリン 783
L-イソロイシン 780
イダルピシリン塩酸塩 745
イドクスウリジン 747
イドクスウリジン点眼液 747
イフェンプロジル酒石酸塩 748
イブプロフェン 744
イプラトロピウム臭化物水和物 776
イミプラミン塩酸塩 751
イミプラミン塩酸塩錠 752
イミベネム水和物 749
イレイセン 1274
インジゴカルミン 754
インジゴカルミン注射液 755
インスリン 758
インスリン亜鉛水性懸濁注射液 763
インスリン注射液 762
インチンコウ 1258
インデノロール塩酸塩 753
インドメタシン 755
インドメタシンカプセル 756
インドメタシン坐剤 757
インフルエンザ HA ワクチン 758
インヨウカク 1284

ウ

ウイキョウ 1285
ウイキョウ末 1285
ウイキョウ油 1286
ウコン 1367
ウヤク 1313
ウラビジル 1217
ウリナスタチン 1215
ウルソデオキシコール酸 1220
ウロキナーゼ 1219
ウワウルシ 1262

ウワウルシ流エキス 1371

工

エーテル 641
 エイジツ 1346
 エイジツ末 1346
 液状フェノール 986
 エコチオパートヨウ化物 610
 エスタゾラム 631
 エストラジオール安息香酸エステル 631
 エストラジオール安息香酸エステル水性懸濁注射液 633
 エストラジオール安息香酸エステル注射液 632
 エストリオール 633
 エストリオール錠 635
 エストリオール水性懸濁注射液 634
 エタクリン酸 636
 エタクリン酸錠 636
 エタノール 638
 エタンブトール塩酸塩 637
 エチオナミド 643
 エチゾラム 652
 エチドロン酸二ナトリウム 649
 エチドロン酸二ナトリウム錠 650
 エチニルエストラジオール 642
 エチニルエストラジオール錠 642
 L-エチルシステイン塩酸塩 645
 エチルモルヒネ塩酸塩水和物 648
 エチレフリン塩酸塩 650
 エチレフリン塩酸塩錠 651
 エチレンジアミン 648
 エテンザミド 640
 エデト酸ナトリウム水和物 598
 エトスクシミド 644
 エトドラク 653
 エトポシド 653
 エドロホニウム塩化物 611
 エドロホニウム塩化物注射液 612
 エノキサシン水和物 616
 エピリゾール 621
 エピルピシン塩酸塩 622
 エフェドリン塩酸塩 619
 エフェドリン塩酸塩錠 621
 エフェドリン塩酸塩注射液 619
 エフェドリン塩酸塩 10% 620
 エペリゾン塩酸塩 618
 エリスロマイシン 627
 エリスロマイシンエチルコハク酸エステル 629
 エリスロマイシンステアリン酸塩 630
 エリスロマイシンラクトビオン酸塩 629
 エルカトニン 612
 エルゴカルシフェロール 624
 エルゴタミン酒石酸塩 627
 エルゴメトリンマレイン酸塩 625
 エルゴメトリンマレイン酸塩錠 626
 エルゴメトリンマレイン酸塩注射液 625

塩化亜鉛 1244
 酸化亜鉛 1245
 塩化インジウム(111In)注射液 755
 塩化カリウム 1009
 塩化カルシウム水和物 390
 塩化カルシウム注射液 390
 塩化タリウム(201Tl)注射液 1158
 塩化ナトリウム 1097
 10% 塩化ナトリウム注射液 1098
 エンゴサク 1279
 塩酸 725
 塩酸キニーネ 1052
 塩酸ドパミン注射液 602
 塩酸モルヒネ注射液 901
 塩酸リモナーデ 727
 エンビオマイシン硫酸塩 616
 エンフルラン 615

オ

オウギ 1260
 オウゴン 1358
 オウゴン末 1359
 黄色ワセリン 982
 オウセイ 1336
 オウバク 1330
 オウバク・タンナルピン・ビスマス散 1332
 オウバク末 1331
 オウレン 1277
 オウレン末 1278
 オキサゾラム 948
 オキサピウムヨウ化物 947
 オキサプロジン 948
 オキシコドン塩酸塩水和物 951
 オキシテトラサイクリン塩酸塩 956
 オキシトシン 958
 オキシトシン注射液 960
 オキシドール 954
 オキシブプロカイン塩酸塩 951
 オキシメトロン 956
 オキセサゼイン 949
 オクスプレノロール塩酸塩 950
 オフロキサシン 938
 オリブ油 939
 オルシブレナリン硫酸塩 946
 オレンジ油 946
 オンジ 1335
 オンジ末 1335

カ

カイニン酸・サントニン散 790
 カイニン酸水和物 789
 カオリン 795
 カカオ脂 386
 加香ヒマシ油 415
 カゴソウ 1341
 カシユウ 1336
 加水ラノリン 807
 カッコン 1341
 葛根湯エキス 1308
 過テクネチウム酸ナトリウム(99mTc)

注射液 1105
 果糖 689
 果糖注射液 689
 カナマイシン一硫酸塩 793
 カナマイシン硫酸塩 794
 カノコソウ 1306
 カノコソウ末 1307
 カフェイン水和物 387
 カプセル 403
 カプトプリル 403
 過マンガン酸カリウム 1013
 加味逍遙散エキス 1309
 カモスタットメシル酸塩 400
 カリジノゲナーゼ 790
 カリ石ケン 1007
 カルテオロール塩酸塩 412
 カルナウバロウ 411
 カルバゾクロムスルホン酸ナトリウム水和物 405
 カルバマゼピン 404
 カルビドパ水和物 406
 L-カルボシステイン 407
 カルメロース 408
 カルメロースカルシウム 409
 カルメロースナトリウム 410
 カルモナムナトリウム 412
 カルモフル 411
 カロコン 1369
 カンキョウ 1341
 乾燥はぶウマ抗毒素 716
 乾燥まむしウマ抗毒素 841
 乾燥ジフテリアウマ抗毒素 596
 乾燥ボツリヌスウマ抗毒素 374
 乾燥亜硫酸ナトリウム 1109
 乾燥甲状腺 1171
 乾燥酵母 1241
 乾燥細胞培養痘そうワクチン 1091
 乾燥弱毒生おたふくかぜワクチン 902
 乾燥弱毒生風しんワクチン 1074
 乾燥弱毒生麻しんワクチン 843
 乾燥水酸化アルミニウムゲル 288
 乾燥水酸化アルミニウムゲル細粒 289
 乾燥組織培養不活化狂犬病ワクチン 1054
 乾燥炭酸ナトリウム 1096
 乾燥痘そうワクチン 1091
 乾燥日本脳炎ワクチン 786
 乾燥破傷風ウマ抗毒素 1156
 乾燥硫酸アルミニウムカリウム 292
 乾燥 BCG ワクチン 335
 カンゾウ 1295
 カンゾウエキス 1296
 カンゾウ粗エキス 1297
 カンゾウ末 1296
 カンテン 1252
 カンテン末 1253
 dl-カンフル 402
 d-カンフル 401
 肝油 538
 カンレノ酸カリウム 1008
 ガジュツ 1372

ガスエソウマ抗毒素 696
 β -ガラクトシダーゼ(アスペルギルス)
 694
 β -ガラクトシダーゼ(ペニシリウム)
 695
 含糖ペプシン 1074

キ

希塩酸 726
 キキョウ 1334
 キキョウ末 1335
 キキョウ流エキス 1334
 キクカ 1271
 キササゲ 1271
 キシリトール 1240
 キシリトール注射液 1241
 キジツ 1302
 キタサマイシン 798
 キタサマイシン酒石酸塩 800
 キタサマイシン酢酸エステル 799
 キニーネエチル炭酸 1051
 キニーネ硫酸塩水和物 1053
 キニジン硫酸塩水和物 1050
 吸水軟膏 265
 キョウカツ 1321
 キョウニン 1257
 キョウニン水 1257
 希ヨードチンキ 769
 金チオリンゴ酸ナトリウム 1092
 牛脂 336

ク

クエン酸ガリウム(67Ga)注射液 696
 クエン酸ナトリウム 1099
 クエン酸水和物 515
 クコシ 1315
 クジン 1364
 クジン末 1364
 苦味重曹水 1364
 苦味チンキ 1266
 クラブラン酸カリウム 1010
 クラリスロマイシン 516
 クラリスロマイシン錠 517
 クリノフィブラート 522
 クリンダマイシンリン酸エステル
 521
 クリンダマイシン塩酸塩 519
 クリンダマイシン塩酸塩カプセル
 520
 クレオソート 544
 クレゾール 544
 クレゾール水 545
 クレゾール石ケン液 545
 クレマスチンフマル酸塩 519
 クロカブラミン塩酸塩水和物 523
 クロキサシリンナトリウム水和物
 533
 クロキサゾラム 534
 クロコナゾール塩酸塩 546
 クロスカルメロースナトリウム 546
 クロチアゼパム 531

クロトリマゾール 532
 クロナゼパム 528
 クロニジン塩酸塩 529
 クロフィブラート 525
 クロフィブラートカプセル 526
 クロフェダノール塩酸塩 524
 クロペラスチン塩酸塩 530
 クロミフェンクエン酸塩 526
 クロミフェンクエン酸塩錠 527
 クロミプラミン塩酸塩 528
 クロム酸ナトリウム(51Cr)注射液
 1099
 クロモグリク酸ナトリウム 1100
 クロラムフェニコール 487
 クロラムフェニコールコハク酸エステ
 ルナトリウム 489
 クロラムフェニコールパルミチン酸エ
 ステル 488
 クロルジアゼポキシド 490
 クロルジアゼポキシド散 491
 クロルジアゼポキシド錠 492
 クロルフェニラミン・カルシウム散
 497
 クロルフェニラミンマレイン酸塩
 498
 クロルフェニラミンマレイン酸塩散
 499
 クロルフェニラミンマレイン酸塩錠
 500
 クロルフェニラミンマレイン酸塩注射
 液 499
 クロルフェネシンカルバミン酸エステ
 ル 496
 d-クロルフェニラミンマレイン酸塩
 501
 クロルプロパミド 504
 クロルプロパミド錠 505
 クロルプロマジン塩酸塩 502
 クロルプロマジン塩酸塩錠 503
 クロルプロマジン塩酸塩注射液 503
 クロルヘキシジングルコン酸塩液
 493
 クロルヘキシジン塩酸塩 493
 クロルマジノン酢酸エステル 494
 クロロブタノール 495
 グアイフェネシン 714
 グアナベンズ酢酸塩 715
 グアネチジン硫酸塩 716
 グアヤコールスルホン酸カリウム
 1011
 グラミシジン 712
 グリシン 704
 グリセオフルビン 713
 グリセリン 702
 グリセリンカリ液 704
 グリベンクラミド 699
 グルコン酸カルシウム水和物 391
 グルタチオン 701

ケ

ケイガイ 1353
 経口生ポリオワクチン 1005

ケイ酸マグネシウム 836
 軽質無水ケイ酸 1087
 軽質流動パラフィン 967
 ケイヒ 1272
 ケイヒ末 1273
 ケイヒ油 1273
 ケタミン塩酸塩 795
 結晶セルロース 477
 結晶性インスリン亜鉛水性懸濁注射液
 765
 血清性性腺刺激ホルモン 710
 ケツメイシ 1271
 ケトチフェンフマル酸塩 797
 ケトプロフェン 796
 ケノデオキシコール酸 486
 ケンゴシ 1330
 滅菌精製水 1236
 ゲンタマイシン硫酸塩 698
 ゲンチアナ 1290
 ゲンチアナ・重曹散 1290
 ゲンチアナ末 1290
 ゲンノショウコ 1291
 ゲンノショウコ末 1291

コ

コウカ 1348
 硬化油 735
 コウジン 1342
 コウブシ 1280
 コウブシ末 1280
 コウボク 1315
 コウボク末 1316
 コカイン塩酸塩 535
 コムギデンプン 1237
 コメデンプン 1346
 コリスチンメタンスルホン酸ナトリウ
 ム 540
 コリスチン硫酸塩 541
 コルチゾン酢酸エステル 543
 コルヒチン 538
 コレカルシフェロール 506
 コレステロール 507
 コレラワクチン 506
 コロンボ 1267
 コロンボ末 1268
 コンズランゴ 1276
 コンズランゴ流エキス 1277
 合成ケイ酸アルミニウム 290
 ゴオウ 1325
 ゴシツ 1252
 ゴシユ 1285
 705
 ゴボウシ 1267
 ゴマ油 1084
 ゴミシ 1352

サ

サイクロセリン 551
 サイコ 1266
 サイシン 1259
 柴苓湯エキス 1349

酢酸 268
 酢酸ナトリウム水和物 1091
 サッカリン 1075
 サッカリンナトリウム水和物 1076
 サフラン 1349
 サラシ粉 494
 サラシミツロウ 336
 サラゾスルファピリジン 1077
 サリチル・ミョウバン散 1078
 サリチル酸 1079
 サリチル酸ナトリウム 1108
 サリチル酸メチル 881
 サリチル酸精 1080
 サリチル酸絆創膏 1080
 サルブタモール硫酸塩 1078
 酸化カルシウム 393
 酸化チタン 1178
 酸化マグネシウム 835
 サンキライ 1364
 サンキライ末 1364
 三酸化ヒ素 316
 サンシシ 1288
 サンシシ末 1288
 サンシュユ 1279
 サンショウ 1371
 サンショウ末 1371
 酸素 955
 サンソウニン 1307
 サントニン 1081
 サンヤク 1282
 サンヤク末 1282
 ザルトプロフェン 1242
 ザルトプロフェン錠 1243

シ

シアナミド 548
 シアノコバラミン 548
 シアノコバラミン注射液 549
 歯科用アンチホルミン 310
 歯科用トリオジンク pasta 1210
 歯科用パラホルム pasta 969
 歯科用フェノール・カンフル 987
 歯科用ヨード・グリセリン 771
 シクラシリン 507
 シクロスポリン 509
 シクロペントラート塩酸塩 549
 シクロホスファミド水和物 550
 シコン 1313
 シゴカ 1283
 シスプラチン 513
 シソマイシン硫酸塩 1090
 シタラピン 552
 シッカニン 1086
 シツリシ 1369
 シプロヘプタジン塩酸塩水和物 551
 シメチジン 513
 シャクヤク 1328
 シャクヤク末 1329
 シャゼンシ 1334
 シャゼンソウ 1334
 臭化カリウム 1008
 臭化ナトリウム 1095

シュクシャ 1256
 シュクシャ末 1256
 酒石酸 1141
 ショウキョウ 1291
 ショウキョウ末 1292
 硝酸イソソルビド 785
 硝酸イソソルビド錠 786
 硝酸銀 1088
 硝酸銀点眼液 1088
 ショウズク 1271
 消毒用エタノール 640
 消毒用フェノール 986
 消毒用フェノール水 987
 ショウマ 1272
 シラスタチンナトリウム 510
 シロスタゾール 511
 シロスタゾール錠 512
 シロップ用ファロペネムナトリウム 659
 シンイ 1317
 親水ワセリン 981
 親水軟膏 735
 診断用クエン酸ナトリウム液 1100
 ジアスターゼ 567
 ジアスターゼ・重曹散 567
 ジアゼパム 567
 ジエチルカルバマジンクエン酸塩 574
 ジエチルカルバマジンクエン酸塩錠 574
 ジオウ 1343
 ジギトキシシ 576
 ジギトキシシ錠 576
 ジクロキサシリンナトリウム水和物 573
 ジクロフェナクナトリウム 570
 ジクロフェナミド 571
 ジクロフェナミド錠 572
 ジコッピ 1315
 ジゴキシシ 577
 ジゴキシシ錠 580
 ジゴキシシ注射液 578
 次硝酸ビスマス 369
 ジスチグミン臭化物 599
 ジスチグミン臭化物錠 600
 ジスルフィラム 600
 ジソピラミド 598
 ジドロゲステロン 609
 ジドロゲステロン錠 610
 ジノスタチン スチマラマー 1247
 ジノプロスト 592
 ジヒドロエルゴタミンメシル酸塩 583
 ジヒドロエルゴトキシシメシル酸塩 584
 ジヒドロコデインリン酸塩 581
 ジヒドロコデインリン酸塩散 10% 582
 ジヒドロコデインリン酸塩散 1% 582
 ジピリダモール 597
 ジフェニドール塩酸塩 575
 ジフェンヒドラミン 593

ジフェンヒドラミン・バレリル尿素散 593
 ジフェンヒドラミン・フェノール・亜鉛華リニメント 595
 ジフェンヒドラミン塩酸塩 594
 ジフテリアトキシソイド 596
 ジフテリア破傷風混合トキシソイド 596
 ジブカイン塩酸塩 569
 ジベカシン硫酸塩 569
 次没食子酸ビスマス 368
 ジメモルファンリン酸塩 588
 ジメルカプロール 590
 ジメルカプロール注射液 590
 ジメンヒドリナート 588
 ジメンヒドリナート錠 589
 ジモルホラミン 591
 ジモルホラミン注射液 591
 弱アヘンアルカロイド・スコポラミン注射液 945
 ジャッシュウシ 1275
 ジュウヤク 1302
 常水 1235
 ジョサマイシン 786
 ジョサマイシンプロピオン酸エステル 788
 ジラゼプ塩酸塩水和物 586
 ジルチアゼム塩酸塩 586
 人全血液 1238

ス

水酸化カリウム 1012
 水酸化カルシウム 392
 水酸化ナトリウム 1101
 スキサメトニウム塩化物水和物 1137
 スキサメトニウム塩化物注射液 1138
 スクラルファート水和物 1118
 スコポラミン臭化水素酸塩水和物 1083
 ステアリルアルコール 1117
 ステアリン酸 1116
 ステアリン酸カルシウム 400
 ステアリン酸ポリオキシル 40 1007
 ステアリン酸マグネシウム 837
 ストレプトマイシン硫酸塩 1117
 スピラマイシン酢酸エステル 1114
 スピロノラクトン 1116
 スペクチノマイシン塩酸塩水和物 1114
 スルタミシリントシル酸塩水和物 1134
 スルチアム 1136
 スルバクタムナトリウム 1122
 スルピリド 1131
 スルピリドカプセル 1132
 スルピリド錠 1132
 スルピリン水和物 1133
 スルピリン注射液 1134
 スルファジアジン銀 1124
 スルファメチゾール 1125
 スルファメトキサゾール 1126
 スルファモノメトキシシ水和物 1126

スルフィンピラゾン 1127
 スルフィンピラゾン錠 1128
 スルフィソキサゾール 1128
 スルベニシリンナトリウム 1123
 スルホプロモフタレインナトリウム
 1129
 スルホプロモフタレインナトリウム注
 射液 1130

セ

成人用沈降ジフテリアトキソイド
 596

精製セラック 1085
 精製ゼラチン 697
 精製デヒドロコール酸 556
 精製ラノリン 808
 精製水 1236
 精製白糖 1120
 生理食塩液 1098
 石油ベンジン 982
 セタノール 484
 セッコウ 1297
 セトラキサート塩酸塩 485
 セネガ 1360
 セネガシロップ 1361
 セネガ末 1361
 セファクロル 415
 セファクロルカプセル 416
 セファクロル細粒 417
 セファクロル複合顆粒 419
 セファゾリンナトリウム 426
 セファゾリンナトリウム水和物 428
 セファトリジンプロピレンジリコール
 425
 セファドロキシル 421
 セファピリンナトリウム 424
 セファレキシン 422
 セファロチンナトリウム 423
 セフィキシム 442
 セフェピム塩酸塩水和物 439
 セフォジジムナトリウム 447
 セフォゾプラン塩酸塩 457
 セフォタキシムナトリウム 449
 セフォチアムヘキセチル塩酸塩 453
 セフォチアム塩酸塩 455
 セフォテタン 451
 セフォペラゾンナトリウム 448
 セフカペン ピボキシル塩酸塩細粒
 432
 セフカペン ピボキシル塩酸塩錠 433
 セフカペン ピボキシル塩酸塩水和物
 430
 セフジトレン ピボキシル 437
 セフジトレン ピボキシル細粒 438
 セフジトレン ピボキシル錠 438
 セフジニル 434
 セフジニルカプセル 435
 セフジニル細粒 436
 セフスロジンナトリウム 464
 セフタジジム水和物 466
 セフチゾキシムナトリウム 471
 セフチブテン水和物 470

セフテラムピボキシル 468
 セフテラム ピボキシル細粒 469
 セフトリアキソンナトリウム水和物
 472
 セフピラミドナトリウム 458
 セフピロム硫酸塩 460
 セフペラゾンナトリウム 429
 セフポドキシムプロキセチル 461
 セフミノクスナトリウム水和物 446
 セフメタゾールナトリウム 445
 セフメノキシム塩酸塩 443
 セフロキサジン水和物 462
 セフロキシムアキセチル 474
 セフロキシムナトリウム 476
 セラセフェート 480
 セラペプターゼ 1083
 セルモロイキン(遺伝子組換え) 481
 センキュウ 1275
 センキュウ末 1276
 センコツ 1321
 センソ 1368
 センナ 1361
 センナ末 1362
 センブリ 1365
 センブリ・重曹散 1367
 センブリ末 1366
 ゼラチン 696

ソ

ソウジュツ 1261
 ソウジュツ末 1261
 ソウハクヒ 1320
 ソボク 1352
 ソヨウ 1329
 ソルビタンセスキオレイン酸エステル
 1111
 D-ソルビトール 1112
 D-ソルビトール液 1113

タ

タイソウ 1307
 タウリン 1142
 タクシャ 1253
 タクシャ末 1253
 タムスロシン塩酸塩 1140
 タランピシリン塩酸塩 1138
 タルク 1139
 炭酸カリウム 1009
 炭酸ナトリウム水和物 1096
 炭酸マグネシウム 834
 炭酸リチウム 826
 炭酸水素ナトリウム 1093
 炭酸水素ナトリウム注射液 1094
 単シロップ 1089
 単軟膏 1089
 タンニン酸 1141
 タンニン酸アルブミン 282
 タンニン酸ジフェンヒドラミン 595
 タンニン酸ベルベリン 352
 ダイオウ 1344
 ダイオウ末 1345

大黃甘草湯エキス 1280
 ダイズ油 1113
 ダウノルビシン塩酸塩 553
 ダントロレンナトリウム水和物 553

チ

チアマゾール 1159
 チアマゾール錠 1160
 チアミラルナトリウム 1163
 チアミン塩化物塩酸塩 1160
 チアミン塩化物塩酸塩散 1162
 チアミン塩化物塩酸塩注射液 1161
 チアミン硝化物 1162
 チアラミド塩酸塩 1171
 チアラミド塩酸塩錠 1172
 チアントール 1165
 チオテパ 1168
 チオベンタールナトリウム 1166
 チオリダジン塩酸塩 1168
 チオ硫酸ナトリウム水和物 1109
 チオ硫酸ナトリウム注射液 1110
 チクセツニンジン 1326
 チクセツニンジン末 1327
 チクロピジン塩酸塩 1173
 チザニジン塩酸塩 1179
 窒素 928
 チニダゾール 1175
 チペビジンヒベンズ酸塩 1176
 チペビジンヒベンズ酸塩錠 1177
 チメビジウム臭化物水和物 1174
 チモ 1256
 チモール 1170
 チモロールマレイン酸塩 1174
 注射用アセチルコリン塩化物 271
 注射用アムホテリシン B 304
 注射用アモバルビタールナトリウム
 300
 注射用イダルビシン塩酸塩 746
 注射用イミペネム・シラスタチンナト
 リウム 750
 注射用スキサメトニウム塩化物 1137
 注射用セフェピム塩酸塩 441
 注射用セフォゾプラン塩酸塩 458
 注射用セフォチアム塩酸塩 456
 注射用チアミラルナトリウム 1164
 注射用チオベンタールナトリウム
 1167
 注射用テセロイキン(遺伝子組換え)
 1148
 注射用バンコマイシン塩酸塩 1223
 注射用ヒト絨毛性性腺刺激ホルモン
 708
 注射用ヒドララジン塩酸塩 724
 注射用ピペラシリンナトリウム 998
 注射用ファモチジン 656
 注射用フェニトインナトリウム 992
 注射用フロモキシセフナトリウム 666
 注射用プレドニゾロンナトリウムコハ
 ク酸エステル 1025
 注射用ホスホマイシンナトリウム
 687
 注射用血清性性腺刺激ホルモン 711

注射用水 1235
 注射用硫酸ビンプラスチン 1227
 チョウジ 1274
 チョウジ末 1274
 チョウジ油 1275
 チョウトウコウ 1370
 チョレイ 1336
 チョレイ末 1337
 チンク油 1245
 沈降はぶトキソイド 716
 沈降ジフテリア破傷風混合トキソイド 596
 沈降精製百日せきジフテリア破傷風混合ワクチン 596
 沈降精製百日せきワクチン 980
 沈降炭酸カルシウム 389
 沈降破傷風トキソイド 1156
 沈降 B 型肝炎ワクチン 716
 チンピ 1273

ツ

ツバキ油 400
 ツボクラリン塩化物塩酸塩水和物 1212
 ツボクラリン塩化物塩酸塩注射液 1213
 ツロブテロール塩酸塩 1213

テ

テイコプラニン 1150
 低置換度ヒドロキシプロピルセルロース 738
 テオフィリン 1158
 テガフル 1149
 テストステロンエナント酸エステル 1154
 テストステロンエナント酸エステル注射液 1154
 テストステロンプロピオン酸エステル 1155
 テストステロンプロピオン酸エステル注射液 1155
 テセロイキン(遺伝子組換え) 1143
 テトラカイン塩酸塩 1156
 テトラサイクリン塩酸塩 1157
 テルブタリン硫酸塩 1153
 テレピン油 1214
 天然ケイ酸アルミニウム 289
 テンマ 1289
 テンモンドウ 1259
 デキサメタゾン 560
 デキストラン硫酸エステルナトリウムイオウ 5 564
 デキストラン硫酸ナトリウムイオウ 18 565
 デキストラン 40 561
 デキストラン 40 注射液 562
 デキストラン 70 562
 デキストリン 565
 デキストロメトルファン臭化水素酸塩水和物 566

デスラノシド 559
 デスラノシド注射液 560
 デヒドロコール酸 556
 デヒドロコール酸注射液 557
 デフェロキサミンメシル酸塩 554
 デメチルクロルテトラサイクリン塩酸塩 558

ト

トウガシ 1264
 トウガラシ 1268
 トウガラシ・サリチル酸精 1270
 トウガラシチンキ 1269
 トウガラシ末 1269
 トウキ 1305
 トウキ末 1305
 トウニン 1327
 トウニン末 1327
 トウヒ 1265
 トウヒシロップ 1325
 トウヒチンキ 1325
 トウモロコシデンブソ 542
 トウモロコシ油 542
 トコフェロール 1181
 トコフェロールコハク酸エステルカルシウム 1183
 トコフェロールニコチン酸エステル 1184
 トコフェロール酢酸エステル 1182
 トコン 1303
 トコンシロップ 1304
 トコン末 1303
 トチュウ 1284
 トドララジン塩酸塩水和物 1185
 トフィソパム 1186
 トブラマイシン 1180
 トラガント 1369
 トラガント末 1369
 トラザミド 1187
 トラネキサム酸 1191
 トラネキサム酸カプセル 1192
 トラネキサム酸錠 1193
 トラネキサム酸注射液 1193
 トラピジル 1194
 トリアムシノロン 1195
 トリアムシノロンアセトニド 1196
 トリアムテレン 1197
 トリクロホスナトリウム 1202
 トリクロホスナトリウムシロップ 1203
 トリクロルメチアジド 1198
 トリクロルメチアジド錠 1199
 トリコマイシン 1201
 L-トリプトファン 1211
 トリヘキシフェニジル塩酸塩 1204
 トリヘキシフェニジル塩酸塩錠 1204
 トリメタジオン 1208
 トリメタジオン錠 1209
 トリメタジジン塩酸塩 1207
 トリメタジジン塩酸塩錠 1207
 トリメトキノール塩酸塩水和物 1209
 トリメブチンマレイン酸塩 1206

トルナフタート 1189
 トルナフタート液 1189
 トルブタミド 1188
 トルブタミド錠 1188
 トルペリゾン塩酸塩 1190
 L-トレオニン 1169
 トレビプトン 1195
 トロピカミド 1211
 トロンピン 1170
 豚脂 808
 ドキサプラム塩酸塩水和物 603
 ドキシサイクリン塩酸塩水和物 606
 ドキシフルリジン 604
 ドキシフルリジンカプセル 604
 ドキソルピシン塩酸塩 605
 ドパミン塩酸塩 602
 ドブタミン塩酸塩 601
 ドロベリドール 608

ナ

ナイスタチン 937
 ナタネ油 1055
 ナドロール 904
 ナファゾリン・クロルフェニラミン液 906
 ナファゾリン塩酸塩 907
 ナファゾリン硝酸塩 908
 ナプロキセン 908
 ナリジクス酸 905
 ナロキソン塩酸塩 906

ニ

ニカルジピン塩酸塩 911
 ニカルジピン塩酸塩注射液 912
 ニガキ 1333
 ニガキ末 1333
 ニコチン酸 920
 ニコチン酸アミド 919
 ニコチン酸注射液 921
 ニコモール 917
 ニコモール錠 918
 ニコランジル 919
 ニ酸化炭素 407
 ニセリトロール 916
 ニセルゴリン 913
 ニセルゴリン散 914
 ニセルゴリン錠 915
 ニトラゼパム 925
 ニトレンジピン 926
 ニトレンジピン錠 927
 ニトログリセリン錠 928
 ニフェジピン 922
 日本脳炎ワクチン 786
 乳酸 801
 乳酸カルシウム水和物 393
 乳糖水和物 803
 尿素 1218
 ニルバジピン 923
 ニルバジピン錠 924
 ニンジン 1292
 ニンジン末 1293

ニンドウ 1314

ネ

ネオスチグミンメチル硫酸塩 909
ネオスチグミンメチル硫酸塩注射液 910

ネチルマイシン硫酸塩 910

ノ

濃グリセリン 703
濃ベンザルコニウム塩化物液 50 343
ノスカピン 936
ノスカピン塩酸塩水和物 937
ノルアドレナリン 930
ノルアドレナリン注射液 931
ノルエチステロン 932
ノルゲストレル 933
ノルゲストレル・エチニルエストラジ
オール錠 934
ノルトリプチリン塩酸塩 935
ノルフロキサシン 932

ハ

白色セラック 1085
白色ワセリン 981
白色軟膏 1238
白糖 1121
ハチミツ 1301
ハッカ 1317
ハッカ水 1318
ハッカ油 1318
ハマボウフウ 1294
ハロキサゾラム 719
ハロタン 719
ハロペリドール 717
ハロペリドール錠 718
ハンゲ 1333
バイモ 1287
バカンピシリン塩酸塩 329
バクモンドウ 1324
バクロフェン 331
バクロフェン錠 332
バシトラシン 330
バソプレシン注射液 1223
バメタン硫酸塩 333
L-バリリン 1221
バルビタール 333
バルプロ酸ナトリウム 1110
バレイショデンブン 1014
バンコマイシン塩酸塩 1221
パップ用複方オウバク散 1332
パニペネム 962
パパペリン塩酸塩 965
パパペリン塩酸塩注射液 965
パラアミノサリチル酸カルシウム水和物 395
パラアミノサリチル酸カルシウム顆粒 394
パラオキシ安息香酸エチル 647
パラオキシ安息香酸ブチル 386

パラオキシ安息香酸プロピル 1039
パラオキシ安息香酸メチル 878
パラフィン 966
パラホルムアルデヒド 968
パルナパリンナトリウム 969
パンクレアチン 961
パンクロニウム臭化物 962
パンテチン 964
パントテン酸カルシウム 394

ヒ

ヒトインスリン(遺伝子組換え) 760
ヒト下垂体性性腺刺激ホルモン 708
ヒト絨毛性性腺刺激ホルモン 707
人免疫グロブリン 724
ヒドララジン塩酸塩 724
ヒドララジン塩酸塩散 725
ヒドララジン塩酸塩錠 725
ヒドロキシジンパモ酸塩 739
ヒドロキシジン塩酸塩 739
ヒドロキシプロピルセルロース 736
ヒドロキシコバラミン酢酸塩 736
ヒドロクロロチアジド 727
ヒドロコタルニン塩酸塩水和物 734
ヒドロコルチゾン 728
ヒドロコルチゾン・ジフェンヒドラミ
ン軟膏 730
ヒドロコルチゾンコハク酸エステル
733
ヒドロコルチゾンコハク酸エステルナ
トリウム 732
ヒドロコルチゾンリン酸エステルナト
リウム 731
ヒドロコルチゾン酢酸エステル 729
ヒドロコルチゾン酪酸エステル 730
ヒプロメロース 741
ヒプロメロースフタル酸エステル
743
ヒマシ油 414
ヒメクロモン 740
氷酢酸 269
ビスコジル 367
ビスコジル坐剤 368
ビタミンA油 1229
ビタミンA油カプセル 1230
ビベリデン塩酸塩 366
ビホナゾール 366
ビヤクシ 1257
ビヤクジュツ 1260
ビヤクジュツ末 1260
ビワヨウ 1314
ビンクリスチン硫酸塩 1228
ビンプラスチン硫酸塩 1226
ビンロウジ 1258
ピコスルファートナトリウム水和物
1105
ピブメシリナム塩酸塩 1004
ピベミド酸水和物 996
ピペラシリンナトリウム 997
ピペラジンリン酸塩錠 1000
ピペラジンリン酸塩水和物 999
ピマリシン 994

ピラジナミド 1046
ピラルビシン 1000
ピランテルパモ酸塩 1045
ピリドキシリン塩酸塩 1047
ピリドキシリン塩酸塩注射液 1048
ピリドスチグミン臭化物 1047
ピレノキシリン 1001
ピレンゼピン塩酸塩水和物 1002
ピロールニトリン 1049
ピロ亜硫酸ナトリウム 1108
ピロカルピン塩酸塩 994
ピロキシカム 1003
ピロキシリン 1049
ピンドロール 995

フ

ファモチジン 655
ファモチジン散 657
ファモチジン錠 657
ファロペネムナトリウム錠 660
ファロペネムナトリウム水和物 658
フィトナジオン 993
フェニトイン 991
フェニトイン散 992
フェニトイン錠 992
フェニルブタゾン 990
L-フェニルアラニン 989
フェニレフリン塩酸塩 990
フェネチシリンカリウム 983
フェノール 985
フェノール・亜鉛華リニメント 987
フェノールスルホンフタレイン 988
フェノールスルホンフタレイン注射液
988
フェノール水 987
フェノバルビタール 984
フェノバルビタール散 10% 985
フェンタニルクエン酸塩 661
フェンブフェン 660
複方アクリノール・チンク油 273
複方オキシコドン・アトロピン注射液
953
複方オキシコドン注射液 952
複方サリチル酸メチル精 879
複方サリチル酸精 1080
複方ジアスターゼ・重曹散 567
複方ダイオウ・センナ散 1346
複方チアントール・サリチル酸液
1165
複方ビタミンB散 1230
複方ヨード・グリセリン 770
複方ロートエキス・ジアスターゼ散
1355
フシジン酸ナトリウム 1101
フラジオマイシン硫酸塩 688
フラビンアデニンジヌクレオチドナト
リウム 662
フラボキサート塩酸塩 664
フルオキシメステロン 675
フルオシノニド 672
フルオシノロンアセトニド 670
フルオレセインナトリウム 673

フルオロウラシル 674
 フルオロメトロン 673
 フルシトシン 668
 フルジアゼパム 669
 フルスルチアミン塩酸塩 692
 フルニトラゼパム 670
 フルフェナジンエナント酸エステル
 676
 フララゼパム 677
 フララゼパムカプセル 678
 フララゼパム塩酸塩 678
 フルルビプロフェン 679
 フロセミド 690
 フロセミド錠 691
 フロプロピオン 667
 フロプロピオンカプセル 667
 フロモキシセフナトリウム 664
 粉末セルロース 480
 ブクモロール塩酸塩 378
 ブクリョウ 1337
 ブクリョウ末 1337
 ブシ 1338
 ブシ末 1339
 ブシラミン 377
 ブスルファン 384
 ブチルスコボラミン臭化物 1082
 ブトロピウム臭化物 385
 ブドウ酒 1238
 ブドウ糖 700
 ブドウ糖注射液 701
 ブナゾシン塩酸塩 383
 ブフェキサマク 380
 ブフェキサマククリーム 380
 ブフェキサマク軟膏 381
 ブフェトロール塩酸塩 379
 ブプラノロール塩酸塩 383
 ブメタニド 382
 ブレオマイシン塩酸塩 370
 ブレオマイシン硫酸塩 372
 ブロマゼパム 374
 ブロムヘキシシン塩酸塩 375
 ブロモクリブチンメシル酸塩 376
 ブロモバレリル尿素 377
 プラステロン硫酸エステルナトリウム
 水和物 1107
 プラゼパム 1020
 プラゼパム錠 1021
 プラノプロフェン 1018
 プラバスタチンナトリウム 1018
 プリミドン 1026
 プルラン 1045
 プレドニゾロン 1021
 プレドニゾロンコハク酸エステル
 1024
 プレドニゾロン錠 1022
 プレドニゾロン酢酸エステル 1023
 プロカインアミド塩酸塩 1028
 プロカインアミド塩酸塩錠 1029
 プロカインアミド塩酸注射液 1029
 プロカイン塩酸塩 1030
 プロカイン塩酸塩注射液 1030
 プロカテロール塩酸水和物 1032
 プロカルバジン塩酸塩 1031

プロクロルペラジンマレイン酸塩
 1033
 プロクロルペラジンマレイン酸塩錠
 1033
 プログルミド 1035
 プロゲステロン 1034
 プロゲステロン注射液 1034
 プロタミンインスリン亜鉛水性懸濁注
 射液 766
 プロタミン硫酸塩 1041
 プロタミン硫酸塩注射液 1042
 プロチオナリド 1042
 プロチレリン 1043
 プロチレリン酒石酸塩水和物 1044
 プロテイン銀 1088
 プロテイン銀液 1089
 プロパンテリン臭化 1036
 プロピルチオウラシル 1040
 プロピルチオウラシル錠 1040
 プロピレングリコール 1039
 プロプラノロール塩酸塩 1037
 プロプラノロール塩酸塩錠 1038
 プロベネシド 1027
 プロベネシド錠 1027
 プロメタジン塩酸塩 1036

へ

ヘパリンナトリウム 721
 ヘパリンナトリウム注射液 722
 ヘンズ 1282
 ベカナマイシン硫酸塩 337
 ベクロメタゾンプロピオン酸エステル
 335
 ベザフィブラート 364
 ベザフィブラート徐放錠 365
 ベタネコール塩化物 363
 ベタヒスチンメシル酸塩 353
 ベタヒスチンメシル酸塩錠 354
 ベタメタゾン 355
 ベタメタゾンジプロピオン酸エステル
 358
 ベタメタゾンリン酸エステルナトリウ
 ム 359
 ベタメタゾン吉草酸エステル 360
 ベタメタゾン吉草酸エステル・ゲンタ
 マイシン硫酸塩クリーム 361
 ベタメタゾン吉草酸エステル・ゲンタ
 マイシン硫酸塩軟膏 362
 ベタメタゾン錠 356
 ベニジピン塩酸塩 338
 ベニジピン塩酸塩錠 339
 ベラドンナエキス 1263
 ベラドンナコン 1263
 ベラパミル塩酸塩 1225
 ベラパミル塩酸塩錠 1226
 ベンザルコニウム塩化物 342
 ベンザルコニウム塩化物液 342
 ベンジルアルコール 346
 ベンジルペニシリンカリウム 349
 ベンジルペニシリンベンザチン水和物
 348
 ベンズプロマロン 343

ベンセラジド塩酸塩 340
 ベンゼトニウム塩化物 344
 ベンゼトニウム塩化物液 345
 ベントナイト 341
 ベルベリン塩化物水和物 351
 ペチジン塩酸塩 980
 ペチジン塩酸塩注射液 981
 ペプロマイシン硫酸塩 975
 ペルフェナジン 977
 ペルフェナジンマレイン酸塩 978
 ペルフェナジンマレイン酸塩錠 979
 ペルフェナジン錠 977
 ペンタゾシン 972
 ペントキシペリリングエン酸塩 974
 ペントバルビタールカルシウム 973
 ペンブトロール硫酸塩 972

ホ

ホウ酸 374
 ホウ砂 1095
 抱水クロラール 487
 ホスフェストロール 683
 ホスフェストロール錠 684
 ホスホマイシンカルシウム水和物
 685
 ホスホマイシンナトリウム 686
 補中益気湯エキス 1298
 ホマトロピン臭化水素酸塩 722
 ホミカ 1321
 ホミカエキス 1322
 ホミカエキス散 1323
 ホミカチンキ 1323
 ホモクロルシクリジン塩酸塩 723
 ホリナートカルシウム 391
 ホルマリン 681
 ホルマリン水 682
 ホルモテロールフマル酸塩水和物
 682
 ボウイ 1363
 ボウコン 1302
 ボウフウ 1352
 ボグリボース 1231
 ボグリボース錠 1232
 ボタンピ 1318
 ボタンピ末 1319
 ボレイ 1326
 ボレイ末 1326
 ポビドン 1015
 ポビドンヨード 1017
 ポリスチレンスルホン酸カルシウム
 398
 ポリスチレンスルホン酸ナトリウム
 1106
 ポリソルベート 80 1007
 ポリミキシン B 硫酸塩 1006

マ

マーキュロクロム 862
 マーキュロクロム液 863
 マイトマイシン C 898
 マオウ 1283

マクリ 1280
 マクロゴール軟膏 833
 マクロゴール 1500 831
 マクロゴール 20000 833
 マクロゴール 400 830
 マクロゴール 4000 832
 マクロゴール 6000 832
 マシニン 1298
 麻酔用エーテル 641
 マプロチリン塩酸塩 842
 マルトース水和物 840
 D-マンニトール 841
 D-マンニトール注射液 842

ミ

ミクロノマイシン硫酸塩 893
 ミグレニン 896
 ミコナゾール 892
 ミコナゾール硝酸塩 893
 ミツロウ 336
 ミデカマイシン 894
 ミデカマイシン酢酸エステル 895
 ミノサイクリン塩酸塩 897
 ミョウバン水 293

ム

無晶性インスリン亜鉛水性懸濁注射液 764
 無水アンピシリン 306
 無水エタノール 639
 無水カフェイン 386
 無水クエン酸 514
 無水乳糖 802
 無水リン酸水素カルシウム 396
 ムピロシムカルシウム水和物 902

メ

メキシレチン塩酸塩 891
 メキタジン 861
 メクロフェニキサート塩酸塩 843
 メグルミン 850
 メコバラミン 844
 メシル酸塩ガベキサート 693
 メストラノール 864
 メタンフェタミン塩酸塩 868
 メダゼパム 845
 L-メチオニン 869
 メチ克蘭 884
 メチラポン 890
 メチルエルゴメトリンマレイン酸塩 876
 メチルエルゴメトリンマレイン酸塩錠 877
 dl-メチルエフェドリン塩酸塩 875
 dl-メチルエフェドリン塩酸塩散 10% 876
 メチルジゴキシン 885
 メチルセルロース 871
 メチルテストステロン 882
 メチルテストステロン錠 883

メチルドパ錠 874
 メチルドパ水和物 873
 メチルプレドニゾロン 879
 メチルプレドニゾロンコハク酸エステル 880
 メチルベナクチジウム臭化物 871
 メチルロザニリン塩化物 881
 メテノロンエナント酸エステル 866
 メテノロンエナント酸エステル注射液 866
 メテノロン酢酸エステル 865
 メトキシサレン 870
 メトクロプラミド 886
 メトクロプラミド錠 886
 メトトレキサート 869
 メトプロロール酒石酸塩 887
 メトプロロール酒石酸塩錠 888
 メトホルミン塩酸塩 867
 メトホルミン塩酸塩錠 867
 メトロニダゾール 889
 メトロニダゾール錠 889
 メナテトレノン 855
 メビチオスタン 858
 メビバカイン塩酸塩 859
 メビバカイン塩酸塩注射液 860
 メフェナム酸 847
 メフルシド 849
 メフルシド錠 849
 メフロキシン塩酸塩 848
 メベンゾラート臭化物 857
 メルカプトプリン水和物 861
 メルファラン 855
 メロペナム水和物 863
 dl-メントール 856
 l-メントール 857

モ

モクツウ 1253
 モッコウ 1352
 モノステアリン酸アルミニウム 291
 モノステアリン酸グリセリン 704
 モルヒネ・アトロピン注射液 899
 モルヒネ塩酸塩錠 902
 モルヒネ塩酸塩水和物 900

ヤ

焼セッコウ 1298
 ヤクチ 1265
 薬用石ケン 846
 薬用炭 845
 ヤシ油 535
 ユーカリ油 654

ユ

ユウタン 1262
 輸血用クエン酸ナトリウム注射液 1099
 ユビデカレノン 1214

ヨ

ヨーダミド 767
 ヨーダミドナトリウムメグルミン注射液 854
 ヨード・サリチル酸・フェノール精 772
 ヨードチンキ 769
 ヨードホルム 773
 ヨウ化カリウム 1013
 ヨウ化人血清アルブミン (131I) 注射液 768
 ヨウ化ナトリウム 1102
 ヨウ化ナトリウム (123I) カプセル 1103
 ヨウ化ナトリウム (131I) 液 1103
 ヨウ化ナトリウム (131I) カプセル 1103
 ヨウ化ヒプル酸ナトリウム (131I) 注射液 1103
 葉酸 680
 葉酸錠 681
 葉酸注射液 681
 ヨウ素 768
 ヨクイニン 1276
 ヨクイニン末 1276

ラ

ラウリル硫酸ナトリウム 1104
 ラウロマクロゴール 810
 酪酸リボフラビン 1060
 ラクツロース 804
 ラタモキセフナトリウム 809
 ラッカセイ油 971
 ラナトシド C 805
 ラナトシド C 錠 806
 ラニチジン塩酸塩 1054

リ

リオチロンナトリウム 821
 リオチロンナトリウム錠 822
 リシノプリル錠 824
 リシノプリル水和物 823
 L-リジン塩酸塩 829
 リゾチーム塩酸塩 830
 リトドリン塩酸塩 1067
 リトドリン塩酸塩錠 1068
 リドカイン 818
 リドカイン注射液 818
 リファンピシン 1064
 リファンピシンカプセル 1065
 リボスタマイシン硫酸塩 1063
 リボフラビン 1059
 リボフラビンリン酸エステルナトリウム 1061
 リボフラビンリン酸エステルナトリウム注射液 1062
 リボフラビン散 1060
 リマプロスト アルファデクス 819
 リュウコツ 1313

硫酸アルミニウムカリウム水和物
292

硫酸カリウム 1014

硫酸バリウム 334

硫酸マグネシウム水 839

硫酸マグネシウム水和物 838

硫酸マグネシウム注射液 839

硫酸亜鉛水和物 1246

硫酸亜鉛点眼液 1247

硫酸鉄水和物 661

リュウタン 1306

リュウタン末 1306

流動パラフィン 966

リョウキョウ 1256

荅桂朮甘湯エキス 1347

リンゲル液 1066

リンコマイシン塩酸塩水和物 820

リン酸コデイン散 10% 537

リン酸コデイン散 1% 536

リン酸コデイン錠 537

リン酸コデイン水和物 536

リン酸水素カルシウム水和物 396

リン酸水素ナトリウム水和物 568

リン酸二水素カルシウム水和物 397

レ

レセルピン 1055

レセルピン散 0.1% 1057

レセルピン錠 1057

レセルピン注射液 1056

レチノールパルミチン酸エステル
1059

レチノール酢酸エステル 1058

レナンピシリン塩酸塩 810

レバロルフアン酒石酸塩 813

レバロルフアン酒石酸塩注射液 814

レボチロキシナトリウム錠 817

レボチロキシナトリウム水和物

816

レボドパ 814

レボメプロマジンマレイン酸塩 815

レンギョウ 1286

レンニク 1320

ロ

ロートエキス 1353

ロートエキス・アネスタミン散 1355

ロートエキス・カーボン散 1355

ロートエキス・タンニン坐剤 1357

ロートエキス・パパベリン・アネスタミ
ン散 1356

ロートエキス散 1353

ロートコン 1357

L-ロイシン 812

ロキサチジン酢酸エステル塩酸塩
1070

ロキサチジン酢酸エステル塩酸塩徐放
カプセル 1071

ロキシスロマイシン 1073

ロキソプロフェンナトリウム水和物

828

ロキタマイシン 1069

ロジン 1347

ロラゼパム 827

ワ

ウイルス病秋やみ混合ワクチン 1237

ワルファリンカリウム 1233

ワルファリンカリウム錠 1234